The UK National Culture Collection (UKNCC) Biological Resource: Properties, Maintenance and Management

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Preface

Culture collections have provided a service to the scientific community for over fifty years. The first "service collection" was established by Franticek Král in Prague towards the end of the nineteenth century. In the ensuing years, several service culture collections were established worldwide, not least in the United Kingdom. The traditional role of such collections was to provide the scientific community with access to authenticated cultures and specialist advice on their cultivation and preservation. The *ex situ* conservation of micro-organisms was seen to be essential for ensuring that a source of living cells were readily available for scientific purposes of both a pure and applied nature. This is still the case, especially since micro-organisms isolated from environmental samples cannot always be found again and even if fresh isolates are obtained, they may lack the desired properties expressed by the earlier strains.

In recent years, other services have been added to the curatorial role of service culture collections, such as patent deposit facilities and the supply of cultures for quality control. Indeed, service culture collections have evolved into Biological Resource Centres (BRC's) thereby responding to revolutionary developments in areas such as molecular biology and bioinformatics. The current role of BRC's is to provide the scientific world with access to properly maintained culturable material (eg animal and plant cell lines, archaea, bacteria, fungi, viruses), replicate parts of these (eg CDNA banks, genomes and plasmids), and associated databases. As a consequence of these developments BRC's form an invaluable part of the infrastructure that underpins the conservation of microbial diversity, developments in microbial technology, and ecological structures linked to the sustainability of life support systems.

The need to further develop collections was recognised by advisors to the UK Government in the 1990s resulting in the establishment of the UK National Culture Collection (UKNCC), bringing together expertise and co-ordinating some essential activities. This has created a critical mass to enable the member collections to achieve much more together. More recently the Organisation for Economic Co-operation and Development (OECD) have been discussing the long-term sustainability and development of todays culture collection into tomorrows BRC to better serve the changing needs of the users of micro-organisms, cell lines and the associated data. It has been recognised by both that high quality services must be provided and international linkages established to share tasks and broaden coverage. The UKNCC has made huge strides towards such goals since its conception and this manual outlines some of the key principles and procedures behind such development.

The traditional expertise and new skills that are becoming stock-in-trade for those working in service collections which form the United Kingdom National Culture Collection (UKNCC) are reflected in this microbiological cornucopia. The manual contains a wealth of invaluable specialist information which will be drawn upon by researchers and practitioners in academia, research institutes and industry. The editors and contributors to the manual are to be commended for breaking new ground and in so doing showing the shape of things to come from Biological Resource Centres.

Professor Mike Goodfellow 15 February 2001

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Safety Considerations

All of the procedures described in this book are designed for use by trained researchers in adequately equipped and supervised laboratories. A complete risk assessment should be carried out for each researcher. Many of the chemicals mentioned are listed as toxic, mutagenic, carcinogenic, harmful and/or irritant, while electophoretic procedures use high voltage electricity. Safety awareness is essential when undertaking these methods.

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Chapter 1

Culture collection operation and management

David Smith

1.1 Introduction

The basic elements in microbiology and cell biology are the living organisms or cells themselves, they are the research tools, the producer of compounds, fuel and food and the basis for teaching and education in microbiology. These cells are grown and utilised in huge numbers, in laboratories around the world and are the key to many research programmes, industrial processes and training courses. These organisms must be maintained without change to ensure reproducibility and sustainability. However, they are often placed in the back of a refrigerator, or kept in the incubator until they are needed again. If research is carried out using deteriorated, contaminated or incorrect strains large investments in time, human resources and finance can be lost. Microbiologists often establish their own laboratory collections to ensure their key strains are maintained for future use and for confirmation of results. There are many such collections world-wide maintaining organisms for use in their laboratories and, on occasion, sharing with other researchers in the field. The organisms in such collections are stored by many methods of preservation and the selection of the right method to suit the purpose can often be difficult. Often equipment and facilities are not available and are expensive. Collections must be efficient, yet cost effective, strains must be retained without change yet often the resources are not always to hand.

Biological resource collections range from small private collections through to large service collections, and have widely differing policies and holdings. There are relatively few collections that attempt to maintain organisms solely for the scientific community in general, or have a remit for *ex-situ* conservation of biodiversity. Collections of organisms are normally linked to their use in operations related to those of the parental organisation activities. For example, screening for exploitable metabolites or enzymes, direct use as food, or food modification, as biocontrol agents, waste bioconversion, or waste detoxification.

The need for total stability of an organism's abilities stems from the continuing discoveries of new uses and natural products. A collection may be asked to provide representative strains that may express a property not considered when the strain was first deposited. If strains are not maintained appropriately then these as yet unknown properties could be lost. Methods normally used for long-term stability of organisms are freeze-drying and cryopreservation, either in, or above, liquid nitrogen, or in a low temperature freezer (Smith & Onions, 1994; Smith & Kolkowski, 1996). Methods should be optimised for the cells being preserved and understanding the science of low temperature chemistry and physics and the ability to observe what happens to cells is essential for technique design (Smith, 1992, 1993).

A wealth of knowledge and experience has been built up in culture collections, which is often shared through publications. This book collates key information and shares with the reader protocols and procedures developed over decades by the UK national culture collections and that currently operate in the UK National Culture Collection (UKNCC). The UK public service collections have specialised in specific areas of expertise and kept duplication to a minimum since the network of national culture collections was established in 1947. Some of the UK national collections operated before that time and holdings date back to the end of the nineteenth century.

1.2 The role of public service collections

Public service culture collections are charged with several tasks, which are influenced by access legislation. They are in a unique position as custodians of *ex situ* genetic resources and therefore have a key role to play in the conservation of genetic resources (Kirsop & Hawksworth, 1994). Biologists who collect organisms for their research and publish information on them should make their most important strains available for confirmation of results and future use by depositing them in public service collections. This will aid collections in their major roles of:

- The *ex situ* conservation of organisms
- Custodians of national resources
- Provision of living resources to underpin the science base
- Reception of deposits subject to publication
- Safe, confidential and patent deposit services

To operate such functions collections must follow standard procedures to offer products and services of a consistent nature. Organisations have been established to try and co-ordinate collection activities and aid the sharing of knowledge and experience in maintaining collections of organisms, their safe handling and distribution.

1.3 Culture collection organisations

There are several levels at which co-ordination, collaboration and discussion on approaches to microbial resource collection establishment and organisation is carried out. Organisations exist for the support of collection activities on national, regional, and international bases. These include national federations such as the United Kingdom Federation for Culture Collections (UKFCC), United States Federation for Culture Collections (USFCC), and the Japanese Federation for Culture Collections (JFCC). At the regional level the European Culture Collection Organisation (ECCO), and at the international level, the World Federation for Culture Collections (WFCC), Microbial Strain Data Network (MSDN), and Microbial Resource Centres (MIRCENs) operate. Further information on these organisations can be found in Hawksworth & Kirsop (1988). Collections have also been drawn together to operate at a more intimate level through national and international affiliations such as the Belgium Co-ordinated Collection of Micro-organisms (BCCM), the UK National Culture Collection (UKNCC) and Common Access to Biological Resources and Information (CABRI).

1.3.1 The World Federation of Culture Collections (WFCC)

The WFCC was founded in 1968 and is a multidisciplinary commission of the International Union of Biological Sciences (IUBS) and since the separation of the International Union of Microbiological Societies (IUMS) from IUBS in 1979 it has operated as an inter-union commission. It seeks to promote and foster activities that support the interests of culture collections and their users.

The WFCC has published guidelines agreed by its Executive Board (Hawksworth *et al.,* 1990) for the establishment and operation of collections. These Guidelines are updated regularly and can be viewed on the WFCC web site (http://wdcm.nig.ac.jp/wfcc/index.html). In order that a collection's user can rely upon the organisms supplied, and the services provided, it is imperative that the collection follows good practices. Acceptance of a collection as a member of WFCC offers a limited form of accreditation, but in the future a more formal accreditation scheme may be desirable (Stevenson & Jong, 1992).

Member collections of the WFCC register with the World Data Center for Micro-organisms (WDCM) and there are currently *c.* 500 in 60 countries (Sugawara & Miyazaki, 1999). A congress is held every four years to discuss advances in technology and common policies with regard to biodiversity and the role of culture collections. The WFCC keeps its members informed on matters relevant to collections in its Newsletter and has standing committees reporting on patent depositions, postal, quarantine and safety regulations, safeguard of endangered collections, education, publicity, standards and biodiversity.

1.3.2 The World Data Center for Micro-organisms (WDCM)

Since 1986, the WFCC has overseen the activities of the World Data Center for Micro-organisms (WDCM) and it is now the data center for the WFCC and Microbial Resource Centers (MIRCENs) Network. The WDCM is supported by UNEP and UNESCO and the database holds information on collections, the species they hold and details on their specialisation. It was established in 1968 and produced the first hard copy volume of the *World Directory of Collections of Cultures of Micro-organisms* in 1972, whilst based at the University of Queensland, Australia. The WDCM relocated in 1986 to RIKEN, Saitama, Japan and then again in 1999 to the National Institute of Genetics, Japan. The *World Directory* (Sugawara & Miyazaki, 1999) illustrates some of the data held; it has indexes by country, main subjects studied, cultures held, the culture availability, their staff, and services offered. These data can also be accessed on internet – http://www.wdcm.ac.jp.

The WDCM collections hold in excess of 800 000 strains, 44% are fungi, 43% bacteria, 2% viruses, 1% live cells, and 10% others (including plasmids, plant, animal cells and algae). Of the total, 35% of the holdings are maintained by only 10 collections. Statistics provided by Sugawara, based on information in Sugawara *et al.*. (1993) show that European collections hold approximately 36% of the strains with over 52 000 available for exchange, 49 000 for a fee and 46 000 free. In comparison the USA provide access to 31% and Asia to 15% of the strains registered in the WDCM.

1.3.3 The Microbial Strain Data Network (MSDN)

The MSDN is a non-profit organisation providing an information and communication network for microbiologists and biotechnologists facilitating electronic mail, bulletin boards, computer conferences and databases. It is a distributed network linking databases and shares its activities with different groups worldwide. MSDN has servers at the Tropical Data Base, Campinas, Brazil, the World Data Centre for Micro-organisms, the Bioinformatics Distributed Information Center, India and the Microbial Information Network, China.

Around 60 laboratories housing microbial resource collections are indexed, collections from over 10 countries have their catalogues on-line through the MSDN and there are links to related resources, http://panizzi.shef.ac.uk. The secretariat is based at 63 Wostenholm Road, Sheffield, UK, msdn@sheffield.ac.uk.

1.3.4 Microbial Resource Centres (MIRCENs)

In 1974 UNEP, UNESCO and ICRO established the MIRCEN network and in 1978 the first evaluation of MIRCEN activities was carried out. This led to the establishment of MIRCENs in industrialised countries and the production of MIRCEN News in 1980 to help publicise the activities of the network. In 1985 Oxford University Press published the MIRCEN Journal of applied microbiology and biotechnology and UNESCO has recommended consolidation and expansion of the MIRCEN network (Da Silva, 1988).

The objectives of the worldwide network of MIRCENs are to preserve and exploit microbial gene pools, make them accessible to developing countries and to carry out research and development in environmental microbiology and biotechnology. Sixteen centers are listed by Kirsop & DaSilva (1988). The MIRCEN network carries out various activities to meet these ends including training and the provision of information. Further details can be obtained from The MIRCEN secretariat, Division of scientific research and higher education, United Nations Educational Scientific and Cultural Organisation (UNESCO), 7 Place de Fontenoy, 75700 Paris, France, Tel: 010 331 4568 3883 Fax: + 331 430. Information on the MIRCEN fellowships is available on http://www.unesco.org/science/life/life1/rcenform.htm

1.4 The United Kingdom National Culture Collection (UKNCC)

The United Kingdom National Culture Collection (UKNCC) co-ordinates the activities, marketing and research of the UK national service collections. It was established through the implementation of the UK government's strategy for UK microbial collections in 1996. This UK initiative brings together 9 national collections (one on two sites) under the UK National Culture Collection (UKNCC). The Office of Science and Technology (OST) established the UKNCC following the Government's response to an independent review of UK microbial culture collections. The UKNCC addressed three specific initiatives: to improve the profile and marketing of the collections, to fund a molecular

characterisation programme and to establish an animal virus collection. The Biotechnology and Biological Sciences Research Council (BBSRC) administered the first phase through the Culture Collection Advisory Group (CCAG), a steering group selected to advise and support these activities. The UKNCC is now operating independently advised by a Steering group and a Commercial opportunities group and the day to day activities co-ordinated by the Curators' group.

The UKNCC collection holdings number more than 73000 (*c* 2300 algae and protozoa, 20000 animal cell lines, 25000 bacteria, 25000 fungi including yeasts, plus actinomycetes, cyanobacteria, nematodes, mycoplasma and viruses). The supply of these organisms, associated services and expertise of the 9 national culture collections that make up the UKNCC are available to support your microbiological and cell culture needs. The services and products provided by the member collections are detailed on the UKNCC web site where access is given to the information at one point (a one-stop-shop): http://www.UKNCC.co.uk. The collections primary interests are generally in identification and preservation of their holdings but they and their parental organisations offer many more services.

Key activities

- A single point of contact: The UKNCC web site
- Establishment of a distributed electronic database
- A unified marketing strategy
- Implementation of a UKNCC quality management system
- Collaborative research to enhance expertise and strain data

Such activities have made UK biological resources more accessible but to facilitate biosciences further such activities must develop globally. There are similar initiatives in Belgium where the Belgian Co-ordinated Collection of Micro-organisms (BCCM) (http://www.belspo.be/bccm) has been established and on a European scale the Common Access to Biological Resources and Information (CABRI) project has been developed (http://www.cabri.org).

1.5 Culture collection quality management

1.5.1 Background

It is crucial that the organisms for use in biotechnology are maintained in a way that will retain their properties. Biological resource collections must ensure a quality product providing standard reference material that will give reproducible results. To achieve this collections must apply quality control and assurance measures to maintain these standards taking into account the needs of users and of the facilities and resources available.

The need for common standards is evident as the task of maintaining representative samples of microbial diversity cannot be achieved by one collection alone. It is therefore essential that a world-wide network of collections exists to provide the coverage the user requires. For example there are an estimated 1.5 million species of fungi (Hawksworth, 1991). However, there is not one strain of the majority of these species that can represent the full spectrum of morphology and physiology expressed

within that species and therefore there is a requirement to retain a range of representative strains. To make any impression on such an enormous task with the fungi, and other micro-organisms, there must be a focus for each collection and a sharing of tasks between them. In order that a customer of such a network would get a consistent level of service and quality it is necessary to set standards for all collections to attain. These standards would also provide a useful target for new collections to achieve. Although there are guidelines set for the establishment and operation of collections *(*http://wdcm.nig.ac.jp/wfcc/index.html*)*, they do not cover all protocols or procedures, nor do they set minimum requirements. Standards are necessary to increase the quality in collections and to provide a sound footing to ensure they offer the service to science and industry that is required today and provide stable reference material for the future.

1.5.2 Biological resource collection standards

Examples of existing standards for collections are:

- The WFCC *Guidelines for the establishment and operation of collections of micro-organisms (*http://wdcm.nig.ac.jp/wfcc/index.html).
- The Microbial Information Network for Europe (MINE) project standards for the member collections (Hawksworth & Schipper, 1989).
- Genebank standards (Anon, 1994). Food and Agriculture Organisation of the United Nations, Rome, International Plant Genetic Resources Institute, Rome.
- UKNCC quality management system (http://www.ukncc.co.uk).
- Common Access to Biological Resources and Information (CABRI) guidelines (http://www.cabri.org).

There are more specific standards set for microbiology laboratories such as Good Laboratory Practice (GLP), British Standard 5750, UK Accreditation Service (UKAS) formerly the National Measurement Service (NAMAS) - ISO Guide 25 and ISO 9000. Industry are expressing the need for quality control and standards within collections and although publications on collection management and methodology give information on protocols and procedures (Hawksworth & Kirsop, 1988; Kirsop & Kurtzman, 1988; Kirsop & Doyle, 1991; Smith & Onions, 1994) the UKNCC quality management system goes further toward setting minimum standards. A Quality assurance scheme exists for plant genetic resources. In 1993 the Commission on Plant Genetic Resources endorsed *The Genebank Standards* for use as the international reference in national, regional and international genebanks (Anon, 1994). The international network of genebanks have the responsibility to hold, for the benefit of the international community, plant genetic resources and make them available without restriction. The genebank standards are solely concerned with the storage of seeds of orthodox species that can survive very considerable desiccation and for which longevity is improved by reducing seed storage moisture content and/or temperature. It is evident that microbial resource collections could benefit from the general application of similar standards. The genebank standards provide targets for participating institutes to achieve with the ultimate objective of long-term safe and sustainable conservation. A tiered system is described providing both minimal and preferred higher standards. They acknowledge differences in the storage of original material, not usually for distribution directly to the user, and that

of collections where the material is immediately available, laying down criteria including the number of generations acceptable between the original material and that supplied to the end users.

The standards demand set treatments and conditions in many areas:

- Control of environmental conditions
- Standardised drying protocols
- Seed cleaning and health standard criteria for storage containers
- Defined seed storage conditions
- Size of sample preserved
- Viability monitoring
- Minimum information
- Standards for exchange
- Adherence to quarantine and other regulations
- Personnel and training
- Safety and security of the germplasm

There are critical elements to standard procedures in the handling, storage, characterisation and distribution of micro-organisms and cell lines and to the handling of associated information. Mechanisms for ensuring reproducibility and monitoring such procedures lead to the establishment of common standards.

Identified, authenticated strains

The collection of identified, authenticated strains is essential. Collecting material without descriptive data will cause unnecessary duplication and waste resources. An organism is not very useful if nothing is known about it. If the organism cannot be fully identified then descriptive data, photomicrographs, metabolic profiles, sequencing data are extremely useful. Collections must store characterised strains and the methods used to record such data must be standardised. There are many collection databases to be used as models one in particular is the MINE database (Gams *et al.,* 1988; Stalpers *et al.,* 1990).

Purity

Strains must be pure or mixtures defined or noted. The purity of strains should be checked and recorded before preservation, immediately after and during storage. The preferred standard would be that no contamination at all is accepted. There would be exceptions where strains cannot be grown without their symbiont, host, or food organisms but it would be imperative that this is recorded and defined.

Viability

The viability of strains should be checked and recorded before preservation, immediately after and during storage. The programme for testing depends upon the methods used. The data obtained will demonstrate if a strain is deteriorating during storage. For fungi the preferred level of viability might be the germination and subsequent development of in excess of 75% of propagules/cells although an acceptable level may be set at 50% or possibly lower for some cell types. Obviously such levels may

not be possible to attain with some cells and standards would have to be set accordingly. Any deviation from the standard would need to be explained and recorded.

Stability of strain properties

A programme of tests to ensure stability of strains must be put in place. Known properties can be checked periodically but full metabolic profile checks are seldom necessary on a regular basis. However, to be able to judge stability a less stable property should be selected to indicate how well a strain is being maintained.

Methodology

Optimised techniques and standard procedures should be adhered to. It is necessary that procedures are documented, so that all staff can follow them and that in the future the methods and the treatments used can be traced. This would include all measuring and recording techniques from viability and purity checks through preservation methods to characterisation and the checking of properties. The accepted level of deviation from measurable parameters must be set and records maintained to show that performance is within the accepted limits. A procedures manual is essential to ensure continuity and new staff must be trained to ensure the attainment of the standards set. Training must include how to carry out methods, but also the accepted levels of result, monitoring and what must be recorded and where data is maintained.

Equipment

All equipment used in the collection must be regularly maintained and calibrated and must operate to set limits. All details must be recorded so that you can ensure traceability and reproducibility.

Long-term security

There is little point in establishing a collection without considering the long-term security of its holdings. If procedures are put in place that cannot be maintained in the future then a considerable waste of time and resources is inevitable. Freeze-dried collections need little maintenance. A frozen collection needs only to be kept cold. A collection must ensure that there is a back-up, organisms should be stored by a minimum of two techniques, and that both working and security stocks are maintained. At least all “important” strains should be stored on another site with all the information about them as a “disaster” measure.

Auditing/ monitoring

It is essential that adherence to set standards is monitored at every level. The appointment of auditors from other departments, or even from outside the organisation (required for many accreditation schemes), is beneficial. The recording of such monitoring is vital to demonstrate competence and self-checks should also be part of a good management system.

Compliance with legislation

There are many regulations that apply to the work of collections from the collecting, through the handling to their dispatch and transport (Smith *et al.,* 1999). Collection workers must be aware of such legal requirements not only in their own countries but internationally. Examples of the areas covered by regulations are:

- Packaging, shipping and transport
- Quarantine
- Health and safety
- Patenting
- Access to national genetic resources

Accreditation

There are several national and international schemes that can be followed to give a standard of quality assurance accepted by a collection's customers. Particularly relevant are ISO 9000 and BS 5750. Another that can be gained is the UKAS accreditation for particular services the laboratory provides, for example testing of materials for microbial resistance. There is no accreditation scheme that has been specifically designed for culture collections but the aforementioned are well recognised and can be applied. It is becoming more common for a collection to be asked what accreditation scheme or protocols they follow to ensure quality control of the product.

High standards are required to meet the requirements of the users of microbial resource collections of today. At the very least this requires set methods and levels of acceptability, recording of results and an independent monitoring system to enable the long-term security and sustainability of holdings.

References

Anon (1994). *Genebank standards.* Food and Agriculture Organisation of the United Nations, Rome, International Plant Genetic Resources Institute, Rome. ISBN 92 9043 236 5.

Da Silva, E. (1988). UNESCO, Microbial Resources and the GIAMS Twenty Five Years: A Brief Review. In: *Recent Advances in Biotechnology and Applied Biology* (edited by S.T.Chang, K.Y.Chan and N.Y.S. Wood) pp 89-101. Hong Kong: The Chinese University Press.

Gams, W., Hennebert, G.L., Stalpers, J.A., Jansens, D., Schipper, M.A.A., Smith, J., Yarrow, D. & Hawksworth, D.L. (1988). Structuring strain data for the storage and retrieval of information on fungi and yeasts in MINE, Microbial Information Network Europe. *Journal of General Microbiology* **134**, 1667-1689.

Hawksworth, D.L. (1991). The fungal dimension of biodiversity; magnitude, significance and conservation. *Mycological Research* **95**, 641-655.

Hawksworth, D.L., Sastramihardja, I., Kokke, R. & Stevenson, R. (1990). *Guidelines for the Establishment and Operation of Collections of Cultures of Micro-organisms*, pp 16. WFCC Secretariat, Brazil: WFCC.

Hawksworth, D.L. & Kirsop, B.E. (eds) (1988). *Living Resources for Biotechnology*: *Filamentous Fungi*, pp 209. Cambridge: Cambridge University Press.

Hawksworth, D.L. & Schipper, M.A.A. (1989). Criteria for consideration in the accreditation of culture collections participating in MINE, the Microbial Information Network Europe. *MIRCEN Journal* **5**, 277-281.

Kirsop, B.E. & DaSilva, E.J. (1988), Organisations of resource centres. In: *Living Resources for Biotechnology: Filamentous Fungi* (edited by D.L. Hawksworth and B.E. Kirsop), pp. 173 - 187. Cambridge: Cambridge University Press.

Kirsop, B.E. & Doyle, A. (eds) (1991). Maintenance of Micro-organisms and Cultured Cells: *A Manual of Laboratory Methods*, pp 308. London: Academic Press.

Kirsop, B. & Hawksworth, D.L. (1994). *The Biodiversity of Micro-organisms and the role of microbial resource centres. Brauschweig,* Germany: World Federation for Culture Collections (WFCC).

Kirsop, B.E. & Kurtzman, C.P. (eds.) (1988). *Living Resources for Biotechnology: Yeasts*, 234 pp., Cambridge, UK: Cambridge University Press.

Smith, D. (1992). Optimising preservation. *Laboratory Practice* **41**, 25-28.

Smith, D. (1993). Tolerance to freezing and thawing. *In*: Tolerance of fungi. (edited by D.H. Jennings). pp.145-171. New York, USA: Marcel Dekker Inc.

Smith, D. & Kolkowski, J. (1996). Fungi *In*: Preservation and Maintenance of cultures used in Biotechnology and Industry, pp 101-132. USA: Academic Press.

Smith, D. & Onions A.H.S. (1994). The Preservation and Maintenance of Living Fungi. Second edition. IMI Technical Handbooks No. 2, pp 122. Wallingford, UK: CAB INTERNATIONAL.

Smith, D. Rhode, C. & Holmes, B. (1999). Handling and distribution of micro-organisms and the law. *Microbiology Today* **26**, 14-16.

Stalpers, J.A., Kracht, M., Jansens, D., De Ley, J., Van der Toorn, J., Smith, J., Claus, D. & Hippe, H. (1990). Structuring strain data for storage and retrieval of information on bacteria in MINE, Microbial Information Network Europe. *Systematic and Applied Microbiology* **13**, 92-103.

Stevenson, R.E. & Jong, S.C. (1992). Application of good laboratory practice (GLP) to culture collections of microbial and cell cultures. *World Journal of Microbiology and Biotechnology* **8**, 229-235.

Sugawara, H. & Ma, J., Miyazaki, S. (eds) (1999). *World Directory of Collections of Cultures of Micro-organisms*. Fifth edition, pp 140. Japan: WFCC World Data Center on Micro-organisms.

Sugawara, H., Ma, J., Miyazaki, S., Shimura, J. & Takishima, Y. (eds) (1993) *World Directory of Collections of Cultures of Micro-organisms*. Fourth edition, pp 1148. Japan: WFCC World Data Center on Micro-organisms.

Chapter 2

Legislation affecting the collection, use and safe handling of micro-organisms

David Smith and Sarah Clayton

2.1 Introduction

The collection, isolation, handling, maintenance and distribution of micro-organisms and cell lines are controlled by law at the national, regional and international levels. Microbiologists and in particular the providers of living biological resources, must be aware of such legislation and operate within it. This presents additional responsibilities for the collection worker and the consequences for not keeping up to date as legislation continues to develop and change can be catastrophic (Smith, 1996, 2000).

The Convention on Biological Diversity (CBD), signed in Rio de Janeiro in 1992 and which came into force at the end of 1993 (CBD, 1992) has now been ratified by more than 170 countries (CBD web site - http://www.biodiv.org/). The CBD provides sovereign rights over genetic resources to the country of origin and controls access to *in situ* organisms. In the simplest of terms, the CBD requires a biologist who wishes to collect genetic resources to seek prior informed consent from the relevant authorities and to negotiate fair and equitable sharing of benefits that may arise from their use before access can be granted. The conditions agreed should include whether strains can be deposited and distributed from public service collections. As a result the biological resource collection receiving the strain is obliged to inform recipients of cultures of their conditions of its use. The collection is therefore placed in a critical position to ensure there is a link maintained between the genetic resource and the user so that the CBD operates as it was intended. The Conferences of the Parties (COP) to the CBD continue to discuss access to genetic resources and the situation is ever changing. The Convention and national legislation on access to genetic resources place an enormous duty on the shoulders of the collector, they are not intended to prevent the advancement of science.

Organisms of hazard groups 2, 3 and 4 (see Section 2.4.1 for definitions of hazard groups) are hazardous substances under the UK Control of Substances Hazardous to Health (COSHH) legislation (COSHH, 1988). They fall under the EU Biological agents directives and are dangerous goods as defined by the International Air Transport Association (IATA) Dangerous goods regulations (IATA, 2000), where requirements for their packaging are defined. In addition, there are restrictions on distribution imposed by national postal authorities and some prohibit the transport of Infectious, Perishable Biological Substances (IPBS) and, in some cases, Non-infectious Perishable Biological Substances (NPBS), including hazard group 1 organisms. The Universal Postal Union (UPU, 1998) publishes such information. Irrespective of whether organisms are shipped by mail, courier or by hand and whether between or within countries, thought must be given to the regulations that control these matters. The World Federation for Culture Collections (WFCC) Committee on postal, quarantine and safety (http://wdcm.nig.ac.jp/wfcc/index.html) attempts to keep abreast of the continuously evolving regulations and inform their membership through newsletters and reports of new and changing rules

(Smith, 1996). The EC Directives 93/88/EEC and 90/679/EEC on biological agents set mandatory control measures for laboratories requiring that risk assessments are carried out on all organisms handled. This necessitates the assignment of each strain to a hazard group following a thorough risk assessment including a positive inclusion into hazard group 1 when they are not categorised in hazard groups 2, 3 or 4. Copies of EC Directives are available from the Office for official publications of the European Communities, L-2985 Luxembourg. The risk assessment should include an assessment of all hazards involved, including the production of toxic metabolites and the ability to cause allergic reactions. Organisms that produce volatile toxins or aerosols of spores or cells present a greater risk. It is the responsibility of the microbiologist to provide such assessment data to a recipient of a culture to ensure its safe handling and containment.

Health and safety precautions are not limited to the laboratory in which the organisms are handled they extend to all those who may come in contact with substances and products from that laboratory. An organism in transit will potentially put carriers, postal staff, freight operators and recipients at risk, some organisms being relatively hazard free whilst others are quite dangerous. It is essential that safety regulations such as COSHH, and shipping regulations be followed to ensure safe transit. Sound packaging and correct labelling and information must be used to minimise risk.

2.2 Ownership of Intellectual Property Rights (IPR)

Organisms originating from different habitats all over the world are deposited in collections. On deposit the issue of ownership of intellectual property associated with them must be addressed. The CBD bestows sovereign rights over genetic resources to the country of origin, but intellectual property rights covering their use in processes is another matter. The CBD requires that the country of origin has a share in benefits accruing from such use, but there may be several other stakeholders. These may include the landowner where the organism was isolated, the collector, those involved in purification and growing the organism, the discoverer of the intellectual property, the collection owner where the organism was preserved and the developer of the process. It is clear that all stakeholders do not all have an equal stake, this will depend upon the input of each one to the discovery or process. This has implications for the sharing of benefits arising from exploitation of the genetic resource. The collection has a role to play in the protection of IPR even if it is merely informing the recipient of any existing material transfer agreement or the citation of the strain in a patent. The implementation of the CBD is still being discussed by delegates from the countries signatory to it who meet at the Conference of the Parties and their workgroups. Information on the progress of these discussions can be found on the CBD web site (http://www.biodiv.org/).

The general principles of international patent law require that details of an invention must be fully disclosed to the public. Inventions that utilise living organisms present problems of disclosure as a patented process often cannot be tested following the publication of a written description alone. Organisms are not patentable in their natural state or habitat, new species are discoveries not inventions. Although genetically manipulated micro-organisms are usually considered as a human

invention and are therefore patentable. If a process involving an organism has novelty, inventiveness, utility or application and sufficient disclosure it can be subject to patent (Kelley & Smith, 1997). The invention of a product, a process of manufacture or a new use for a known product is an intellectual property owned by the inventor whether it involves an organism or not.

In many cases the organism involved must be part of the disclosure and many countries either recommend or require by law that a written disclosure of an invention involving the use of organisms be supplemented by the deposit of the organism into a recognised culture collection. Most patent lawyers recommend that the organism is deposited, regardless of it being a requirement, to avoid the possibility of the patent being rejected. To remove the need for deposit of organisms in a collection in every country where patent protection is desired, the "Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for the Purpose of Patent Procedure" was concluded in 1977 and came into force towards the end of 1980. This recognises named culture collections as "International Depository Authorities" (IDA) and a single deposit made in any one is accepted by every country party to the treaty. Any collection can become an IDA providing it has been formally nominated by a contracting state and meets certain criteria. There are 29 IDAs around the world and 6 in the UK which accept patent deposits of human and animal cell lines, algae, bacteria, cyanobacteria, fungi, nematodes, non-pathogenic protozoa, plant seeds and yeasts (Table 2.1).

Table 2.1 Collections designated as IDA's in the UKNCC

CABI Bioscience UK Centre (Egham)	Bacteria, fungi, nematodes, yeasts
Culture Collection of Algae and Protozoa	Algae, cyanobacteria and protozoa
European Collection of Cell Cultures	Animal cells and hybridomas; animal viruses; DNA probes
National Collections of Industrial, Food and Marine Bacteria	Bacteria; phages; plasmids; plant seeds; yeasts
National Collection of Plant Pathogenic Bacteria	Phytopathogenic bacteria
National Collection of Type Cultures	Bacteria

The Budapest Treaty provides an internationally uniform system of deposit and lays down the procedures which depositor and depository must follow, the duration of deposit and the mechanisms for the release of samples. Thirty-six states and the European Patent Office are now party to the Budapest Treaty.

The World Intellectual Property Organisation (WIPO) publishes data on the numbers of micro-organisms deposited in collections under the terms of the Budapest Treaty (1977). Since the treaty's inception, there were 24 712 deposits up to December 1994 (Anon, 1996a). Patent protection is covered in Article 16 of the CBD under which parties must co-operate. However, this is subject to national legislation and international law, to ensure patents and other intellectual property rights are

supportive of, and do not contravene, the objectives of the Convention (Fritze, 1994). This remains an area of dispute as the article leaves open the possibility that the CBD takes priority over national patent law. Patent law and the CBD are generally compatible but can conflict in cases where exploitation may endanger the resource. In many cases where organisms are grown artificially there is no threat to the existence of the species. Details of the requirements for a collection that relate to the deposit of an organism can be obtained directly from IDA collections and are summarised by Kelley & Smith (1997).

It is quite clear that every intermediary in an improvement or development process is entitled to a share of the IPR, which adds another dimension to ownership. Therefore, it is critical that clear procedures on access, mutually agreed terms on fair and equitable sharing of benefits and sound material transfer agreements are in place to protect the interested parties.

2.3 The Convention on Biological Diversity (CBD)

The CBD aims to encourage the conservation and sustainable utilisation of the genetic resources of the world and has a number of articles that affect biologists. These cover:

- Development of national strategies for the conservation and sustainable use of biological diversity
- Identification, sampling, maintenance of species and their habitats and the production of inventories of indigenous species
- Encouragement of *in situ* and in-country *ex situ* conservation programmes
- Adoption of economically and socially sound measures to encourage conservation and sustainable use of genetic resources
- Establishment of educational and training programmes and the encouragement of research
- Commitment to allow access to genetic resources for environmentally sound uses on mutually agreed terms and with prior informed consent
- Fair and equitable sharing of benefits and transfer of technology resulting from exploitation of genetic resources
- Exchange of information
- Promotion of technical and scientific co-operation

The CBD requires that Prior Informed Consent (PIC) be obtained in the country where organisms are to be collected before access is granted. Terms, on which any benefits will be shared, must be agreed in advance. The benefit sharing may include monetary elements but may also include information, technology transfer and training. If the organism is passed to a third party it must be under terms agreed by the country of origin. This will entail the use of material transfer agreements between supplier and recipient to ensure benefit sharing with, at least, the country of origin. Many biological resource centres or culture collections have operated benefit sharing agreements since they began, giving organisms in exchange for deposits and re-supplying the depositor with the strain if a replacement is required. However, huge rewards that may accompany the discovery of a new drug are illusory as the

hit rate is often reported as less than 1 chance in 250 000. In the meantime, access legislation and the hope for substantial financial returns from isolated strains are restricting the free deposit in public service collections and the legitimate free movement of strains. An EU DG XII project, Micro-organisms Sustainable Use and Access Regulation International Code of Conduct (MOSAICC) is developing mechanisms to allow traceability and enable compliance with the spirit of the CBD and with national and international laws governing the distribution of micro-organisms, whilst not restricting scientific goals (Davison *et al.,* 1998). The development of such common procedures is an evolutionary process and the co-ordinators of this project have placed the document on their web site and amend it as it develops (http://www.bccm.belspo.be).

There are many concerns that exist and these will take time to resolve. In the meantime, countries are developing legislation to control access to their genetic resources and biologists are struggling to comply. The International Union for the Conservation of nature (IUCN) has produced a guide to designing legal frameworks to determine access to genetic resources (Glowka, 1998) which examines the convention and national access legislation. In the Philippines, Executive order 247 puts in place a mechanism to ensure it controls access to and use of its genetic resources. The Andean countries have also developed their own regulations and procedures. The CBD secretariat offers information on mechanisms to attain workable regulations (http://www.biodiv.org/).

The Convention should not affect the functions of public service collections (see Chapter 1) but it increases the importance and extent of their role. However, to date little guidance has been given to collections to determine actions necessary to comply with the CBD. Collections have therefore developed several approaches independently.

- Statements are prominently displayed on accession forms and on information accompanying delivery of strains, explaining the implications of, and requesting compliance with, the convention. This draws attention to the requirements, but does not protect the sovereign rights of the country of origin nor any other stakeholder.
- A requirement for depositors to declare in writing that PIC has been obtained and that this includes unrestricted distribution of the materials to third parties or has clearly defined conditions on distribution.
- A requirement for a signed material transfer agreement on supply of material including mutually agreed terms.

These are minimum requirements and should be followed by all. Difficulty lies in defining the beneficiaries and what is a fair and equitable sharing of benefits. It is also difficult at this stage of implementation of the CBD for collections and depositors alike to comply, as in most countries a PIC authority is difficult to identify. In such cases, proof can be provided to demonstrate that a depositor has made reasonable efforts to get permission to collect from landowners and a Government Office.

Several organisations have addressed issues on IPR and the CBD and have developed and published their policies. These organisations include large national collections, international organisations and

industrial companies. For example, CAB International (CABI), an intergovernmental organisation established by treaty, dedicated to improving human welfare through the application of scientific knowledge in support of sustainable development world-wide, with emphasis on agriculture, forestry, human health, conservation of natural resources and with particular attention to the needs of developing countries (http://www.cabi.org). The CABI Genetic Resources Collection (GRC) is based at CABI Bioscience in Egham, UK and is a member collection of the UKNCC. It is tasked with the collection of organisms to provide a resource for the scientific programmes of CABI and to underpin biotechnology, conservation and science in its member countries. CABI maintains extensive collections that originate from many different countries and has introduced policy and procedures to ensure compliance with the requirements of the CBD. This policy was agreed by member country representatives and published in the 13th Review conference proceedings (Anon, 1996b).

The CABI policy offers an example of a mechanism to enable compliance with the CBD. CABI complies with national legislation of member country governments concerning rights over natural resources and access to genetic material and operates in a manner consistent with the CBD. It protects the interests of the source country of each element of biodiversity. The work of CABI adds value to the material held, particularly by ensuring authoritative identifications. It makes its reference collections and the information on them available to institutions in the countries of origin and the wider scientific community. CABI and the UKNCC keep the rapidly changing situation under review and will adopt procedures required to maintain operations within the spirit of the convention.

Supply agreements are often put in place but new deposits are equally controlled. For example before strains can be accepted into the CABI collections, confirmation is required from the depositor that the collector has made reasonable efforts to obtain PIC to collect the organisms and also has permission to deposit them in a public service culture collection. This confirmation forms part of the accession form that must accompany the deposit. Collections also need to know whether there is any restriction on further distribution and if there are conditions that must be included in any material transfer agreement that may accompany the samples when they are passed to a third person. Such information is required from all depositors regardless of the country of origin of the material or the collector.

Biological Resource Collections, like the UKNCC public service collections, often add value to received and collected biological material. This is done through purification, expert preparation, authoritative identification, description, determination of biochemical and other characteristics, comparison with related material, safe and effective storage/preservation, evaluation of value for specified uses and indication of importance of beneficial and detrimental attributes. They often provide samples of deposited organisms free of charge to the depositor and participate in capacity building projects to help establish facilities and expertise in-country to maintain *ex situ* collections. This plays a role in the utilisation of genetic resources and defines a collection as a stakeholder.

There are several problems that can impede the development of procedures for compliance with the CBD and these will need some time to resolve.

- Definition of the precise role and responsibilities of public service collections within the CBD
- Clarification is required on ownership, intellectual property rights and benefit sharing
- Identification of country authorities who can grant prior informed consent
- Identification of stakeholders and assessment of the value of their input
- Establishing, a clear, simple and flexible approach that avoids impractical bureaucracy
- Monitoring and enforcement of procedures put in place
- Keeping up to date with legislation

The CBD is not an opportunity for all to benefit financially and prospects of accruing huge profits from exploiting an organism for the country of origin are small. Additionally, the process from sampling to market can take from 8-15 years, therefore nothing will be achieved quickly and is likely to require considerable investment. The CBD was negotiated to protect genetic resources and thus ensure their sustainable use.

The agreement on Trade-related Aspects of Intellectual Property Rights (TRIPs) is thought to conflict with the CBD where there is concern that developing countries are required to allow companies to take out patents on products and processes of biotechnology. There are several forms of intellectual property rights that are relevant to the convention in addition to patents, for example copyright, trade secrets and plant breeder's rights. The CBD requires that terms for technologies subject to IPR protection should recognise and be consistent with adequate and effective protection of IPR (Glowka, 1998). In reality, so long as there is an agreement on mutually agreed terms for benefit sharing with the country of origin, the TRIPs agreement and patenting do not run contrary to the CBD.

2.4 Health and safety

Organisms can present several challenges to health and safety including infection, poisoning and allergies (Anon, 1993a, b; Stricoff & Walters, 1995). Handling, distribution and use of organisms are therefore controlled by regulations. Operators of microbiology laboratories or culture collections must follow the basic requirements needed to establish a safe workplace are should provide:

- Adequate risk assessment
- Provision of adequate control measures
- Provision of health and safety information
- Provision of appropriate training
- Establishment of record systems to facilitate safety audits
- Implementation of good working practices

Good working practice requires assurance that correct procedures are being followed and this requires a sound and accountable safety policy.

The UK Management of Health and Safety at Work (MHSW) Regulations 1992 (Anon, 1992) are all-encompassing and general in nature but overlap and lead into many specific pieces of legislation. The Control of Substances Hazardous to Health (COSHH) regulations require that every employer makes a suitable and sufficient assessment of the risks to health and safety to which any person, whether employed by them or not, may be exposed through their work (Anon, 1996d). These assessments must be reviewed regularly, additionally when changes in procedures or regulations demand, and must be recorded when the employer has more than five employees. The distribution of micro-organisms to others outside the workplace extends these duties to protect others. Such assessments of risk are extended to other biological agents, such as entomopathogenic nematodes, through EC council directives on biological agents (90/679/EEC; 93/88/EEC).

The Control of Substances Hazardous to Health (COSHH) regulations aim to stimulate and enforce an improvement in health and safety in the workplace (Simpson & Simpson, 1991). All principles embodied in the COSHH regulations are contained in the UK Health and Safety at Work Act 1974. COSHH formalises, enforces and in some instances, extends certain sections of this act. The COSHH regulations (1988) require a suitable and sufficient risk assessment for all work that is liable to expose an employee to any substance that may be hazardous to health. This UK legislation has equivalents in other countries and at the European level, but in common with all health and safety legislation, is not comprehensive and leaves much open to interpretation.

2.4.1 Assessment of risk

Organisms present different levels and kinds of hazard, evaluation of which represents an enormous, but necessary, task for biologists. A risk assessment for example, must take into account the production of potentially hazardous toxins. Ultimately, a safe laboratory is the result of applying good techniques, a hallmark of technical excellence. Containment level 2 (Anon, 1996c) is easily achievable and should be standard practice in all laboratories handling organisms that present a risk of infection or of causing other harm. Good aseptic techniques applied by well-trained personnel will ensure pure and clean cultures and will minimise contact with the organism. However, the possibility of accidents must also be taken into account when assessing risks. The employment of good laboratory practice and good housekeeping, workplace and equipment maintenance, together with ensuring that staff have relevant information and training, will minimize the risk of accidents (Smith & Onions, 1994). The establishment of emergency procedures to reduce potential harm is an additional and sensible precaution.

Various classification systems exist, including those of the World Health Organisation (WHO); United States Public Health Service (USPHS); Advisory Committee on Dangerous Pathogens (ACDP); European Federation of Biotechnology (EFB) and the European Commission (EC). In Europe, the EC Directive (93/88/EEC) on Biological Agents sets a common base line that has been strengthened and expanded in many of the individual member states. In the UK, the definition and minimum handling

procedures for pathogenic organisms are set by the ACDP who list four hazard groups with corresponding containment levels (Anon, 1996c).

Table 2.2 ACDP Hazard group classification

Group 1	A biological agent that is most unlikely to cause human disease.
Group 2	A biological agent that may cause human disease and which might be a hazard to laboratory workers but is unlikely to spread in the community. Laboratory exposure rarely produces infection and effective prophylaxis or treatment is available.
Group 3	A biological agent that may cause severe human disease and present a serious hazard to laboratory workers. It may present a risk of spread in the community but there is usually effective prophylaxis or treatment.
Group 4	A biological agent that causes severe human disease and is a serious hazard to laboratory workers. It may present a high risk of spread in the community and there is usually no effective prophylaxis or treatment.

The containment level numbers correlate with the risk group in which the organism falls (i.e. organisms in Risk group 1 require containment Level 1 and so forth, see Table 2.3 below).

The Advisory Committee on Genetic Manipulation (ACGM) in the UK prescribe separate but similar regulations for those organisms that have been genetically modified. Similarly, other European countries have advisory committees, in Germany it is the Zentrale Kommission für die Biologische Sicherheit (ZKBS), Robert Koch-Institute, Berlin. The Trade Corporation Association of the Chemical Industry (BG Chemie) advises on how individual Genetically Engineered Micro-organisms (GEMs) should be classified. The assessment of risk in handling GEM or GMOs is more difficult as the hazards of the donor and recipient have to be taken into account, as well as those of the resulting GEM.

The species of bacteria, fungi and other parasites falling into hazard groups 2 and 3 have been defined (Anon, 1996c). Similarly, all bacteria from the Approved List of Bacterial Names (Skerman *et al.,* 1980) have been assigned to an appropriate hazard group in Germany (Anon, 1997a, 1997b, 1998). However, species of fungi have not been assigned to hazard group 1 (Anon, 1996c, 1996d). Medically important fungi have been categorised into relevant hazard groups by de Hoog (1996). To meet the UK and European legislation, all microbiologists will have to make a risk assessment on the organisms with which they work or hold in collections. In the case of fungi, it is recognised that many may infect following traumatic inoculation through the skin, or infect a compromised patient, but do not infect healthy individuals. Most fungi from clinical specimens require Containment level 2 (Anon, 1996c), unless a higher degree of containment is specified (see Table 2.3).

In the UK, Genetically Modified Organisms (GMO's) also require Containment level 2 for handling and all potential work with such organisms must first be referred to the institution's Biological safety officer and/or Biological safety committee. Again, legislation can be different in other countries, for example, in Germany some manipulated organisms can be handled at Containment level 1. The COSHH regulations work well and can be easily applied in establishments with designed laboratories

but may not work as well in an industrial environment where very large volumes and more hazardous techniques may be used. Total containment is rarely applicable.

Table 2.3 Summary of laboratory containment levels for the UK (Anon 1996c)

CONTAINMENT REQUIREMENT	CONTAINMENT LEVEL			
	1	2	3	4
Laboratory site: isolation	No	No	Partial	Yes
Laboratory: sealable for fumigation	No	No	Yes	Yes
	Optional	Optional	Yes	Yes
Ventilation: inward airflow/negative pressure	No	Optional	Optional	No
Ventilation: through safety cabinet				
Mechanical: direct	No	No	Optional	No
Mechanical: independent ducting	No	No	Optional	Yes
Airlock	No	No	Optional	Yes
Airlock: with shower	No	No	No	No
Wash hand basin	Optional	Yes	Yes	Yes
Effluent treatment	No	No	No	Yes
Autoclave site: on site	Yes	-	-	-
in suite	-	Yes	Yes	-
in lab: free standing	-	-	Optional	-
in lab: double ended	-	-	-	Yes
Microbiological safety cabinet/enclosure	No	Optional	Yes	Yes
Class of cabinet/enclosure*	-	Class I	Class I/III	Class I/III

*Guidance on the use of Class II microbiological safety cabinets is given in the ACDP report (Anon 1996c).

Compared to chemicals, organisms are more difficult to name, less predictable and more difficult to enumerate or measure. Virulence and toxicity may vary from strain to strain within a species and additional hazards, such as toxin production and allergenicity must be considered. To meet biological agents legislation and COSHH requirements, a step by step evaluation of a laboratory procedure or an industrial process must be carried out. This is necessary as different organisms present different hazards and different size inocula can be required to cause a problem. The assessment must cover the procedure from the original inoculum or seed culture to the final product or the point where the organism is killed and disposed of. It is not adequate to say that the micro-organism is of ACDP hazard group 2 or less and therefore work can be carried out on the laboratory bench apart from those procedures that may create aerosols. It must be noted that individuals may respond differently to exposure, with some being more sensitive than others. It is therefore critical that the full hazard potential of the organism is considered and that this is related to effects it may have on the particular individual carrying out the work.

Mycotoxins

One of the better known hazards associated with fungi is the ability to produce toxic secondary metabolites. The presence of these in culture media adds to the hazard status of the growing organisms. The toxins produced may be carcinogenic, mutagenic, nephrotoxic, hepatotoxic, haemorrhagic, oestrogenic or cause inflammatory effects. The most commonly known is aflatoxin which is considered to be carcinogenic, hepatotoxic and potentially mutagenic and is produced by strains of *Aspergillus flavus* and *A. parasiticus*. Table 2.4 lists some mycotoxins that may be present in growth media and present additional problems in both use and disposal. Mycotoxicoses are poisonings caused by the ingestion of food contaminated (and sometimes rendered carcinogenic) by toxin producing microfungi. Toxins are also produced by many other fungi, for example, citreoviridin, citrinin, islanditoxin and patulin by species of *Penicillium*, ochratoxin by *Aspergillus* and trichothecenes and zearelenone by species of *Fusarium*, and various other compounds including cochliodinol by *Chaetomium*. It should always be remembered that many fungi have not been studied chemically and because mycotoxins are not reported for a species does not mean it does not produce them. The handling of materials contaminated by these toxins can lead to their ingestion and subsequent poisoning. Inhalation of mycotoxins can also be dangerous. Toxins from *Aspergillus* and *Fusarium* species have caused problems in patients when inhaled. The first major poisonings of man were reported in 1974 in India when over 1000 cases were diagnosed and 300 deaths occurred (Krishnamachuri, *et al.,* 1975).

Bacterial toxins

As infection patterns caused by bacterial pathogens are so different and depend on the bacterial pathogen and the individual host, every infection is an extremely individual process. Diseases caused by bacteria may be grouped as follows (Anon, 1998b):

- Local infections: Manifestation of the pathogen in a localised tissue.
  Examples: *Staphylococcus aureus, Neisseria gonorrhoeae.*
- Local infections with production of a potent toxin: Low invasiveness as above, but general diffusion of the toxin via the lymphatic and blood stream.
  Examples: *Clostridium tetani, Corynebacterium diphtheriae.*
- Acute generalised infections: Usually highly invasive distribution of the pathogens after infection leading to possible septic-toxic shock. Sometimes tissue specific (organotroph) manifestation, examples: plague, typhoid, brucellosis, some types of tuberculosis.

The virulence of strains of pathogenic bacterial species is determined by their invasiveness, production of aggressins and toxigenicity. Most bacterial toxins are capable of damaging or killing normal host cells and are effective upon infection. In contrast, most mycotoxins are effective without invasion or infection. The role played by bacterial invasiveness in damaging the host varies widely: sometimes infection can be extremely localised (e.g., *Corynebacterium diphtheriae*), the toxin diffuses and reaches almost all tissues. Alternatively, pathogens may invade and need to multiply in order to generate enough toxin to cause damage to the host (e.g., *Bacillus anthracis*). Two classes of bacterial toxins have been designated which can be distinguished by their chemical nature. The first are protein-like

exotoxins (examples are diphtheria, tetanus, botulinum toxins and enterotoxins) and the second are endotoxins which are molecular complexes containing protein, lipid and polysaccharide components.

Table 2.4 Some common mycotoxins and examples of fungi producing them

Mycotoxin	Fungus
Aflatoxin	*Aspergillus flavus, A. parasiticus*
Aflatrem	*Aspergillus flavus*
Altenuic acid	*Alternaria alternata*
Alternariol	*Alternaria alternata*
Austdiol	*Aspergillus ustus*
Austamide	*Aspergillus ustus*
Austocystin	*Aspergillus ustus*
Bentenolide	*Monographella nivalis*
Brevianamide	*Aspergillus ustus*
Citrinin	*Aspergillus carneus, A. terreus, Penicillium citrinum, P. hirsutum, P. verrucosum*
Citreoviridin	*Aspergillus terreus, Penicillium citreoviride*
Cochliodinol	*Chaetomium cochliodes*
Crotocin	*Acremonium crotocinigenum*
Cytochalasin E	*Aspergillus clavatus*
Cyclopiazonic acid	*Aspergillus versicolor*
Destruxin B	*Aspergillus ochraceus*
Fumagilin	*Aspergillus fumigatus*
Fusarin	*Fusarium moniliforme*
Gliotoxin	*Aspergillus fumigatus*
Islanditoxin	*Penicillium islandicum*
Malformin	*Aspergillus niger*
Maltoryzine	*Aspergillus* spp.
Moniliformin	*Fusarium moniliforme, F. oxysporum, F. equiseti*
Ochratoxin	*Aspergillus ochraceus, Penicillium viridictum*
Oxalic acid	*Aspergillus niger*
Patulin	*Aspergillus clavatus, Penicillium expansum, P. roquefortii, P. claviforme, P. griseofulvum*
Penicillic acid	*Aspergillus ochraceus*
Penitrem	*Penicillium crustosum*
Roridin	*Myrothecium roridum, M. verrucaria, Dendrodochium* spp., *Cylindrocarpon* spp., *Stachybotrys* spp.
Rubratoxin	*Penicillium rubrum*
Rubroskyrin	*Penicillium* spp.
Rubrosulphin	*Penicillium viridicatum*
Rugulosin	*Penicillium brunneum, P. kloeckeri, P. rugulosum*
Satratoxin	*Stachybotrys chartarum*
Slaframine	*Rhizoctonia leguminicola*
Sterigmatocystin	*Aspergillus flavus, A. nidulans, A. versicolor, Penicillium rugulosum*
Trichodermin	*Trichoderma viride*
Trichothecin	*Trichothecium roseum*
Trichothecenes T2 toxin deoxynivalenol (vomitoxin) nivalenol diacetoxyscirpenol fusarenone 3-acetyldeoynivalenol 15-acetyldexoynivalenol	*Fusarium acuminatum, F. roseum, F. sporotrichioides*
Tryptoquivalene	*Aspergillus clavatus*
Verrucarin	*Myrothecium verrucaria, Dendrodochium* spp.
Verruculogen	*Aspergillus fumigatus*
Viopurpurin	*Trichophyton* spp., *Penicillium viridicatum*
Viomellein	*Aspergillus* spp., *Penicillium aurantiogriseum, P. crustosum, P. viridicatum*
Viriditoxin	*Aspergillus fumigatus*
Xanthocillin	*Eurotium chevalieri*
Zearalenone	*Fusarium culmorum, F. graminearum, F. oxysporum, F. roseum*

Data from the CABI Bioscience Genetic Resource Collection database and Smith & Moss (1985).

Table 2.5 Some bacterial exotoxins and examples of bacterial producers (after Stanier *et al.*, 1987)

Toxin	Bacterium
Neurotoxins	
Botulinum toxins: A, B, C1, C2, D, E, F, G	*Clostridium botulinum*
Tetanospasmin	*Clostridium tetani*
Tetanolysin	
Cytotoxins	
α lecithinase	*Clostridium perfringens*
Necrotic factors	
Hemolysin	
Collagenase	
Diphtheria toxin	*Corynebacterium diphtheriae*
Streptolysin O	*Streptococcus pyogenes*
Streptolysin S	
Enterotoxins	
Enterotoxin	*Clostridium perfringens*
α toxin	*Staphylococcus aureus*
"Enterotoxin"	
Shiga toxin	*Shigella dysenteriae*
Cholera toxin	*Vibrio cholerae*
"Guinea pig toxin"	*Yersinia pestis*
Heat-labile enterotoxin (LT)	*Escherichia coli*
Heat-stable enterotoxin (ST)	*Escherichia coli*

Generally, endotoxins are relatively non-specific, are derived from the outer layers of cell walls of Gram-negative bacteria and released after bacterial lysis. Cells of nearly all Gram-negative pathogenic bacteria are intrinsically toxic. The best known endotoxins exhibiting pyrogenicity and toxicity are those of the enteric bacteria of the genera *Escherichia, Salmonella* and *Shigella*. Endotoxins are also inflammatory agents increasing capillary permeability. Aggressins are enzyme-like substances e.g., proteases, collagenases, lipases, phospholipases or neuraminidases which usually support the invasion of a pathogen by damaging host tissue. A complete list of all known bacterial toxins cannot be given here, some examples are given in Table 2.5 and further toxin producers can be found in Annexe III, Community Classification of the EU Directive 90/679/EEC. In addition to those bacteria that produce toxins during infection there are also those that are non-infectious toxin producers, of these the cyanobacteria of which there are *ca.* 2000 species and are currently considered as hazard group 1. Within these groups of organisms there are three main classes of toxins: liposaccharide toxins, (generally considered to be of low toxicity); peptide hepatotoxins (possessing significant risk on both short-term high level and long-term low-level exposure) and alkaloid neurotoxins, (generally highly toxic). Toxicity of individual strains may vary with environmental conditions but toxicity is invariably associated with bloom formation and generally involves strains of *Microcystis aeruginosa*, *Anabaena flos aquae*, *Oscillalonia* (*Planktothrise*) *agardhii* or occasionally other species. Further information can be found on bacterial toxins in Collier *et al.,* (1998).

2.5 Regulations governing distribution of cultures

The distribution of organisms is controlled by numerous regulations and some of these are discussed below. These include postal and shipping regulations, requirements for packaging aimed at protecting handlers and recipients of organisms and quarantine legislation to protect plant health.

The International Air Transport Association (IATA) Dangerous Goods Regulations lay down rules on the shipping of organisms by air (IATA, 2000). There are several other regulations that impose export restrictions on the distribution of micro-organisms, these include control of distribution of agents that could be used in biological warfare (EC Council Regulation 3381/94/EEC) and the control of export of dual-use goods (Official J. L 367, p1). Most countries are currently implementing access regulations to genetic resources under the CBD. It is critical that microbiologists are aware of, and follow such legislation. Some cultures represent a health hazard and for post and packaging purposes these are placed into four classes by the UPU see Table 2.6.

For further details consult *Packaging and Shipping of Biological Materials at ATCC* (Alexander & Brandon, 1986) and *Shipping of infectious, non-infectious and genetically modified biological materials, International Regulations* DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (1998) and the IATA Dangerous Goods Regulations (IATA, 2000).

Table 2.6 Shipping classes

Class 1.	Agents of no recognised hazard under ordinary conditions of handling. Unrestricted distribution for *bona fide* teaching, research industry, etc.
Class 2.	Agents of ordinary potential hazard. Distribution is restricted to professional investigators.
Class 3.	Pathogens involving special hazard. Distribution is restricted to professional investigators.
Class 4.	Agents of potential danger to the public health, animal health or of hazard to laboratory personnel requiring special facilities for their containment.

In Europe non-pathogenic biological materials of risk group 1 are transported according to EN 829 requirements. Transport by road is regulated by the Accord Européen relatif au transport international des merchandises dangereuses par routes (ADR). This clearly separates class 6.2 into two subclasses, A: highly infectious material (hazard groups 3 and 4) and B: other infectious material. The two groups have different packaging requirements although currently the UN specification containers for class 6.2 materials must be used for both subclasses. The EU have made an attempt to co-ordinate member state laws on transport of dangerous goods by road with the 'ADR-Directive' EC Council Directive 94/55/EC of 21 November 1994 on the approximation of the laws of the member states on the transport of dangerous goods by road (EC, 1996). The basis for all regulations governing the safe transport of goods for all carriers are laid down in the Orange book, *Recommendations on the transport of dangerous goods* (Anon, 1997c).

Some service culture collections such as the National Collection of Type Cultures (NCTC) and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) maintain registers of persons authorised by their employer to request hazardous pathogens. This measure is designed to protect the customer by ensuring orders are authorised by a responsible person who will ensure that the hazardous

micro-organisms are handled by appropriate staff under suitable conditions of containment. Requests for such organisms are accepted only when countersigned by one of the authorised signatories.

Most countries have their own regulations governing the packaging and transport of biological material in their domestic mail. International postal regulations regarding the postage of human and animal pathogens are very strict on account of the obvious safety hazard they present. There are several organisations that set regulations controlling the international transfer of such material. These include the International Air Transport Association (IATA), International Civil Aviation Organisation (ICAO), United Nations Committee of experts on the transport of dangerous goods, the Universal Postal Union (UPU) and the World Health Organisation (WHO). It is common place to send micro-organisms by post, as this is more convenient and much less expensive than airfreight. However, many countries prohibit the movement of biological substances through their postal services. The International Bureau of the UPU in Berne publishes all import and export restrictions for biological materials by national postal services. This information can also be found in the countries table published in the DSMZ *Shipping of infectious, non-infectious and genetically modified biological materials. International Regulations* brochure (Anon, 1998a). Some countries will not accept human pathogens through the post for carriage overseas and this now includes the UK. A list, which changes from time to time, of these countries can also be obtained from the Post Office (Anon, 1998a; Smith, 1996).

It is probably not uncommon for cultures to be transported personally by scientists, however, this is a practice that should be resisted. Such an act contravenes public transport regulations and where aircraft are concerned cultures are considered dangerous goods under the IATA regulations with the possibility of heavy penalties imposed on those caught. Carriage on the person also circumvents all the controls designed to promote safety.

The IATA Dangerous Goods Regulations (DGR) require that shippers of micro-organisms of hazard groups 2, 3 or 4 must be trained by IATA certified and approved instructors. They also require shippers declaration forms, which should accompany the package in duplicate and that specified labels are used for organisms in transit by air (IATA, 2000). It is critical that microbiologists are aware of, and follow, such legislation. Further details can be found in Alexander & Brandon (1986), *Shipping of infectious, non-infectious and genetically modified biological material, International Regulations* (Anon, 1998) and IATA Dangerous Goods Regulations (IATA, 2000).

2.6 Packaging

IATA Dangerous Goods Regulations (DGR) require that packaging used for the transport of hazard group 2, 3 or 4 must meet defined standards according to IATA packing instruction 602 (class 6.2) (IATA, 2000). Relevant guidelines for the shipping of micro-organisms and updates it on a regular basis are provided by the German national culture collection *DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH* (Anon, 1998a) and its web site (http://www.gbf.de/dsmz/shipping/shipping.htm). Packaging must meet EN 829 triple containment

requirements for hazard group 1 organisms (Anon, 1996b). However, micro-organisms that qualify as dangerous goods (class 6.2) and are sent by air must be in UN certified packages. These packages must be sent by airfreight or courier if the postal services of the countries through which it passes do not allow the organisms in their postal systems. IATA (2000) Sections 2.4.1, 2.4.2 and 2.4.2.1 state that the carriage of dangerous goods in the mail is forbidden by UPU except as permitted in Section 2.4.2.1 which states: *Infectious substances, provided a "Shipper's Declaration" accompanies the consignment, and Carbon dioxide, solid (dry ice) when used as a refrigerant for infectious substances.* They can only be sent airmail if the national postal authorities accept them. There are additional costs above the freight charges and package costs if the carrier does not have its own fleet because the package and documentation will need to be checked at the airport DGR centre. There are currently very few private carriers that transport dangerous goods internationally. These private carriers do provide assistance in completing the shipper's declaration forms. The shipper is exclusively responsible for the shipment, its correct packaging, documentation, marking and labelling. Ready to use, re-usable packaging can be obtained from Air Sea Containers Ltd. at www.air-sea.co.uk and SAF-T-Pak Inc. at www.saftpak.com and both can provide useful information for the shipper of dangerous goods.

2.7 Quarantine regulations

Clients in the UK who wish to obtain cultures of non-indigenous plant pathogens must first obtain a MAFF plant health license and provide a letter of authority. Under the terms of such a license the shipper is required to see a copy of the Ministry permit before such strains can be supplied. Such licenses can be applied for in England and Wales from the Ministry of Agriculture, Fisheries and Food, Room 340, Foss House, Kings Pool, 1-2 Peace Holme Green, York YO1 2PX and in Scotland from the Plant Health Section, Agricultural Science Agency, East Craigs, Edinburgh EH12 8NJ. Non-indigenous tree pathogens can only be supplied if the customer holds a current permit issued by The Forestry Commission: Forestry Commission Headquarters, 231 Corsthorphine Road, Edinburgh EH12 7AP.

All shipments to Canada and the USA for plant pathogens must be accompanied by import mailing labels, without which entry of cultures to these countries is refused. Applications for these labels, stating the names of the organisms and the purpose for which they are required, should be made for Canada to the Chief of the Plant Protection Division, Agriculture Canada' Science Division, Science Service Building, Ottawa, Ontario, Canada K1AS 0C5 and for the USA to USDA Agricultural Research Service, Plant Protection & Quarantine, Room 764, 6505 Belcrest Road, Hyattsville, Maryland 20782, USA.

The specified Animal pathogens order 1998 makes it an offence to possess or spread a listed animal pathogen (e.g., *Brucella*) within Great Britain without a license. It is supplemented by the importation of Animal Pathogens Order 1980 which makes it an offence to import any animal pathogen, or potential or actual carrier, of an animal pathogen from an non-EC country, except under license. Both the supplier and recipient must hold the appropriate licenses and undergo regular inspections from

MAFF. Requests for strains must be refused where the requestor is unable to produce a copy of the appropriate license. Such licenses can be obtained in the UK from MAFF, AHDC Branch C, Tolworth (Toby Jug), Hook Rise, South Tolworth, Surbiton, Surrey KT6 7NF. Information on the transport of plant pathogens throughout Europe can be obtained from the European and Mediterranean Plant, Protection Organisation (EPPO), 1 rue le Nôtre, 75016 Paris, France. EC Council Directive (77/93/EEC) on protective measures against the introduction of harmful organisms and of plant or plant products, also provides useful information.

2.8 Control of dangerous pathogens

There is considerable concern over the transfer of selected infectious agents capable of causing substantial harm to human health. There is potential for such organisms to be passed to parties not equipped to handle them or to persons who may make illegitimate use of them. Of special concern are pathogens and toxins causing anthrax, botulism, brucellosis, plague, Q fever, tularemia and all agents classified for work at Biosafety level 4 (hazard group 4). The 'Australia Group' have strict controls for movement outside their group of countries but has lower restrictions within. The UKNCC has implemented a system involving the registration of customers to ensure *bona fide* supply (see http://www.ukncc.co.uk). The USA have rules that include a comprehensive list of infectious agents, registration of facilities that handle them and requirements for transfer, verification and disposal. Contravention of the rules entails criminal and civil penalties. In the UK, all facilities handling hazard groups 2, 3 or 4 must be registered. Strict control of hazard group 3 and 4 organisms is in place. The UK Department of Trade and Industry (DTI) require that certain infectious agents are exported to members of the 'Australia Group' under an Open General Export License (OGEL) which is granted only to organisations registered with the DTI. Exports of these agents outside the 'Australia Group' require an Individual Export License (IEL) and only individuals nominated by their senior management and who are registered with the DTI may submit an application for an IEL. Failure to comply with these requirements is a criminal offence. Persons being supplied with these infectious agents should not avoid these regulations by providing subcultures to third parties.

In Germany, permission to import, distribute, store and handle micro-organisms allocated to risk group 2 and higher (pathogenic or "hazardous" biological material able to multiply) are subject to restrictions laid down in the Federal German Infectious Diseases Act of December 1979 with its amendments in on micro-organisms pathogenic to humans. A laboratory must be registered with the local health authority. Furthermore, the scientific leader of the responsible institution whether industry, hospital, university etc. or the head of the laboratory must have a personal permit issued by the local health authority. It is not sufficient for an institution to have registered laboratories, additionally there must be at least one authorised qualified person registered. If the person leaves the institution, a new authorised person must be registered. However, the person does not loose authorisation (personal authorisation is transferable to another institution). Furthermore, in Germany, handling of micro-organisms which are exclusively pathogenic to animals, is subject to restrictions according to the Federal infectious diseases of animals enactment. The position is similar to that with human pathogens, the institution has to have registered

laboratories and at least one authorised person. However it is the district authority that is responsible for granting permission in this case. The district authority is also responsible for permits to laboratories working on genetically manipulated micro-organisms. A similar registration is necessary for handling Genetically Engineered Micro-organisms (GEMs) allocated to safety level 1, the laboratory must be registered, there must be a deputy biological safety officer (authorised person as above) and a project leader who is responsible for the genetic engineering project. Additionally each project involving GEMs must be registered separately with the district authority.

2.9 Safety information provided to the recipient of micro-organisms

A safety data sheet must be dispatched with an organism, indicating to which hazard group it belongs and the containment and disposal procedures required. In the UK, micro-organisms are covered by the Control of Substances Hazardous to Health (COSHH) regulations (1988), HSW Act (Anon, 1974) s.6(4)(c) and subject to the Approved Code of Practice Biological Agents (Anon, 1996d). *Substances for use at work: the provision of information* (1985) provides details of the safety data that must be provided. A safety data sheet accompanying a micro-organism must include:

- The hazard group of the organism being dispatched as defined by EC Directive 90/679/EEC *Classification of Biological Agents* and by the national variation of this legislation for example, in the UK, as defined in the Advisory Committee on Dangerous Pathogens (ACDP) *Categorisation of biological agents*, 4 edition (Anon, 1996c), and the Approved Code of Practice (ACOP) for Biological Agents (Anon, 1996d).
- A definition of the hazards and assessment of the risks involved in handling the organism.
- Requirements for the safe handling and disposal of the organism.
  - Containment level
  - Opening cultures and ampoules
  - Transport
  - Disposal
  - Procedures in case of spillage

Such information is absolutely essential to enable the recipient of organisms to handle and dispose of the organisms safely.

Summary

Legislation controls the safe handling and use of organisms and biologists must ensure they keep abreast of existing, new and changing regulations. Misuse and abuse of rules will inevitably result in even more restrictive legislation that will make the exchange of organisms for legitimate use even more difficult. Health and safety, packaging and shipping and controlled distribution legislation may be extensive and sometimes cumbersome, but it is there to protect both scientists and the wider community. Biologists wishing to collect organisms, characterise them and investigate their roles in nature must remember that many rules and regulations govern their actions. If the organisms or their products are to be exploited, then the country of origin must be taken into account. If agreements are in place, including permission to collect and how the organism may be used, and a suitable risk

assessment is completed as soon as practicable, the process of compliance with the law is made much simpler. In the interests of the progress of science, biologists must be able to exchange the organisms upon which their hypotheses and results are based, but they must do this in a way that presents minimum risk to those who come into contact with the organism. Further information can be found in a paper on the Society for General Microbiology web site (http://www.socgenmicrobiol.org.uk).

References

Alexander, M.T. & Brandon, B.A. (eds.) (1986). *Packaging and shipping of biological materials at ATCC*. Rockville, Maryland: American Type Culture Collection.

Anon (1974). *Health and Safety at Work etc. Act 1974*. 117 pp. London: HMSO.

Anon (1992). *The UK Management of Health and Safety at Work (MHSW) Regulations 1992*. Health and Safety Executive. Sudbury: HSE, Books.

Anon (1993a). Chemicals (Hazard Information and Packaging) Regulations 1993: Approved Supply List: Information approved for the classification and labeling of substances and preparations dangerous for supply. Health and Safety Executive. Sudbury: HSE Books.

Anon (1993b). CHIP for Everyone. Chemicals (Hazard Information and Packaging) Regulations 1993). Health and Safety Executive. Sudbury: HSE Books.

Anon (1996a). Industrial Property Statistics, 1994, Part II. *Geneva: World Intellectual Property Organisation (WIPO).*

Anon (1996b). *CAB INTERNATIONAL 13th Review Conference Proceedings*. Wallingford, UK: CAB INTERNATIONAL.

Anon (1996c). *Categorisation of pathogens according to hazard and categories of containment*. Fourth edition. Advisory Committee on Dangerous Pathogens (ACDP). London: HMSO.

Anon (1996d). *COSHH (General ACOP), Control of Carcinogenic substances, Biological Agents: Approved Codes of Practice (1996).* London: HSE Books.

Anon (1997a). Sichere Biotechnologie, Eingruppierung biologischer Agenzien: Bakterien. *Berufsgenossenschaft der chemischen Industrie. Merkblatt B 006e, 2/97, ZH 1/346.* 69021 Heidelberg: Jerdermann-Verlag.

Anon (1997b). Sichere Biotechnologie, Eingruppierung biologischer Agenzien: Fungi. *Berufsgenossenschaft der chemischen Industrie. Merkblatt B 007e, 2/97, ZH 1/346.* 69021 Heidelberg: Jerdermann-Verlag.

Anon (1997c). *Orange Book, Recommendations on the Transport of Dangerous Goods, Tests and Criteria*. 10th edition. New York: UNO.

Anon (1997d). *EH40/73 Occupational exposure limits 1993*: Sudbury: HSE Books.

Anon (1998a). *Shipping of infectious, non-infectious and genetically modified biological materials, International Regulations* DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

Anon (1998b) Sichere Biotechnologie, Eingruppierung biologischer Agenzien: Bakterien. Merkblatt B006, 1998, Germany: BG Chemie.

CBD (1992). The Convention on Biological Diversity **31** I.L.M. 822.

Collier, L., Balows, A. & Sussman, M. (eds) (1998). Topley and Wilson's Microbiology and Microbial Infections. 9th edition. London: Arnold.

COSHH (1988). *Statutory Instruments 1994 No. 3246. Health and Safety: The Control of Substances Hazardous to Health (Amendment) Regulations 1994*. London: HMSO.

Davison, A. Brabandere, J. de., & Smith, D. (1998). Microbes, collections and the MOSAICC approach. *Microbiology Australia* **19**, 36-37.

de Hoog, G.S. (1996). Risk assessment of fungi reported from humans and animals. *Mycoses* **39**, 407-417.

EC Council Directive 77/93/EEC on protective measures against the introduction into the Member States of harmful organisms of plant or plant products. *Official Journal of the European Communities* **20**, 20-54 (1977).

EC Council Directive 90/679/EEC. Protection of workers from risks related to biological agents *Official Journal of the European Communities* **L374**, 31 (1990).

EC Council Directive 93/88/EEC Directive amending Directive 90/679/EEC on the protection of workers from risks related to exposure to biological agents. *Official Journal of the European Communities* **L307** (1993).

EC Council Directive 94/55/EC on the approximation of the laws of Member States on the transport of dangerous goods by road. *Official Journal of the European Communities* **L319**, 7 (1994).

EC Council Regulation 3381/94/EEC on the control of export of dual-use goods. *Official Journal of the European Communities*. **L 367**, 1 (1994).

EC (1996). Annexes of EC Council Directive 94/55/EC on the approximation of the laws of Member States on the transport of dangerous goods by road. *Official Journal of the European Communities* **L275** (1996).

Fritze, D. (1994). Patent aspects of the Convention at the microbial level. *In*: The biodiversity of Micro-organisms and the Role of Microbial Resource Centres. *pp 37-43. Germany: World Federation for Culture Collections (WFCC).*

Glowka, L. (1998). *A guide to designing legal frameworks to determine access to genetic resources*. pp98. Gland, Switzerland, Cambridge and Bonn: IUCN.

IATA - International Air Transport Association. (2000). *Dangerous Goods Regulations*. 41st edition. Montreal; Geneva: IATA

Kelley, J. & Smith, D. (1997). Depositing Micro-organisms as part of the Patenting Process. In: *European BioPharmaceutical Review* **2**, 52-56. London, UK: Ballantyne Ross Ltd.

Krishnamachuri, K.A., Bhat, R.V., Nagarajan, V. & Tilak, T.B.G. (1975). Hepatitis due to aflatoxicosis. *Lancet* **1**, 1061-1063.

Simpson, D. & Simpson, W.G. (1991). The COSHH Regulations: A practical Guide. Cambridge, UK: The Royal Society for Chemistry. pp192.

Skerman, V.B.D., McGowan, V. & Sneath, P.H.A. (1980). Approved Lists of bacterial names. *International Journal of Systematic Bacteriology* **30**, 225-420.

Smith, D. (ed.) (1996). *Committee on postal, quarantine and safety regulations report 1996, Postal, quarantine and safety regulations: status and concerns*. pp39. Braunschweig, Germany: World Federation for Culture Collections (WFCC).

Smith. D. (2000). Legislation affecting the collection, use and safe handling of entomopathogenic microbes and nematodes. In Bioassays of Entomopathogenic Microbes and Nematodes (edited by A. Navon and KRS Ascher). Wallingford, UK: CAB International. p295-313.

Smith, D. & Onions A.H.S. (1994). The Preservation and Maintenance of Living Fungi. Second edition. IMI Technical Handbooks No. 2, pp 122. Wallingford, UK: CAB INTERNATIONAL.

Smith J.E. & Moss, M.O. (1985). Mycotoxins: Formation, analysis and significance. Chichester, New York: John Wiley.

Stanier R.Y., Ingraham, J.L., Wheelis, M.L. & Painter, P.R. (eds) (1987). General Microbiology, 5th edition pp. 621. Houndmills, Basingstoke, Hampshire, UK: Macmillan Education Ltd.

Stricoff, R.S. & Walters, D.B. (1995). *Handbook of Laboratory Health and Safety*. Second edition. pp 462. New York: John Wiley & Sons.

Substances for use at work: the provision of information. (1985). HSE booklet HS(G)27. London: HMSO.

UPU (1998). *Universal Postal Convention, Compendium of Information*. Edition of 1 January 1996 - last update 15.06.98. Berne: Universal Postal Union, Berne (International Bureau)

NB: The EU/EC Directives are available from the Office for Official Publications of the European Communities, 12 rue Mercier, L-2985 Luxembourg.

Chapter 3

General hints on growing microbes and animal cell lines

David Smith, Matthew Ryan, Sarah Clayton, John Day and Peter Green

This chapter contains general, but essential, advice on how to grow and maintain microbes and cell lines for immediate use in the laboratory. Details about conditions of light, temperature, pH and aeration along with helpful advice and suggestions are described for each type of organism. Methods used for longer-term maintenance and preservation are given in Chapter 4.

3.1 Algae and cyanobacteria

Algae and cyanobacteria have quite specific growth requirements. Following isolation from the environment, strains are maintained under largely artificial conditions of media composition, light and temperature. Maintaining cultures can be problematic as the imposition of an artificial environment on a cell population that previously survived under complex, fluctuating conditions inevitably causes a period of physiological adaptation and/or selection. Cultures are often maintained in unialgal non-axenic stocks or in axenic culture. The correct maintenance of algal strains is dependent on choice of media, temperature and light conditions.

3.1.1 Preparation of cultures for subculturing

Once algae have been isolated from their natural environment, they are then maintained under artificial conditions in the laboratory. Cultures can either be maintained as uni-algal or axenic stocks, although isolation requires considerable skill and patience (Pringsheim, 1946b; Droop, 1967). Alternatively, strains can be maintained with bacterial, fungal, protozoan, invertebrate or other algal contaminants, where a micro-environment involving predation/symbiosis, competition and other inter-relationships will develop, affecting the physiological status of the population. While contaminated cultures have previously been satisfactory for certain applications and experiments, modern experimental methods and applications require axenic cultures where the taxonomy and growth characteristics of strains are defined. Once established, cultures enter a period of logarithmic growth, followed by a stationary phase. During the latter period, depletion of nutrients and dissolved gases and accumulation of waste products will cause deterioration and ultimate loss of the culture. Therefore, it is essential that a sub-sample of viable material in late exponential or early stationary phase is transferred to fresh growth medium.

3.1.2 Media

The choice of culture medium for an algal strain is dependent on nutritional, morphological and taxonomic characteristics of the organism. The first choice to make is between a liquid or solid medium as many algal strains will grow successfully on both. Development and refinement of media composition for laboratory-maintained algal cultures has been the object of research for several decades, resulting in many different media ‘recipes’ being reported in the literature. Media can be

classified as being defined or undefined (Turner & Droop, 1978). In defined media, all of the constituents are known and can be assigned a chemical formula which is essential for nutritional studies. However, undefined media: contain one or more natural or complex ingredients, for example agar, liver extract or seawater. The composition of these ingredients is unknown and may vary. Defined and undefined media may be further subdivided into freshwater and marine media.

When selecting or formulating a medium, it may be important to decide whether it is likely to promote bacterial growth. Richly organic media should be avoided unless the cultured algae are axenic. If cultures are contaminated, mineral media should be used. This may contain small amounts of organic constituents, such as vitamins or humic acids which provide insufficient carbon for contaminating organisms to outgrow the algae. Examples of media suitable for growing algae are included in Appendix B.

Considerations when selecting appropriate algal media

Many different media formulations have been proposed for algae. Attempts have been made to rationalise the number and standardise formulae for algal strain maintenance. In particular, the use of undefined biphasic media (soil/water mixture) is declining, due to lack of reproducibility in media batches and occasional contamination of the media from soil samples. There are several considerations that must be taken into account when selecting appropriate media, the organism itself, the water used, preparation method, culture vessels and the need for solid media.

Organism Type

Photoautotrophic algae require a medium containing a nitrogen source (generally nitrate), phosphate, major inorganic trace elements, and sometimes organic micronutrients. A typical medium used is 'Jaworski's medium, see Appendix B for formula and method of preparation. Obligate heterotrophs often require an external carbon source, generally supplied as acetate or glucose. Similarly, Cryptophytes, volvocalean flagellates and euglenoid flagellates may require a carbon source. The heterocystous forms of Cyanobacteria (nitrogen fixing bacteria) should generally be maintained on media free of, or deficient in, combined nitrogen, otherwise strains may loose heterocyst function or structure (Castenholz, 1988). The diatoms require an external source of silica, this is usually supplied as Na_2SiO_3 a medium providing this is ASP2 (see Appendix B). For specific industrial applications, it may be necessary to grow algae under heterotrophic conditions.

Water

Natural water should be used to prepare freshwater and marine medium. However, constituents can also be dissolved in distilled water. Filtered natural seawater is excellent for maintenance medium; but may demonstrate seasonal variation in its ability to support growth. Localised chemical pollution in water samples may remain undetected.

Preparation of media

Media may be prepared by combining concentrated stock solutions, which are not combined before use, to avoid precipitation or contamination. The ingredients of defined marine media may be mixed and dried prior to long-term storage.

Culture vessels

Borosilicate glass conical flasks are standard for liquid culture and test tubes standard for agar. Vessels are capped by non-absorbent cotton wool plugs, which will allow aeration but prevent entry of microbial contaminants. Re-usable silicosen rubber bungs [Jencons (Scientific) Ltd.] can be used as they also allow efficient gaseous transfer. Although expensive, they are practical and easy to use. These are sterilised by autoclaving at 121 °C (15 lb./in^2) before filter sterilised* heat labile compounds are added to the media. (*Nucleopore Filters, 0.2 μm diameter).

Solid media

These are usually prepared using 1.0-1.5% agar, the tubes being rested at a 30° angle during agar gelation to form a slope that increases the surface area available for growth.

3.1.3 Subculturing

A large majority of algal strains are maintained through serial subculturing of living stocks. The use of cryopreservation and other preservation techniques is still relatively rare for algal cultures and therefore subculturing becomes the main method of maintenance of many collections. Subculturing is performed using aseptic microbiological techniques. Intervals between routine subcultures vary between 2 weeks and 6 months, depending on the alga, type of medium and environmental parameters. Sterility testing of axenic cultures should be made at each transfer. A successful protocol for routine maintenance of axenic algae is to keep a set of three or four cultures of each strain. If the subculture interval is 2 months, a new culture is established from the culture, which is 2 months old. A sterility test can then be carried out on the culture which is 1 month old; a loopful of material is inoculated into a richly organic liquid medium and this is incubated in the dark at 20°C to allow heterotrophic contaminants to grow up. If the sterility test medium is clear and uncontaminated after incubation, the culture tested can be used as an inoculum. If contamination has occurred, an older uncontaminated culture is used as an inoculum.

The main disadvantages of frequent transfer are:

- Risk of contamination from air-borne or mite-carried bacterial, fungal or yeast cells.
- Time consuming and expensive.
- Diatoms decrease in size in each successive division, eventually leading to an inability of some cultures to divide [leads to the loss of the culture unless auxospores can be produced, (Jaworski *et al.,* 1988)].
- Genetic changes may occur over long periods due to continual selection pressure in an artificial growth environment.

The main advantages of frequent transfer are:

- Cultures are immediately available for distribution.
- No need for elaborate, expensive equipment.

3.1.4 Temperature

The optimal growth temperature is specific for individual algal strains. Therefore, it can be difficult to provide optimal temperatures for all strains. It is often desirable to maintain cultures at a sub-optimal growth temperature to prolong intervals between subculturing. Generally cultures are maintained at 15°C, with minimum and maximum temperatures being 10 and 20°C respectively. The use of lower temperatures to maintain cultures has been suggested; Umebeyashi (1972) reported that several strains of marine diatoms can be maintained for many months at 5°C without subculture, providing cells were given short light periods several times a day.

3.1.5 Light

Artificial light from fluorescent tubes (warm white or cool white) is the preferred means of energy supply to photoautotrophic and mixotrophic algal cultures. Illumination is typically 50-100µmol $m^{-2}s^{-1}$, although taxon vary in optimal light intensity requirements. Many strains of cyanobacteria require lower light intensity (25µmol $m^{-2}s^{-1}$) whilst some diatoms thrive under light conditions of 200µmol $m^{-2}s^{-1}$. In large collections, an appropriate way of providing different levels of illumination is by shading glass shelves using mesh or paper. Day-night light cycles can be controlled using a 24-hour timer (16h light and 8h dark are common used cycles).

3.1.6 Maintenance of industrially exploited microalgae

The economic importance of the algae is not always recognised (Lembi and Waaland, 1988). Algae are utilised for food or in food products, as plant fertilisers, in cosmetics, in manufacturing processes, in biomedical research and many other products and processes. Microalgal biotechnology has to date involved a relatively small number of organisms (Table 3.1) and techniques of culture maintenance used, are largely traditional, with primary cultures maintained by serial transfer on agar slopes incubated in temperature controlled incubators under a 16h light/8h dark cycle. In general, the final production stage of photoautotrophic processes are non-axenic; however, care is taken to ensure culture purity is maintained to the 1-20 litre stage in inoculum build-up. A number of processes have been developed on the basis of growing microalgae under heterotrophic conditions. Starter cultures are maintained under heterotrophic or more commonly mixotrophic conditions; this ensures that any enzymes associated with substrate uptake/utilisation are constitutive within the primary inoculum, also culture densities are much greater than autotrophic culture, giving a larger initial inoculum size.

Table 3.1 Microalgae currently produced on an industrial scale.

Organisms	**Product**
Spriulina platensis	Biomass, health food and pigments
Chlorella spp.	Biomass, health food
Haematococus pluvialis	Astaxanthin
Dunaliella bardawil/salina	β-carotene

It must be stressed that, to date, the majority of microalgae products are produced using wild-type strains. At present, a great deal of strain development is being carried out, resulting in valuable patented strains. It is unlikely that serial transfer will prove to be satisfactory for the maintenance of such production strains or mutants, whether generated by conventional mutagenesis or genetic engineering. Alternative means of maintaining cultures include cryopreservation (the most widely accepted), air-drying and freeze-drying.

3.1.7 Maintenance of algae for aquaculture

Algal cultures are used extensively as hatchery feed for juvenile fish and crustaceans, and for mature shellfish. The principles of algal culture used by the aquaculture industry are little different from those employed by research workers and culture collections. Scaling up presents some problems, especially regarding contamination. Ideally, small starter cultures should be unialgal and axenic, and should be maintained separately from large-scale feed cultures. Cultures larger than 5 litres are difficult to maintain under axenic conditions because of difficulties with autoclaving or filter-sterilising large volumes. In practice, optimal growth conditions of light, temperature and gas supply for the algal strains are employed, to ensure that the relative size of contaminant populations is minimised. Natural waters generally form the basis of bulk media for aquaculture, and pre-filtering is essential (Helm *et al.,* 1979). Chemical sterilisation using hypochlorite solution, followed by neutralisation with sodium thiosulphate, and passage through ultra-violet sterilisation systems may also be used in appropriate situations (Baynes *et al.,* 1979).

Hundred-litre plastic bags and tanks of various materials are used as vessels for growing algae indoors on a mass scale (De Pauw & Pruder, 1986). In such hatcheries, artificial indoor lighting systems comprising of banks of fluorescent tubes are employed. Hatcheries located in climates where sunlight quality and duration are assured, grow feed algae outdoors under non-axenic conditions. Choice of media varies from fully defined media to natural water (with trace elements and vitamins added) and natural water (with no additives) where nutrient quality can be assured. Continual on-site production of strains is subject to contamination and is costly in labour and equipment. Some hatcheries have in the past replaced live cultures with heterotrophically-grown, spray-dried algal cells, for example *Tetraselmis suecica* and *Cyclotella cryptica* (Algal 161™, Algal 262™, Cell Systems Ltd.) or alternatively algal paste concentrates are in fairly common use (Day *et al.,* 1999).

3.2 Bacteria

Bacteria are diverse organisms, and therefore generalisations cannot be made regarding pH values, media, and incubation temperatures that will support optimum growth. However, the majority of organisms will grow at pH values near neutrality, at temperatures between 20 - 30°C and in a medium containing an energy source (e.g., glucose) and an organic nitrogen source (e.g., peptone.) Many UK collections supply information on media and growth conditions in their catalogues or with the strains supplied and will provide advice on an individual basis.

3.2.1 Media

Due to the extreme diversity, no one medium is appropriate for all bacteria. However, many laboratories use Nutrient Agar (NA), as the majority of bacteria are able to grow on this, even if not optimally. Preferences for growth on particular media are normally developed over many years and are the result of experience. Some physicochemical factors affecting bacterial growth are controlled primarily by the constituents of the culture medium. These include hydrogen ion activity (pH), water activity, osmotic pressure and viscosity. Other factors are controlled by the external environment and include temperature, oxygen, light and hydrostatic pressure.

The oxidation-reduction potential is controlled by both the medium and the environment. All of these factors can influence the growth rate, cell yield, metabolic pattern, and chemical composition of bacteria. The control of hydrogen ion activity, temperature, and oxygen supply is important with every bacterial culture and in some cases critical. The control of oxidation-reduction potential is of major importance in culturing obligately anaerobic bacteria. Further information can be found in 'Methods for General and Molecular Bacteriology, eds., Gerhardt *et al.* (1994).

3.2.2 Recommended growth conditions for some bacteria

Many bacteria will grow in the laboratory under general conditions (aerobically at room temperature). However, there are some bacteria that inhabit extreme environments, and therefore have very specific growth requirements. A few examples are:

- Autotrophs that may not tolerate organic compounds and require low pH, e.g., *Nitrobacter, Thiobacillus.*
- Strict thermophiles that require temperatures higher than 55°C, e.g., *Bacillus acidocaldarius.*
- Obligate anaerobes that do not tolerate the presence of any oxygen, e.g., *Clostridium*, *Butyrivibrio* and *Bacteriodes*.
- Parasitic organisms that need suitable hosts to grow on, e.g., *Bdellvibrio, bacteriphage*.
- Halophiles and marine organisms that grow only in media containing high salt concentrations.
- Recombinant organisms that grow in media formulated to maintain inserted characteristics.
- Nutritionally fastidious organisms that require a number of nutrients such as vitamins, blood components, or specific organic compounds, e.g., *Lactobacillus* spp. and several pathogens, e.g., *Neisseria* spp.

3.2.3 Temperature

Incubation temperature dramatically affects the growth rate of bacteria, because it affects the rates of all cellular reactions. Temperature may also affect the metabolic pattern, nutritional requirements and composition of bacterial cells. The number of generations per hour can be plotted against temperature for any strain to determine the optimum temperature for growth. Although all bacteria have an optimal temperature at which they function best, their growth range is often quite wide, e.g., marine bacteria isolated at 10°C can often grow in the range 5 - 30°C and clinical organisms isolated at 37°C can often grow at or below 20°C. Generally bacteria not only grow more slowly but die rapidly at temperatures markedly above the optimum for growth. Consequently, incubation of a bacterial culture at temperatures above its optimum requires precise thermostatic control. For safety, cultures should be incubated at a temperature below its optimum to an extent determined by the variability of the incubator. Organisms that prefer to grow at low temperatures (<20°C) are called psychrophiles, those that grow at ambient (20-37°C), mesophiles and those that prefer higher growth temperatures (≥45°C) are thermophilic.

3.2.4 Light

Light is of primary importance in the cultivation of photosynthetic bacteria. Different photosynthetic bacteria contain a variety of light-absorbing pigments. As a group, the phototrophs absorb light in virtually all regions of the visible spectrum, as well as light in the near-infrared region. Photosynthetic growth in the laboratory requires selection of appropriate light sources and measurement of the quality and quantity of light used for illumination. For more information on light measurement and light sources and filters see, 'Methods for General and Molecular Bacteriology', eds. Gerhardt *et al*. (1994).

3.2.5 Aeration

Gases frequently constitute substrates for bacteria, either serving as an oxidizable energy source (e.g., H_2, CH_4, CO), a terminal electron acceptor of aerobic respiration (e.g., O_2) or a source of nitrogen (e.g., N_2). Consequently, the metabolism and growth rates of bacteria are often dependent on the concentration of gas in solution. Among the most prevalent concerns in this regard are aerobic and facultative bacteria, where growth rate and yield may depend critically on the concentration of oxygen in solution. There are a number of ways in which the availability of oxygen can be maximised, including ensuring that culture vessels have wide openings. For large volumes of culture, air can be forced through the liquid. See, 'Methods for General and Molecular Bacteriology', eds. Gerhardt *et al*. (1994) for more details.

Bacteria can also grow in the absence of oxygen, a condition known as anaerobiosis. Anaerobic organisms are defined as bacteria that are unable to grow in the presence of oxygen, and fall into two classes:

1. Non-stringent anaerobes – these are able to grow on the surface of agar plates with low but significant levels of oxygen in the atmosphere (Gordon *et al.,* 1953).
2. Stringent anaerobes – these die, or are inhibited almost immediately on exposure to an environment containing oxygen.

References containing general principles for growing anaerobic bacteria include: Holdeman *et al*. (1977); Hungate (1969); Jacob (1970); Ljungdahl & Wiegel (1986); Morris (1975); Sutter *et al*. (1980); Gerhardt *et al*. (1994).

3.2.6 pH

Most known bacteria grow over a relatively narrow pH range (usually near neutrality at pH 7.0). However, an ever increasing number of extremophiles continue to be recognised and isolated from the environment (Schlegel & Jannasch, 1992). Many grow optimally at very low (acidophiles) or very high pH (alkaphiles). Accordingly, for precise definition of the pH optimum for growth of such bacteria, it is important to keep in mind the factors that affect the hydrogen ion concentration. Among these factors are temperature, ionic strength, ion charge, dielectric constant and the physical size of the various ions in the solution. In practice, the influence of such factors on pH is taken into account by standardising the pH meter with a pH reference solution whose composition and temperature are as close as possible to those of the solution being measured e.g., the bacterial culture fluid (Gerhardt *et al*. 1994).

3.2.7 Water activity

All organisms require water for metabolism and growth, but the amount required varies widely. However, the mere presence of water in a medium does not ensure its availability, which is determined by the water activity (a_w) of the medium. The majority of bacteria need high levels of available water, but some specialised bacteria have specific requirements. For example, **halophiles** grow optimally on media containing high concentrations of sodium chloride. Variations in the levels of available water may affect growth rates, cell composition and metabolic activities (Gerhardt *et al*. 1994).

3.2.8 Subculturing

In general, cultures to be preserved should be grown under optimum conditions into late log or early stationary phase. Optimal growth conditions should elicit the best titre to ensure survival during preservation. Spores survive preservation conditions well and so wherever possible (not all bacteria form spores) organisms should be grown on media that will elicit sporulation (media usually low in nutrients so as to initiate the spore forming survival mechanism of some bacteria, e.g., *Clostridium* and *Bacillus*). One of the oldest and most traditional methods for maintaining bacterial culture is continuous subculturing. Organisms have to be grown on their optimum medium; some species require transfer after days or weeks, whereas others may be transferred after several months or years.

The main disadvantages of frequent transfer are:

- Change of characteristics. Subculturing can lead to the loss, reduction or intensifying of characteristics. Changes probably occur most frequently among strains where the intervals between transfers are short.
- Mislabeling. Cultures may be labeled with the incorrect name or number. Labels may become distorted and unrecognisable.
- Danger of contamination by air-borne spores or mite carried infections.
- Requires constant specialist supervision to ensure that the bacterium is not replaced by a contaminant.
- Failure to recover cultures happens from time to time, and is probably more common with the more 'delicate' organisms.

The main advantages of frequent transfer are:

- Collections can be kept viable for many years if supervised by a specialist.
- The method is cheap in terms of capital investment requiring no specialised equipment and for a small collection the time involved is not great (it is very labour intensive for a large collection).
- Retrieval is very easy.

3.3 Fungi

Generally, fungi grow best on media formulated from the natural materials from which they were originally isolated. CABI Bioscience utilises extracts from soil and plant materials such as leaves, stems or seeds placed on solid agar. Optimisation of growth conditions is important. Avoidance of selection of variants from within the population, strain deterioration and contamination are important when growing strains for use and essential when maintaining cultures in this way for the long-term. The major factors affecting growth are medium, temperature, light, aeration, pH and water activity.

3.3.1 Media

The growth requirements for fungi may vary from strain to strain, although cultures of the same species and genera tend to grow best on similar media. The source of isolates can give an indication of suitable growth conditions. For example, isolates from jam can be expected to grow well on high-sugar media, species from leaves may sporulate best in light, those from marine situations may require salt and those from hot deserts and the tropics may prefer high growth temperatures.

Cultures are usually maintained on agar slopes in test-tubes or culture bottles. The majority of fungi can be maintained on a relatively small range of media. However, some fungi deteriorate when kept on the same medium for prolonged periods, so the medium should be alternated from time to time. Most laboratories prefer not to keep a large stock of different media and the majority of isolates can be maintained on a relatively small range depending on the specialisation of the collection, e.g., medical isolates tend to grow well on Sabouraud's medium. Experience at CABI is that cultures grow more satisfactorily on freshly prepared media, especially natural media such as vegetable decoctions. These

are usually easy and relatively cheap to prepare and require few facilities. Small quantities can be sterilised using a domestic pressure cooker and if necessary, the pH can be adjusted using drops of hydrochloric acid or potassium hydroxide and measured using pH papers. However, proprietary media are often useful and can be very important in replicating work of others. Some media for special purposes such as assay work will require very careful preparation. A wide range of media are used by different workers and most mycologists have preferred media. For example, Raper & Thom (1949) used Czapek's Agar, Steep Agar and Malt Extract Agar for the growth of penicillia and aspergilli, while Pitt (1980) in his monograph on penicillia recommended Czapek Yeast Autolysate (CYA) and Malt Extract Agar (MEA). Preferences for growth on particular media are normally developed over many years and are the result of experience. The standardisation of defined media formulae is necessary for most work. Media will affect colony morphology and colour, whether particular structures are formed and may affect the retention of properties. Most fungi can be grown on Potato Carrot Agar (PCA) or Malt Agar (MA). However, others have specified growth requirements. Some dermatophytes survive best on hair (Al-Doory, 1968), some water moulds are best stored in water with the addition of plant material (Goldie-Smith, 1956) and other more sensitive water moulds, may require aeration (Clark & Dick, 1974; Webster & Davey, 1976). Examples of particular preferences are given below and in Smith & Onions (1994).

- *Mucorales* grow well on Malt Agar (MA) and will not grow in Czapek Agar (CZ) as they lack the enzymes to digest sucrose.
- Many fungi thrive on Potato Dextrose Agar (PDA), but this can be too rich, encouraging the growth of mycelium with ultimate loss of sporulation, so a period on Potato Carrot Agar (PCA), a starvation medium, may encourage sporulation.
- *Fusarium* species grow well on Potato Sucrose Agar (PSA).
- Wood inhabiting fungi and dematiaceous fungi often sporulate better on Cornmeal Agar (CMA) and Oat Agar (OA) both of which have less easily digestible carbohydrate.
- Cellulose destroying fungi and spoilage fungi, such as *Trichoderma*, *Chaetomium* and *Stachybotrys* retain their ability to produce cellulase when grown on a weak medium such as TWA or PCA with a piece of sterile filter paper, wheat straw or lupin stem placed on the agar surface.
- All sorts of vegetable decoctions are possible and apart from the advantages of standardisation it is reasonable to use what is readily available, e.g., yam media might be preferable to potato media in the tropics.
- *Entomophthora* species can be grown in culture on several media but are reported to do best on an egg yolk medium.
- The introduction of pieces of tissue, such as rice, grains, leaves, wheatstraw or dung, often produces good sporulation. The use of hair for some dermatophytes has proved very successful (Al-Doory, 1968). Animal hair or feathers should be de-fatted in organic solvents first to ensure good growth.

3.2.2 Temperature

The majority of filamentous fungi are mesophilic, growing at temperatures within the range of 10-35°C, with optimum temperatures between 15 and 30°C. Some species (e.g., *Aspergillus fumigatus*, *Talaromyces avellaneus*) are thermotolerant and will grow at higher temperatures, although they are still capable of growth within the 20-25°C range. A small number of species (e.g., *Chaetomium thermophilum, Penicillium dupontii, Thermoascus aurantiacus*) are thermophilic and will grow and sporulate at 45°C or higher but fail to grow below 20°C. A few fungi (e.g., *Hypocrea psychrophila*) are psychrophilic and are unable to grow above 20°C, while many others (e.g., a wide range of *Fusarium* and *Penicillium* species) are psychrotolerant and are able to grow both at freezing point and at mesophilic temperatures (Smith & Onions, 1994).

3.2.3 Light

Many species grow well in the dark, but others prefer daylight and some sporulate better under near ultraviolet light (see Section 3.3.7). Most leaf- and stem-inhabiting fungi are light sensitive and require light stimulation for sporulation. At CABI Bioscience, most cultures are grown in transparent glass-fronted or illuminated incubators. However, some fungi are diurnal and require the transition from periods of light to dark to initiate sporulation.

3.2.4 Aeration

Nearly all fungi are aerobic and cultured in tubes or bottles and obtain sufficient oxygen through cotton wool plugs or loose bottle caps. Care should be taken to see that bottle caps are not screwed down tightly during the growth of cultures. A few aquatic *Hyphomycetes* require additional aeration, in this case air is bubbled through liquid culture media to enable normal growth and sporulation to occur.

3.2.5 pH

Most common fungi grow well over the range pH3 to 7, although some can grow at pH2 and below (e.g., *Moniliella acetoabutans, Aspergillus niger, Penicillium funiculosum*).

3.2.6 Water activity

All organisms need water for growth, but the amount required varies widely. Although the majority of filamentous fungi require high levels of available water, a few are able to grow at low water activity (e.g., *Eurotium* species, *Xeromyces bisporus*). Fungi isolated from preserves or salt-fish, will only grow well on media containing high concentrations of sugar, along with other xerophiles, or salt, the halophiles.

3.2.7 Near ultraviolet light (black light)

Fungi that require near ultraviolet light (near UV or black light, BL - wavelength 300-380nm) for sporulation must be grown in plastic Petri dishes or plastic Universal bottles for 3-4 days before irradiation. Glass is not suitable, as it is often opaque to ultraviolet light. Rich growth media should be

avoided, as they may give rise to excessive growth of mycelium; nutritionally weak media such as potato carrot agar (PCA) are more suitable for inducing sporulation. At CABI Bioscience, three 1.22m fluorescent tubes (a near ultraviolet light tube, Phillips TL 40 W/08, between two cool white tubes, Phillips MCFE 40 W/33) are placed 130mm apart. A time switch gives a 12h on/off cycle. The cultures are supported on a shelf 320mm below the light source and are illuminated until sporulation is induced.

3.2.8 Subculturing

The simplest method of maintaining living fungi is by serial transfer from used to fresh (solid or liquid) media and then incubation under appropriate conditions for the individual isolate. Many fungi can be maintained in this way for several years by maintenance on suitable media (see Section 3.2.1), although it is not ideal. Successful maintenance is dependent upon ensuring that contaminants or genetic variants do not replace the original strain. Such methods are labour intensive and time consuming when large collections are involved. The time period between transfers varies from fungus to fungus, some require transfer every 2-4 weeks, the majority every 2-4 months. Although Chu (1970) maintained several representatives of forest tree pathogens for 1 year at 5°C most organisms were best transferred after much shorter periods.

The main disadvantages of frequent transfer are:

- Danger of variation, loss of pathogenicity or other physiological or morphological characteristics.
- Danger of contamination by air-borne spores or mite carried infections.
- Requires constant specialist supervision to ensure that the fungus is not replaced by a contaminant or subcultured from an atypical sector.

The main advantages of frequent transfer are:

- Collections can be kept viable for many years if supervised by a specialist.
- The method requires no specialised equipment, is inexpensive and, for a small collection, the time involved is not great.
- Retrieval is very easy.

3.2.9 Mite infestation prevention

Fungal cultures are susceptible to infestation with mites, commonly *Tyrophagus* and *Tarsonemus*, which occur naturally in soil and on organic material. They can be brought into the laboratory on fresh plant material, decaying mouldy products, on shoes, on the bodies of flying insects or in cultures received from other laboratories. The damage mites cause is two-fold: Firstly they eat the cultures; a heavy infestation can completely strip the colonies from an agar plate. Secondly, they carry fungal spores and bacteria on and in their bodies and as they move from one culture to another the cultures can become contaminated and heavily infected with other fungi and bacteria.

The mites commonly found associated with fungal cultures are about 0.25mm in length. They can be seen by the naked eye as tiny white dots, almost at the limit of vision, so infestation can easily go

undetected. Given favourable conditions of high humidity and temperature they breed rapidly and spread quickly. Many cultures can be infested before they are noticed. Infested cultures have a deteriorated look and this is often the first indication of their presence. General hygiene and preventative precautions are better than having to control an outbreak. All incoming material should be examined when it enters the laboratory and a separate room for checking and processing dirty material is desirable. The sealing of incoming cultures, storage in a refrigerator or some form of screening and quarantine system can be helpful, as it is possible for cultures with only a light infestation at the time of receipt to develop a heavy infestation later. Methods of control used by different workers are various and a combination of precautions may be appropriate.

a. Hygiene

Hygiene coupled with quarantine procedures is perhaps the best protection. All work surfaces must be kept clean and cultures protected from aerial and dust contamination. The workbenches and cupboards should be regularly washed with an acaricide, especially as soon as infestation is suspected. The procedure at CABI Bioscience is to wash down with a non-fungicidal acaricide (Actellic 25EC, Fargro Ltd) which is left for sufficient time to have an effect (15min) and then cleaned off with alcohol. Actellic (25EC) is of moderate toxicity and irritating to skin, all contact must be avoided. In the concentration at which it is used [3%(v/v): 30ml of stock to 1l distilled water] it is much less toxic. The benches are then re-polished with a cloth if desired. The acaricide must not be allowed to remain on the work surface as it is a skin irritant. Plastic gloves and a vapour filter mask should be worn during handling. As mites appear to become resistant to some chemicals the acaricide should be changed from time to time. When mites are detected, affected cultures should be removed immediately and sterilised. All cultures in the immediate area should be checked and isolated from the collection.

b. Fumigation

This method is used as a last resort or when moving into new premises and should be carried out by a licensed specialised company. Where large numbers of important cultures or specimens are involved these items can be fumigated off site in specialised equipment. If this is necessary it is advised that specialist contractors are employed. Such fumigation generally involves the use of chemicals that are toxic to fungi, therefore unaffected cultures should be removed or protected.

c. Mechanical and chemical barriers

Many physical methods of prevention of infestation and spread of mites have been evaluated. The culture bottles, tubes or plates can be mounted on a platform surrounded by water or oil, or on a surface inside a barrier of petroleum jelly or other sticky material. These methods may provide protection against crawling mites, but not against mites carried by insects or on the hands and clothes of laboratory workers. A method useful to protect cultures in Universal bottles and cotton wool plugged tubes is the cigarette paper method first described by Snyder & Hansen (1946). The pores of the paper allow free passage of air but are too small for mites. Care must be taken to ensure that the paper is not

damaged through handling and that a good seal is made. It has the advantage that it not only keeps mites out, but it also keeps them in - thus preventing spread of infestation.

Method

i. Cut cigarette papers in half and sterilise in an oven at 180°C.

ii. Stick a cigarette paper onto the universal bottle using copper sulphate gelatin glue (20g gelatin dissolved in 100ml water and then add 2g copper sulphate).

iii. Burn the excess cigarette paper up to the outer edge of the tube or bottle.

Sealing of culture containers such as Petri dishes and Universal bottles with sticky tape (Sellotape™, Scotch tape™ or Parafilm™) may reduce penetration but will not act as a complete barrier. Mites eventually find their way through cracks and wrinkles.

d. Protected storage

The various methods of long term storage of cultures used in culture collections prevent infestation and spread of mites, but are of little use for day to day growth of cultures. Cold storage at 4-8°C reduces the spread of mites, which are almost immobile at this temperature. However, on removal from the refrigerator, mites rapidly become active again. Storage of infested cultures in the deep freeze (<-20°C) for at least 3 days gives better control. The cultures usually remain viable, whereas the mites are usually killed. The fungus will have to be re-isolated from the original culture as the contaminants introduced by the mites will eventually grow. Covering cultures with mineral oil prevents mites escaping should they get into the culture vessel, although contamination of the culture may occur due to the growth of spores and bacteria carried by the mites. Cultures stored in silica gel are kept in sealed tubes or in bottles with the caps screwed down so penetration cannot occur. Freeze dried ampoules being completely sealed are impermeable to mites and they cannot penetrate ampoules stored at the ultra-low temperatures of liquid nitrogen.

3.4 Protozoa

Heterotrophic protozoa can be cultured using a variety of methods. Strains of protozoa are in increasing demand for use in teaching, research and industry. References giving details of isolation and identification and culture methods for freshwater protozoa are given by Finlay *et al.* (1988) and Warren *et al.* (1995). A useful and comprehensive specialist account, which includes cultivation details for specific groups of protists is given by Margulis *et al.* (1989). Formulations for selected, commonly used media can be found in Tompkins *et al.* (1988) and on the UKNCC web site (http://www.ukncc.co.uk). The laboratory conditions to which protozoan batch cultures are subjected, and their consequent growth response are determined largely by temperature, medium type and food availability. Convenient incubation temperatures lie between 15 and 20 °C for the majority of laboratory cultivated strains; where possible, reduced temperatures (e.g., 7°C for cyst formers) can significantly reduce the frequency of subculture required.

3.4.1 Culture observations

Laboratory cultures of protozoa need to be carefully examined to ensure that they are growing well and free of microbial contamination using a range of microscopical techniques. An inverted microscope with low-power, dark-ground or bright-field objectives (x4 to x10) is useful and suited to the observation of larger ciliates and amoebae. The higher magnifications provided by x20 / x40 phase-contrast objectives are essential for examining cultures of microflagellates and small (<30μm) protozoa. The distinct advantage of an inverted microscope is its combination of long working distance with good optics and a wide range of objectives, which permits direct examination of protozoan cultures maintained in a wide variety of culture vessels. Nevertheless, a hand lens is often adequate for assessing culture densities of ciliates, and a binocular stereomicroscope can used for general observations of strains in culture.

3.4.2 Culture vessels

The choice of culture vessel is dependent on a number of parameters, i.e. type of media, strain of organism and how frequently the protozoa require fresh food. 'Pyrex' rimless culture tubes (150 x 16mm, *c*.15ml capacity) are suitable for the culture of many free-swimming protozoa (e.g., most bacterivorous ciliates, euglenid flagellates) and are ideal for axenically-grown strains where media can be dispensed and autoclaved in individual tubes. Disposable screw-capped sterile plastic tubes (10-20ml) can be used to achieve a longer culture 'shelf-life' for strains maintained in axenic media at sub-optimal growth temperatures (<20°C). Sterile plastic Petri dishes are a suitable alternative for many culture strains, especially raptorial ciliates, suctorians, heliozoans, and some others that require frequent addition of food protozoa. Sterile plastic tissue culture flasks (e.g., Bibby, Nunc) are well suited to the maintenance of many ciliate, flagellate and amoebae species, particularly marine isolates that can be maintained in either artificial or natural seawater with added sterile wheat or rice grains.

3.4.3 Cyst forming protozoans

Under appropriate conditions a variety of protozoans form cysts (e.g., Gymnamoebae, terrestrial ciliates and flagellates) and these can be conveniently stored on agar slopes. Encystment and production of active (trophic) forms is induced by the addition of liquid and/or food bacterium. For example, amoebae excystment is induced by introducing excised agar blocks (with a dense surface layer of cysts) onto fresh plates streaked with food bacteria such as *E. coli*. Alternatively, cysts or trophic amoebae may be washed from agar plate surfaces using a stream of a suitable medium e.g., AS (Table 3.3) or diluted (75%) seawater (see Appendix B), and transferred as a cell suspension by pipette. However, care must be taken when working with cultures of *Naegleria* and *Acanthamoebae* as these genera contain species which are pathogenic to man.

3.4.4 Subculturing

As with other cultures, subculturing is performed using aseptic microbiological techniques. The majority of protozoa are grown in liquid culture, but there are different techniques used to transfer the

chosen inoculum into fresh media (Table 3.2). Techniques for strains maintained by surface growth of trophic cells on agar plates are described below for Gymnamoebae. There are no general rules governing the frequency and type of inoculum transfer.

The inoculum itself can often be the most critical consideration for the successful and continued maintenance of healthy culture lines. Only experience will show whether subcultures of any one particular strain are better initiated from a few cells, from a comparatively young culture, or from a larger inoculum transferred from a well-established older culture.

i. Transfer of a few cells from a young culture – this method is generally more applicable for 'purer' types of culture (axenic, monoxenic, dixenic).

ii. Transfer of a larger inoculum from an older culture – this method is used when simultaneous transfer of sufficient food organisms (bacteria, other protozoa, etc.) to initiate new cultures is essential (for cruder agnotobiotic or polyxenic cultures).

Table 3.2 Methods of transfer with liquid inocula.

Method of inoculation	**Type of Organism used with**
Sterile loop	Small flagellates
Straight wire	Small flagellates
Micropipette	Small protozoa generally
Standard Pasteur/plastic disposable pipette	Larger protozoa
Pouring from inoculum tube into tubes containing fresh media	All protozoa that grow in liquid media

It should be emphasised that the best solution will inevitably be a compromise between the necessity to have healthy, dense cultures available at any one time and to reduce as far as possible the work involved in culture maintenance. The aim therefore is to reduce the frequency of subculture and this often involves selecting sub-optimal conditions for protozoan growth.

3.4.5 Media

Many protozoa have specific requirements, therefore a wide variety of media formulae are available and are in use today. The so-called 'biphasic' soil/water (S/W) tube-culture method, devised by Pringsheim (1946a), is still one of the most convenient methods for maintaining a large range of protozoan isolates. A selection of commonly used media is given in Table 3.3.

a. Modifications of the S/W formula

Leedale (1967) recommended the use of soils with high clay content for biphasic euglenid cultures. Pringsheim (1946a) suggested adding a diversity of supplementary material (e.g., starch, cheese, etc.) to satisfy more closely the needs of specific flagellate strains, in general at the CCAP a barley grain is added to S/W medium.

Table 3.3 Media used for maintenance of protozoa. (see Appendix B)

Abbreviation	Media
AS	*Amoebae* saline
ASW	Artificial seawater
MW	Mineral water
MP	Modified Pringsheim's solution
NSW	Natural seawater
PM	Polytoma medium
PC	Prescott's and Carrier's
PJ	Prescott's and James'
PPY	Proteose peptone yeast extract
S/W	Soil/water ('biphasic')

b. Culture tube preparation

1. Add approximately 3-4 cm air-dried soil (preferably an untreated garden loam at neutral pH) and a barley grain may be added to each tube.

2. Fill each tube three-quarters full with water.

3. Sterilise by autoclaving. Historically S/W was sterilised twice to ensure all fungal and bacterial spores were killed.

c. Storage of media

Refrigeration of media prevents evaporation and extends shelf-life. Care should be taken to allow temperature acclimation of media to live cultures, or vice versa: likewise, media stocks should be checked for contamination. Contamination of plates may not be immediately obvious in stocks maintained at refrigeration temperatures and removed for immediate use. This may be avoided by allowing the medium to stand at room temperature for 24h or overnight prior to use, thus allowing growth of fungal or other contaminants to become obvious. Wherever possible, all culture media for protozoa should be sterilised by autoclaving or filtration. Examples of particular preferences are given below and in Kirsop & Doyle (1991) and Warren *et al.* (1997).

- 'Biphasic' S/W medium is one of the most convenient methods for maintaining a large range of protozoan isolates, most notably euglenid and chrysomonad flagellates; hymenostome ciliates, scuticociliates and some spirotrichs (e.g., *Spirostomum*).
- Axenic media suitable for routine strain cultivation have been developed and are used for: ◆Amoebae (*Acanthamoebae* and *Naegleria*); ◆Ciliates (*Paramecium, Tetrahymena, Uronema* and *Parauronema*) ◆Flagellates (*Astasia, Chilomonas, Euglena, Polytoma, Polytomella* and *Peranema*).
- Soil extract media, sometimes supplemented with wheat grains, are commonly used for chysomonad and bodonid flagellates. However, better and more consistently balanced media can be made from commercially available dried cereal leaf preparations such as 'Cerophyl'

(International Marketing Corporation) or an equivalent (e.g., Sigma dehydrated cereal leaf preparation C7171) or a Rye grass infusion.

- Inorganic salt solutions such as PJ, AS and MP have been developed for those protozoa that grow less well in the media described above but persist as healthy populations in such solutions when regularly (usually weekly) provided with washed food organisms. Bottled 'still' mineral water (MW) is a suitable alternative to these, and typically has a consistent mineral composition. There are various brands commercially available; 'Volvic' natural mineral water' (Perrier UK Ltd.) has been successfully used for this purpose, and sterilisation by autoclaving does not impair its usefulness.
- **Flagellates:** Many euglenids, e.g., *Astasia, Chilomonas, Distigma, Gyropaigne, Hyalophacus, Khawkinea, Menoidium, Parmidium, Rhabdomonas* and *Rhabdospira* may be maintained successfully using biphasic tube cultures, but they will also grow well in Petri dishes or tissue-culture flasks in inorganic salt solution with added wheat grains. Soil extract or cereal infusion media in tissue culture flasks or Petri dishes, with or without added wheat grains, are suitable media components for the culture of flagellate strains. Further details of methods for flagellate heterotrophs are given in Cowling (1991).
- **Ciliates:** Bacterivorous ciliates grow well on media which include plant infusion's (including Cerophyl-based media), and on soil-extract media. Both culture types may require the addition of either one or more cereal (wheat or rice) grains and/or other protozoa as food to produce dense cell populations. Many formulations for axenic ciliate media have been developed; one of the more commonly used is PPY (see Appendix B) for the culture of *Tetrahymena*.
- **Amoebae:** Many strains of Gymnamoabae may be grown on non-nutrient agar (NNA), or malt-yeast extract agar (e.g., MY75S) plates with a suitable food bacterium (cultured separately on nutrient agar) added by spreading bacteria across the agar surface (Page, 1988). New subcultures are initiated by preparing fresh plates with bacteria onto which is placed a small (<5mm diameter) block of agar excised from a healthy inoculum source plate. *Escherichia coli* is the bacterium commonly used; plates are maintained at 20°C and subcultured at intervals varying from 10 days to 6 weeks. Page (1988) gives further details of such culture methods and media for many amoebae genera.

Gymnamoebae: Some genera (e.g., *Naegleria, Acanthamoebae* and *Paratetramitus*) can be conveniently stored as cysts on agar slopes at a reduced temperature (*c*. 7 °C), they will remain viable for 6 months or longer. Induction of amoebae excystment involves the use of media such as liquid AS or diluted (75%) seawater.

***Larger amoebae*:** Some genera (e.g., *Amoeba* and *Chaos*) can be maintained in tissue culture flasks or dishes of similar capacity (30-40ml) containing PC or MP to which appropriate food organisms are added as washed dense suspensions. Suitable food for these genera are:

Amoeba spp.---------- *Tetrahymena* spp

Chaos spp.---------- *Colpidium* spp

Ploychaos spp.--------- *Chilomonas* spp

Testate amoebae: Culture populations of these, such as the euglyphids *Euglypha, Trinema, Assulina* and *Corythion*, and others such as *Arcella*, may be maintained on CP (Cerophyl-Prescott) agar plates spread with food bacteria and overlain with a shallow liquid medium layer such as Cerophyl or soil extract (Cowling, 1986). Larger forms such as *Difflugia* and *Netzelia* may require the addition of food algae such as *Chlorogonium*, as well as the provision of fine sand particles as shell-building material.

- **Heliozoans:** *Acanthocystis, Actinophrys, Actinosphaerium* and *Raphidiophrys* grow well on autoclaved MW with frequent (weekly or bi-weekly) provision of food ciliates such as *Tetrahymena* and *Colpidium.*

3.5 Yeasts

Yeasts are economically important organisms, especially in the context of brewing and baking where they play a central role in the fermentation process. Growth is optimal when inoculum is introduced into a suitable aqueous environment, containing an adequate supply of nutrients at moderate temperature and pH. Yeasts grow in simple media which contain fermentable carbohydrates to supply energy and 'carbon skeletons' for biosynthesis, adequate nitrogen for protein synthesis, mineral salts and one or more growth factors. Optimisation of conditions for growth and fermentation are the two main factors that are considered here.

3.5.1 Media

Yeasts require food containing many different nutrients, but must include a source of nitrogen, sulphur and minerals. Many yeasts also require certain nutrilites. Complex undefined media such as extracts of malt or fruit juices are generally able to satisfy these demands. However, many of the techniques used in the laboratory for the process of studying and growing yeasts, mean that at times it is necessary to use defined synthetic media that can be guaranteed to have the same composition from batch to batch. Before such media can be prepared it is necessary to determine what chemically defined compounds are potential sources of yeast food. Some utilisable substances are more acceptable in this respect than are others and it is known that antagonistic effects sometimes result from growing yeasts in the presence of a mixture of compounds even though the individual components of the mixture are beneficial to the organism.

a. Sources of carbon

Fruit juices and other plant infusions or decoctions have been used for centuries for the production of alcoholic beverages and some of the earliest microbiological studies revealed that these products were the result of the action of yeasts upon relatively simple carbohydrates contained in such substrates. Investigation has established that two processes are involved in these metabolic activities:

1. The breakdown of sugars to yield alcohol and carbon dioxide.

2. Growth or the incorporation of some of the carbon of the sugars into the cell substance itself.

There are relatively few studies determining exactly what carbon sources yeasts can and cannot utilise for growth. Research is concerned with testing for various compounds for taxonomic purposes. Table 3.5 shows the ability of different yeasts to assimilate particular carbohydrates.

Yeast strain improvement

Occasionally yeasts can't utilise carbon sources in their nutrient medium, or at best only grow slowly in their presence. However, some yeasts can be improved to utilise particular compounds by being repeatedly subcultured in medium containing the compounds of low concentration, subsequent transfers are made to medium containing progressively increasing amounts (Wickerham & Burton, 1948). In addition to the spontaneous or induced mutation methods there are several other ways employed to improve the industrial competence and capacity of micro-organisms. Mutagenisis through radiation, addition of chemical agents, increasing the presence of mutator genes and transposons and the use of other genetic methods have been successful (Crueger & Crueger, 1989).

Table 3.5 A comparison of the ability to ferment and to assimilate different carbohydrates as revealed by various yeasts (adapted from Lodder & Kreger-van Rij, 1952)

Yeast	Carbohydrate fermented					Carbohydrate assimilated				
	Glucose	Galactose	Sucrose	Maltose	Lactose	Glucose	Galactose	Sucrose	Maltose	Lactose
Debaromyces vini	–	–	–	–	–	+	+	+	+	–
Candida lipolytica	–	–	–	–	–	+	–	–	–	–
Candida pulcherrima	+	±	–	–	–	+	±	+	+	–
Cryptococcus laurentii	–	–	–	–	–	+	+	+	+	+
Hansenula anomala	+	±	±	+	–	+	±	+	+	–
Kloeckera africana	+	–	–	–	–	+	–	±	+	–
Saccharomyces cerevisiae	+	+	+	+	–	+	+	+	+	–
Torulopsis ernobii	+	–	–	–	–	+	–	+	+	–
Torulopsis sphaerica	+	+	–	+	+	+	+	+	+	+

– = No reaction; + = positive reaction; ± = weak or variable reaction.
These examples illustrate the ability of yeasts to assimilate particular carbohydrates, but does not imply that they can be fermented.

b. Sources of nitrogen

An extracellular supply of nitrogenous material is essential for the continued production of new protoplasm and yeasts generally derive this element from simple substances such as ammonium salts,

nitrates, amino acids and amides. There is evidence to suggest that dipeptides, or even higher peptides, may be assimilated. Certain yeasts are able to 'fix' nitrogen. Ammonium salts (phosphate, sulphate, and nitrate) have been incorporated in many synthetic media as the sole source of nitrogen and the majority of yeasts will then grow provided that other requirements such as carbon, nutrilites, and mineral salts are satisfied. There is evidence that ammonium salts will support at least as much growth as any other single source of nitrogen. In natural media the most common sources of nitrogen which are easily assimilated by yeasts are amino acids. However, it is important to remember that certain yeasts can only utilise them in the presence of other specific substances. Further information can be found in Yarrow (1999).

c. Nutrilite requirements

Nutrilites are the component factors that make up the "bios" known to be important for yeasts. In general, yeasts fail to grow on synthetic media, even those thought to provide a suitable source of nitrogen, carbon, and mineral salts. However, growth could be induced when extracts of yeasts or the supernatant fluid from old yeast cultures were added to the basically simple medium. These "ill defined" materials were called the "bios" At least six component factors (=nutrilites) of "bios" are known to be important for the growth of yeasts and include: pyroxidine, inositol, nicotinic acid or nicotinamide, biotin, pantothenic acid and thiamine. Nutrilites are known to be important growth stimulators and play an important role in the production of spores. Further information on the isolation growth and maintenance of yeasts is provided by Yarrow (1999).

d. Mineral requirements

The composition of media can be critical to enable reproducibility in yeast morphology, assimilation tests and, for example, the vitamin requirement test. Certain elements have been shown to be essential for growth when present in small amounts whereas others, while not absolutely necessary, can exhibit stimulatory effects. However, many elements are inhibitory at concentrations only slightly in excess of that required for optimal growth. Media should generally contain (almost exclusively) salts of: potassium dihydrogen phosphate, dipotassium hydrogen phosphate, disodium hydrogen phosphate, calcium salts as the chloride carbonate and/or nitrate, magnesium sulphate and sodium chloride. Further detailed information can be found in Yarrow (1999) and chemically defined media are available commercially.

3.5.2 Temperature

The optimum temperature for growing a yeast varies with the strain being investigated. Generally most yeasts grow reasonably between 15 to 30°C. The optimal temperature is usually nearer to the upper limiting temperature. For example, strains of *Saccharomyces cerevisiae* will exhibit limited growth at 10°C and grow optimally at 28°C, yet a rapid decline in growth occurs when the temperature rises above 30°C. However, using another strain of yeast under different cultural conditions growth may increase with temperature up to 36°C but at 40°C growth levels may reduce to normal. Temperature tolerant yeasts are listed by the NCYC in their catalogue of type strains and strains with special

applications (2000). A strain of *Candida kefyr*, three strains of *Kluyveromyces marxianus* and a further three strains of *Pichia angusta* are cited capable of growth at temperatures above 45°C. There are several species of *Candida* cited with the ability to grow at temperatures between 40 and 45°C, strains of *Clavispora lusitaniae*, *Kluyveromyces marxianus*, *Pichia angusta*, *P. farinosa*, *P. membranaefaciens* and *P. mississippiensis* are also capable of doing so.

3.5.3 Light

The effect of light upon the formation of spores by yeasts has received little attention, even though light markedly influences the sporulation process in other fungi. Different dark/light regimes can have an effect on sporulation in different strains of yeast. For further information on the effect of harmful Ultraviolet light see Section 3.5.6 below.

3.5.4 Aeration

Studies have shown that yeast cultures grow more vigorously when aerated. The large-scale industrial production of yeasts meant that the effects of aeration were widely studied. Under strictly anaerobic conditions, the rate of growth is at a minimum, reproduction being limited to 4-5 generations, while the amount of sugar fermented by a unit amount of yeast is at its maximum. The presence of quite small amounts of oxygen results in substantial increases in production of yeast cells, so only small subsequent increases in the amount of growth result from very large increases in the amount of available oxygen. Agitation of the yeast cells can also increase yield, and in a liquid culture, occurs alongside any aeration that is taking place. *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* often require shake culture (Kirsop, 1991). In liquid culture the cells are kept in suspension and therefore staling of local media is avoided.

3.5.5 pH

As with temperature, there is evidence that optimal conditions, with respect to pH may vary somewhat with the strain of yeast being studied. Slightly acid conditions favour the growth of yeasts, but varying the pH over a relatively wide range does not adversely affect growth in nutrient rich media. Usually the range over which most active growth occurs is between pH 4.0 and 6.0. However, yeasts are, surprisingly acid-resistant and many strains are able to tolerate, if not grow in, medium in which the pH is as low as 2.5.

3.5.6 Ultraviolet light

Several investigations have shown that ultraviolet light destroys yeasts in very short time even if they seem somewhat more resistant than bacteria. The detrimental effects of UV light, particularly on DNA, has been extensively investigated (Bainbridge, 1981).

3.5.7 Sporulation

Many yeasts produce spores, and this process is of fundamental importance, not only for taxonomic purposes, but also for genetic analysis. Consequently the ability of yeasts to sporulate makes possible the controlled hybridisation of new strains and leads to a better understanding of the biochemistry of parental strains. Obviously, it is often desirable to be able to induce sporulation more or less at will, but this is not always feasible, because many yeasts tend to lose their ability to form spores after prolonged cultivation in artificial media. Near-starvation and other unfavourable conditions were thought to induce sporulation; however, such circumstances might lead to death. More probably there exists a balance between two sets of conditions, one set favouring vegetative reproduction and the other sporulation. A change in the environment may favour one phase of the life cycle to the detriment of the other.

Yeasts sporulate more readily when growth in a nutrient-rich medium is followed by a transfer to an environment containing little or no nutrient. There is a wide range of presporulation media that have been devised by many workers, a fairly comprehensive coverage of media for inducing different spores in yeasts e.g., ascospores, ballistospores, basidiospores and chlamydospores is given by Yarrow (1999). Wide differences of opinion exist concerning the optimal physical conditions required to stimulate spore formation. There are many other aspects, including moisture, pH, temperature and light, to consider. Bearing these factors in mind along with the huge diversity of yeast strains, it is obvious that optimal conditions will differ with regards to the induction of sporulation. For more information concerning sporulation-inducing media see Yarrow (1999).

3.5.8 Subculturing

If the above factors concerning the vegetative growth phase are satisfied, then new cultures can be propagated simply by transferring a small quantity of the culture to fresh medium. As with other micro-organisms, it is not always convenient to maintain cultures by frequent serial subculture, especially when a large number of strains are being handled. However, subculturing continues to be useful, particularly in the short term. It is simple, quick and relatively inexpensive. A major drawback of this technique is that substantial variation may occur. One investigation found that 10% of strains showed changes in flocculation behaviour following maintenance by subculturing over a period of 10 years; other morphological and physiological properties have been found to show variation to a greater or lesser extent (Kirsop, 1974). If strain stability is desired, subculturing should be minimised.

3.6 Animal cells

Animal cell culture emerged as a valuable research tool in the 1940s and 1950s, with classic work in the development of cell culture media (Kirsop & Doyle, 1991; Earle *et al.,* 1943). Cell-culture media aims to mimic the physiological conditions within tissues. This has a near neutral pH and normally involves the incorporation of serum at concentrations varying from 5 up to 20%, although certain production processes and experimental procedures require the use of serum-free conditions (Mauer,

1992). Antibiotics such as penicillin and streptomycin are used routinely to ensure cultures remain free from contaminants. Current technology allows individuals to isolate, maintain and/or cultivate virtually any tissue cells from any species of interest. However, not all types of animal cell can be propagated *in vitro*. For normal fixed, postmitotic cells such as neurones, heart cells and multinucleated skeletal muscle cells, maintenance *in vitro* is possible but propagation is not (Hunter-Cevera & Belt, 1996). Many other cell types (e.g., fibroblasts, epithelia, lymphoblasts) can be passed to yield secondary, tertiary, and even higher order cultures, increasing the numbers of cells available at each passage. Facilities for the handling of cell lines are covered as this is an important aspect of working with animal cell-cultures.

3.6.1 Facilities for handling cell lines

Cell cultures are used extensively in many areas of biological research. Whereas the hazards to personnel associated with the use of infected tissues are well documented and appropriate safety measures have been introduced to minimise these risks, very little is known about the potential risks associated with the experimental manipulation of primary cultures or established cell lines (Doyle *et al.,* 1998).

Traditionally cell culture facilities have ranged from the minimum, i.e. working on an open bench, up to a specifically designed suite of rooms, built to clean room standard. In fact, the routine use of antibiotics such as penicillin and streptomycin in the medium frequently relates to a lack of properly ventilated cell-line handling facilities where contamination risks are at their highest. To comply with the current safety regulations, a cell-culture laboratory should be fully ventilated, preferably with Hepa filters on the inlets, and equipped with Hepa-filtered workstations where the air flow is directed away from the operator, for example Class II containment cabinets. Ideally, when designing cell culture facilities, they should comply with the requirements for clean rooms used in industry. To comply, equipment must be kept to the minimum required for the job, there must be proper entry facilities and internal surfaces must be easy to clean and dust free. Such facilities are an expensive but necessary investment. A separate cabinet or room for each cell line handled would be the perfect situation. However, using separate rooms for the different types of operation is a more practical approach. Preparation of reagents and media should not be carried out in rooms where cell cultures are handled. A room designated for receiving or starting new cultures should be available to provide isolation prior to testing cultures for microbial contaminants, especially mycoplasma. Only when cell lines have been screened should they be handled in another room for routine culturing. All normal operations should be feasible without the routine use of antibiotics. Good management of the facilities requires that certain standard procedures are followed:

1. Only designated personnel should enter each area and they must be wearing laboratory coats or sterile clothing, including gloves. It may be considered necessary to wear shoe covers, masks and hats.

2. Only sterile, wrapped items (i.e. pipettes, culture flasks, etc.) should enter the rooms, and discarded media, etc., should be removed each day. Germicidal solutions [e.g., 2%(v/v) Tegodor, Goldschmidt Ltd.] should be available for discarding pipettes.
3. No more than one cell line should be handled at a time in each work area. Between the handling of different lines, a 'clearance time' of 10-15 minutes should be allowed and work surfaces should be sprayed with a germicidal solution.
4. Water sources, i.e. water baths and sinks, should be kept to a minimum. Water baths should be cleaned at least twice a week.
5. Eating, drinking, smoking and application of cosmetics must not be allowed in any of the working areas.
6. Ideally, separate bottles of media should be used for each cell line to avoid the risk of cross-contamination by cells from another culture.

3.6.2 Media

As detailed in the introduction, the primary aim of cell-culture medium is to mimic, as closely as possible, the physiological conditions within tissues.

The common components of animal cell-culture media are:

- Serum: the most common source is bovine; either adult, newborn or foetal in origin.
- A complex defined component: comprising a buffer system (phosphate, bicarbonate, CO_2 or Hepes), amino acids (including glutamine), vitamins and glucose.

The choice of a particular type of medium depends upon the cell type, for example hybridomas are usually cultured in DMEM (Dulbecco's Modified Eagles Medium) and human lymphoblastoid cell lines are usually cultured in RPMI 1640 (Roswell Park Memorial Institute 1640).

Availability and testing of media components

All components required for tissue culture media are available from commercial suppliers and can be obtained as powdered medium that requires reconstitution in deionised or distilled water and filter sterilisation, or alternatively in 1x, 5x or 10x concentration in liquid form. Care has to be taken in the selection of serum, and suppliers are willing to provide samples for batch testing before a major purchase. The procedures for testing depend upon the techniques in use. Testing can be as straightforward as the passage of human diploid cells in the test serum, with examination of cells for morphological signs of toxicity and determination of plating efficiency or a more elaborate cloning efficiency study using a hybridoma or tumour cell line (Freshney, 1987). ECACC provide ready to use media intended for use with shipments of cell lines. Serum and glutamate are added to provide the same conditions the cells were grown under prior to shipment (http:// www.camr.org.uk/ecacc.htm).

3.6.3 Subculturing

In order to maintain cell cultures in optimum conditions it is essential to maintain cells in the log phase of growth for as long as practicable. The usual doubling time for animal cells 24-46h. Cells kept for prolonged periods will lose plating efficiency and show a tendency to decline in overall quality and

'healthiness'. Obviously, frequency of subculture is dependent on several factors, which will vary between cell lines, viz. inoculation density, growth rate, plating efficiency and saturation density. Broadly, there are two general types of culture method appropriate for adherent and non-adherent cells and a general outline methodology is given for each. Cultures may be supplied as either growing in tissue culture flasks or frozen in a cryopreserved state.

Materials

Phosphate-buffered saline (PBS); Trypsin-EDTA solution; Growth medium; Tissue culture flasks/Techne strirrers; Graduated pipettes; Centrifuge tubes; Inverted phase-contrast microscope; Centrifuge

The general principles applicable to adherent and non-adherent cell types are given below.

Adherent Cells

Cells are examined routinely using an inverted microscope at 100x magnification, and once confluency is achieved (i.e. the cell sheet is complete) the cells should be subcultured to maintain growth. This requires removal of the culture medium, washing the cell sheet with PBS, removing the cell sheet into suspension using a proteolytic enzyme (usually trypsin) and dispensing cells into new tissue culture flasks. The precise method is given below; it must be emphasised that work must be conducted under strictly aseptic, sterile conditions. Cells may be cultured in tissue culture flasks of 25-175 cm^3, depending upon the yield of cells required. Other methods are available for more large-scale culture of adherent cells in suspension on microcarriers, if large volumes of cells are required (Griffiths, 1992).

Method

1. Discard spent culture medium.
2. Gently pour or pipette a volume of PBS half that of the original culture medium.
3. Swirl the PBS around the flask taking care not to perform this action too vigorously. Discard the PBS.
4. Add sufficient trypsin-EDTA solution to just cover the cell sheet, i.e. 1-2ml per $25cm^2$. Allow it to stand on the cell sheet for 30-45 seconds at room temperature and pour off most of the trypsin-EDTA, leaving behind a small volume (*c*. 0.2ml).
5. Incubate at 37°C and check for cell detachment after 5-10min. Vigorous (but careful) agitation of the flasks may aid cell detachment. Some cell types may take longer to remove.
6. Resuspend cells in a suitable volume of medium; the volume is dependent on the size of the culture vessel but a minimum is required to neutralise the activity of trypsin. If cells show a tendency to clump, it may be necessary to centrifuge the cell suspension and wash in either culture medium or PBS. Such tasks should be carried out with the minimum centrifugal force required to pellet the cells and also avoiding excessive agitation during resuspension, otherwise, considerable damage will be incurred by the cells.

7. Disperse the cells to produce a suspension and perform a viable cell count (see Section 3.6.4).
8. A cell count may not always be necessary if the cell line has a known split ratio, for example 1:2, 1:4, or 1:16, and the appropriate dilution factor can be used.
9. Prepare tissue culture flasks with culture medium pre-warmed to 37°C. The volume of culture media required is 5-7ml for a 25 cm^2 flask, 20-30ml for a 75cm^2 flask and 50-100ml for a 175 cm^2 flask.
10. Gas with a 5% CO_2 and 95% air mixture through an on-line 0.2µm filter for 30-60s.
11. Add the appropriate volume of cells to seed the flask. The size of culture flask used depends entirely on the number of cells required. Saturation densities for adherent cell lines range from about 10^5 cells cm^{-2} for monolayers to 3 to 4 x 10^5 cells cm^{-2} for cells forming multilayers.
12. Incubate at 37°C.

Suspension cell lines

As for adherent cells, cultures are examined microscopically for signs of cell deterioration, i.e. lysis or death, which are signs of overgrowth. Additionally, the colour of the medium can be used as an indicator of cell density: red-orange is normal, whereas yellow indicates that the culture has become too acidic and is at risk of going into stationary phase and decline.

1. Take a sample of the culture and perform a viable cell count (see Section 3.6.4 below).
2. Prepare tissue culture flasks or Techne stirrer flasks with culture medium pre-warmed to 37°C.
3. Gas with a 5% and 95% air mixture through an on-line 0.2µm filter for 30-60s.
4. Add the appropriate volume of cells to the new flask. Incubate at 37°C. Normally, the viability of suspension cells should not be allowed to fall below 90%. However, cultures of lower viability may be recovered by subculture, although it will be necessary to pellet the cells by centrifugation and decant the spent medium.

A typical growing density range for many suspension cells is 10^5-10^6 cells ml^{-1}, although some lines may require higher dilution or may reach higher saturation densities.

3.6.4 Viable cell counts

It is important to estimate the percentage of viable cells whichever preservation method you choose to use. The simplest method is to examine the cells microscopically in a counting chamber (haemocytometer) using a vital stain, trypan blue, although other methods are available (Baserga, 1989).

See Chapter 4 Preservation of Animal cell lines

3.6.5 Temperature, light, aeration, pH and water activity

The majority of animal cell lines can be successfully maintained at 37°C with 5% CO_2 most other environmental parameters are dependent on the specific cell line. The ECACC Cell line catalogue (5th Edition) contains details of the sub-culture routines for each of its accessions that are available to the wider scientific community.

References

Al-Doory, Y. (1968). Survival of dermatophyte cultures maintained on hair. *Mycologia* **60**, 720-723.

Baserga, R. (ed) (1989). Cell Growth and Division. IRL Press, Oxford.

Bainbridge, B.W. (1981). The effects of ultraviolet light radiation on the nuclei of yeasts and filamentous fungi. In *The Fungal Nucleus* (K. Gull and S.G. Oliver, eds), p258. Cambridge University Press, Cambridge.

Baynes, S.M., Emerson, L. & Scott, A.P. (1979). Production of algae for use in the rearing of larval fish. *Fisheries Research Technical Report, Fisheries Research, MAFF* **33**, 13-18.

Castenholz, R.W. (1988). Culture methods for cyanobacteria. In *Methods in Enzymology* (L. Packer and A.N. Glazer, eds), pp. 68-93. Academic Press, New York.

Chu, D. (1970) Forest pathology, storing of agar slants and cultures. *Bi-monthly Research Notes* **26**, 48.

Clark, C. & Dick, M.W. (1974). Long term storage and viability of aquatic oomycetes. *Transactions of the British Mycological Society* **63**, 611-612.

Cowling, A.J. (1986). Culture methods and observations of *Corythion-dubium* and *Euglypha-rotunda* (protozoa, Rhizopoda) isolated from maritime Antarctic moss peats. *Protistologica* **22**, 181-191.

Cowling, A.J. (1991). Free living heterotrophic flagellates: methods of isolation and maintenance, including sources of strains in culture. In *The Biology of Free Living Heterotrophic Flagellates* (D.J. Patterson and J. Larsen, eds), pp 477-492. Oxford University Press, Oxford, UK.

Crueger, W. & Crueger, A. (1989). Biotechnology: a textbook of industrial microbiology. Second edition (English edition edited by T.D. Brock). Sinauer Associates, Inc, Sunderland, MA 01375.

Day J.G., Benson E.E & Fleck RA (1999) *In Vitro* Culture and Conservation Of Microalgae: Applications For Environmental Research, Aquaculture & Biotechnology. *In Vitro Cellular Developmental. Biology - Plant* **35**, 127-136.

De Pauw, N. & Pruder, G. (1986). Uses and production of microalgae as food in aquaculture. In *Realism in Aquaculture: Achievements, Constraints, Perspectives*, pp. 77-106. European Aquaculture Society, Bredane, Belgium.

Droop, M.R. (1967). A procedure for purifying algal cultures with antibiotics. *British Phycological Bulletin* **15**, 295-297. **15**, 295-297.

Earle, W.R., Schilling, E.L., Stark, T.H., Straus, N.P., Brown, M.F., Shelton, E. (1943) Production of malignancy in vitro. IV. The mouse fibroblast cultures and changes seen in the living cells. Journal of the National Institute of Cancer **4**, 165-212.

Finlay, B.F., Rogerson, M. & Cowling, A.J. (1988). *A Beginner's Guide to the Collection , Isolation, Cultivation and Identification of Freshwater Protozoa.* Culture Collection of Algae and Protozoa.

Freshney, R.I. (1987). Culture of Animal Cells: a Manual of Basic Techniques, 2nd edition. Alan R. Liss Inc, New York.

Gerhardt, P., Murray, R.G.E., Wood, W.A. & Kreig, N.R. (1994). Methods for general and molecular bacteriology. pp 803. American Society for Microbiology, Washington DC.

Goldie-Smith, E.K. (1956) Maintenance of stock cultures of aquatic fungi. *Journal of Elisha Mitchell Scientific Society* **72**, 158-166.

Gordon, J., Holman, R.A., and McLeod, J.W. (1953). Further observations on production of hydrogen peroxide by anaerobic bacteria. *Journal of Pathological Bacteriology* **66**: 527-537.

Griffiths, J.B. (1992) Scaling up of animal cell cultures. In: Animal Cell Culture, a Practical Approach. (edited by R.I. Freshney, R.I.), pp. 47-93. Oxford, IRL Press at Oxford University Press.

Helm, M.M., Laing, I. & Jones, E. (1979). The development of a 200l algal culture vessel at Conwy. *Technical Report, Fisheries Research, MAFF* **33** 1-7.

Holdeman, L.V., Cato, E.P., & Moore, W.E.C. (1977). *Anaerobe Laboratory Manual*, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.

Hungate, R.E. (1969). A roll tube method for cultivation of strict anaerobes, pp. 117-132. *In* J.R. Norris & D.W. Ribbons (ed.), *Methods in Microbiology*, vol. 3B. Academic Press, Inc., New York.

Hunter-Cevera J.C. & Belt, A. (1996). Preservation and Maintenance of cultures used in Biotechnology and Industry, pp 263. USA: Academic Press Inc.

Jacob, H.E. (1970). Redox potential. *In* Methods in Microbiology, vol. 2. (edited by J.R. Norris & D.W. Ribbons), pp. 92-123. Academic Press, London.

Jaworski, G.H.M., Wiseman, S.W. & Reynolds, C.S. (1988). Variability in sinking rate of the freshwater diatom *Asterionella formosa*, the influence of colony morphology. *British Phycological Journal* **23**, 167-176.

Kirsop, B. (1974). The stability of biochemical, morphological and brewing properties of yeast cultures maintained by subculturing and freeze-drying. *Journal of the Institute of Brewing* **80**, 565-570.

Kirsop, B.E. (1991). Maintenance of yeasts. In: *Maintenance of Micro-organisms and Cultured Cells: A Manual of Laboratory Methods*, (B.E. Kirsop and Doyle, A. eds) pp 160-182. London: Academic Press.

Kirsop, B.E. & Doyle, A. (eds) (1991). Maintenance of Micro-organisms and Cultured Cells: A Manual of Laboratory Methods. pp 308. London: Academic Press.

Leedale, G.F. (1967). *Euglenoid Flagellates*. pp 242. Prentice-Hall, Englewood Cliff, NJ.

Ljungdahl, L.G. & Wiegel, J. (1986). Working with anaerobic bacteria, pp. 84-96. *In* A.L. Demain & N.A. Solomon (ed.), *Manual of Industrial Microbiology and Biotechnology*. American Society for Microbiology, Washington, D.C.

Lodder, J. & Kreger-van Rij, N.J.W. (1952). The Yeasts', a Taxonomic Study. North Holland Publ. Co., Amsterdam.

Margulis, L., Corliss, J.O.C., Melkonian, M. and Chapman, D.J. (1989). Handbook of Prototista. The structure, cultivation, habitats and life histories of the eukaryotic microorganisms and their descendants exclusive of animals, plants, and fungi. A guide to the algae, ciliates, forminifera sporozoa, water molds, slime molds and other protoctists, pp. 955. Jones and Barlett, Boston.

Maurer, R. (1992) Towards chemically defined serum-free media for mammalian cell culture. In: Animal Cell Culture, a Practical Approach, 2nd edition (edited by R.I. Freshney), pp. 15-46. Oxford, IRL Press at Oxford University Press.

Morris, J.G. (1975). The physiology of obligate anaerobiosis. *Advances in Microbial Physiol*ogy **12**: 169-246.

NCYC (2000). National Collection of Yeast Cultures Catalogue of type strains and strains with special applications. pp118. IFR Enterprises, Norwich.

Page, F.C. (1988). *A New Key to Freshwater Gymnamoebae.* Freshwater Biological Association, Ambleside, U.K.

Pitt, J.I. (1980 ["1979"]) The Genus *Penicillium* and its teleomorphic state: *Eupenillium* and *Talaromyces*. London, New York: Academic Press.

Pringsheim, E.G. (1946a). The biphasic or soil-water culture method for growing algae and flagellata. *Journal of Ecology* **33**, 193-204.

Pringsheim, E.G. (1946b). *Pure cultures of Algae*. pp 119.Cambridge University Press, Cambridge.

Raper, K.B. & Thom, C. (1949) *A Manual of the Penicillia*. Williams and Wilkins: Baltimore.

Schlegel, H.G. & Jannasch, H.W. (1992). Prokaryotes and their habits, pp. 75-125. *In* A. Balows, H.G.

Smith, D. & Onions A.H.S. (1994). The Preservation and Maintenance of Living Fungi. Second edition. IMI Technical Handbooks No. 2, pp 122. Wallingford, UK: CAB INTERNATIONAL.

Snyder W.C. & Hansen H.N. (1946) Control of culture mites by cigarette paper barriers. *Mycologia* **63**, 455-462.

Stacey, G., Doyle, A. & Hambleton, P. (eds) (1998). Safety in cell and tissue culture, pp 244. UK: Kluwer Academic Publishers.

Stelling-Dekker, N.M. (1931). "Die Hefesammlung Des Central-Bureau voor Schimmelcultures," Teil I, Die Sporogen Hefen, Noord Hollandsche Uitgevers Maatschappij, Amsterdam.

Sutter, V.L., Citron, D.M. & Finegold, S.M. (1980). *Wadsworth Anaerobic Bacteriology Manual*, 3rd edition. C.V. Mosby Co., St. Louis.

Tompkins, A.S., Rhodes, J.C. & Pettman, I. (1988). *Culture Collection of Algae and Protozoa: Catalogue of Strains*. CCAP, Windermere

Turner, M.F. & Droop, M.R. (1978). Culture media for algae. In *CRC Handbook Series in Nutrition and Food* (edited by M. Riecheigl Jr.), Section G 3, pp. 287-426.

Umebeyashi, O. (1972). Preservation of some cultured diatoms. *Bulletin of the Tokai Regional Fisheries Research Laboratory* **69**, 55-62.

Warren A., Day J.G. & Brown S. (1997) Cultivation of Protozoa and Algae. In: Manual of Environmental Microbiology. Hurst C.J., Knudsen G.R., McInerney M.J., Stezenbach L.D. and Walter M.V. (Eds.). ASM Press, Washington D.C. pp 61-71.

Webster, J. & Davey, R.A. (1976) Simple method for maintaining cultures of *Blastocladiella emersonii*. *Transactions of the British Mycological Society* **67**, 543-544.

Wickerham, L.J., & Burton, K.A. (1948). Carbon assimilation tests for the classification of yeasts. *Journal of Bacteriology* **56**, 363-371.

Yarrow, D. (1999). Methods for the isolation, maintenance and identification of yeasts. In The Yeasts: a taxonomic study (C.P. Kurtzman and J.W. Fell, eds), Fourth edition, pp 77-100. Elsevier, Amsterdam.

Chapter 4

Preservation methodology

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4.1 Introduction

The primary objective of preserving and storing an organism is to maintain it in a viable state without morphological, physiological, or genetic change until it is required for future use. Ideally, complete viability and stability should be achieved, especially for important research and industrial isolates. However, even teaching or research collections may have to consider additional factors such as simplicity, availability, and cost. Preservation techniques range from continuous growth methods through to methods that reduce rates of metabolism to the ideal situation where metabolism is suspended. There are many methods available for the preservation and storage of micro-organisms and these can be divided into three groups:

1. Continuous growth techniques involve frequent transfer from depleted to fresh nutrient sources, which initially provide optimum growth conditions. The need for frequent sub-culture can be delayed by storing cultures in a refrigerator, freezer (at -10°C to -20°C), under a layer of paraffin oil or in water.

2. Drying of the resting stage (e.g., spores, cysts or sclerotia) of an organism, can be achieved by air drying, in or above silica gel, in soil or sand.

3. Suspension of metabolism normally involves reducing the water content available to cells by dehydration or cryopreservation. Freeze-drying (lyophilisation) is the sublimation of ice from frozen material at reduced pressure and requires storage in an inert atmosphere either under vacuum or at atmospheric pressure in an inert gas. Cryopreservation generally implies storage at temperatures that impede chemical reactions of around -70°C and below. This can be achieved in mechanical deep freezers (some are capable of reaching temperatures of -150°C) or in/above liquid nitrogen. To achieve an adequate suspension of metabolism to a point where no physical or chemical reaction can occur requires storage at temperatures of below -139°C (Morris, 1981).

Desiccation has been used successfully for the preservation of many micro-organisms. The removal of water suspends metabolism of the cell. Fungal spores have a lower water content than vegetative hyphae and are able to withstand desiccation, reviving when water becomes available. Drying can be carried out using many techniques. Air-drying is achieved by passing dry air over the culture or spores, this speeds the drying process by evaporation Air drying has been successful for some *Aspergillus* and *Penicillium* species (Smith & Onions, 1983). Drying can also be achieved using soil, silica gel or other desiccants. Silica gel storage was first employed in CABI in 1970 and organisms stored at this time are periodically tested to determine ultimate shelf life. Soil storage was initiated in the late 60's and over 900 Fusarium and related genera are stored by this method. A third way is to freeze-dry (lyophilise),

first extensively used with fungal cultures by Raper & Alexander (1945). The methods and machinery have developed over the years to produce a reliable and successful preservation technique for sporulating micro-fungi. Stability and long storage periods are the main advantages of freeze-drying though the expense of the modern and quite complex machinery can be a deterrent.

After a suitable preservation technique is selected and the strains successfully stored a distribution and seed stock should be kept. The size of the stock depends upon the anticipated distribution. Enough replicates must be maintained to ensure that preserved strains have undergone a minimum number of transfers from the original. Wherever possible, an original should be preserved without subculturing. The seed stock should be stored separately from the distribution stock. It is also advisable to keep a duplicate collection in another secure building or site as a reserve. An inventory control system should be used to ensure that cultures remain in stock for distribution or use. After preservation, the viability, purity, and identity should be rechecked and compared with the original results before the culture is made available outside the collection. The organism may be sent to the depositor for confirmation of properties.

The viability, purity and stability of strains must be assessed before and after preservation and during storage. Viability is usually assessed by both a percentage recovery of propagules followed by a growth test. The culture should be grown on the most suitable medium, or host, to give optimum growth. Cultures should be monitored to ensure that characteristics have not altered. Careful microscopic examination must be carried out to ensure that the culture is not mixed. It may be necessary to grow the culture under special conditions to determine if there are contaminants present. Cultures from single cell isolations can be prepared to give a better chance of growing a pure culture although this carries a risk of unintentional selection. To determine whether the cells remain stable during storage pre- and post-preservation comparisons should be made. Morphology, pathogenicity, genetic profiles, assay properties, and biochemical properties can be checked where appropriate. All observations must be recorded and retained for future reference.

4.1.2 Fungi

Collectively fungi are ubiquitous; including species able to grow in a wide variety of environments utilising a vast array of natural and man-made substrates. Some are host specific or have unidentified growth requirements and cannot be grown in culture. Generally, fungi grow best on media that are formulated from the natural materials from which they were isolated. Fungi produce structures that enable them to survive adverse conditions (e.g., spores, sclerotia, asci, and thickened hyphae). Such propagules can be readily stored in the laboratory to retain viability. However, methods of storage that allow growth and reproduction may allow the organism to change and adapt to artificial laboratory conditions. Avoidance of selection of variants from within the population, strain deterioration and contamination are important when growing strains for use and essential when maintaining cultures for long periods. There are a number of preservation techniques suitable for fungi, however many criteria should be assessed before preserving an isolate (Ryan *et al.,* 2000). No preservation technique has been

successfully applied to all fungi, although storage in liquid nitrogen appears to approach the ideal. However, changes in physiology and genetic stability may occur in some isolates (Ryan, 1999), so optimal preservation protocols may have to be established (Smith & Thomas, 1998). Most fungi that grow well in culture survive cryopreservation in liquid nitrogen including non-sporulating fungi although isolates that grow poorly tend to do less well. Organisms that have yet to be cultured in the laboratory or those that require growth on their host can also be successfully preserved, for example pathogenic organisms can be preserved in infected tissue. Other techniques, i.e. storage in mineral oil, soil or water may be of use for a wide range of organisms but the period of storage may be quite short. Only sporulating fungi survive well in silica gel storage, and spores with thin walls and high water content or those with appendages do less well. Centrifugal freeze-drying allows only the more robust spores to survive. Some sclerotia and other resting stages, and even in a few cases sterile mycelia, have been known to survive freeze-drying.

4.1.3 Yeasts

In general, yeast cells are considered to be robust, tolerant of unfavourable conditions, nutritionally undemanding, and readily managed in industry. It is incorrectly assumed that they are easy to maintain as a number of preservation and maintenance methods result in poor viability and instability of properties. Factors affecting survival are becoming better understood at the subcellular level, but many strains from a wide range of species remain difficult to preserve. The relative poor performance of yeasts post preservation may be partly attributed to the large size of cells compared with bacteria and the absence of the resistant spore types produced by many of the higher fungi. In the light of present knowledge, high survival rates can best be achieved by careful attention to the techniques used for preservation. For example, growth conditions, suspension media, choice of cryoprotectant and cooling rates.

Percentage survival of the total population, following subculture and drying is generally low, although ‘cultures’ may appear viable (Kirsop, 1974, 1978). Viability following freeze-drying is also frequently poor, but may be improved for some strains by careful selection of the suspension medium (Berny & Hennebert, 1989). In contrast, survival following storage in liquid nitrogen is high, often reaching levels of 100% (Hubalek & Kochova-Kratochvilova, 1978). There is no apparent relationship between survival and taxonomic position, and the factors determining survival are strain specific. Therefore, a preservation method that is satisfactory for one strain of a species may be unsuitable for others. If strain stability is of paramount importance the choice of maintenance method becomes critical. Any method that enables cell division to occur during storage should be rejected. It has been shown that the morphological, physiological and industrial characters of yeasts generally remain unchanged following freeze-drying (Kirsop, 1974), although other authors have found that genetic changes can occur (Souzu, 1973). Some workers have found that yeasts dried on silica gel may show substantial changes (Bassal *et al.,* 1977; Kirsop, 1978), whereas others report satisfactory results (Woods, 1976). Bassal *et al.* (1977) reported that genetically marked strains retain their characteristics after drying on filter paper,

and there is growing evidence that many strains stored in liquid nitrogen remain stable (Wellman & Stewart, 1973; Hubalek & Kochova-Kratochvilova, 1978; Pearson *et al.,* 1990).

4.1.4 Algae

As with other micro-organisms, algae are maintained under largely artificial conditions of media composition, light and temperature. Such conditions may cause selection or physiological adaptation, especially as algae naturally survive under complex and fluctuating conditions that follow a seasonal life cycle (McLellan *et al*., 1991). Compared with other groups of micro-organisms, relatively little research has been carried out on the development of long-term preservation methods for the microalgae. Freeze-drying does not give good recovery (often <1%) of the original population and prolonged storage may result in a further reduction in viability (Day, 1998; Day *et al.,* 1987; Day *et al.,* 2000; Holm-Hansen, 1967; McGrath *et al.,* 1978). The successful lyophilisation of the cyanobacterium *Nostoc muscorum*, using a method similar to that used for bacteria, has been reported, with no observed reduction in viability after storage for 5 years (Holm-Hansen, 1973). This technique has been adopted by a small number of researchers to preserve selected cyanobacterial strains. Cryopreservation has been successfully employed to maintain algae (Day *et al*., 2000; McLellan *et al*., 1991; Morris, 1976; Morris, 1978). Cryopreservation is advantageous because once organisms are cryopreserved by a proven method that yields high cell recovery, subsequent viability becomes independent of storage time. For example, some strains have no significant decline in viability >20 years of storage (Day *et al*, 1997). Using an appropriate protocol, high levels of viability (>90%) may be observed, however, no single protocol has been found to be successful for a wide range of strains.

4.1.5 Bacteria

As with fungi and algae, bacteria can be maintained by sub-culture. However, this is not ideal because of the chances of strain drift that can result from artificial culture conditions and contamination with other bacteria or fungal spores is a significant problem. Freeze-drying is often the method of choice providing a method that gives excellent recovery and ampoules that are easy to distribute and require no special storage conditions. Another method sometimes used to preserve organisms whilst allowing easy distribution is the gelatine disc technique. Occasionally, cultures are distributed as active cultures resuscitated from cryopreservation onto a suitable nutrient media. Cryopreservation is generally only used for isolates that are difficult to preserve. Freezing, especially to ultralow temperatures in or above liquid nitrogen is generally considered to be the least damaging preservation technique for bacteria. The lower the storage temperature the better the long-term survival and stability. Storage at -20°C is not generally recommended and storage in -70°C or -80°C freezers can be used if liquid nitrogen is not available. Many collections preserve plasmids, bacteriophages and genetically modified organisms in liquid nitrogen. Preserving them in this way reduces the chance of potential contamination which can result from aerosols produced during lyophilisation or when opening ampoules.

4.1.6 Standard preservation regimes

Similar techniques are used for the preservation for many different organisms often with special adaptations for the different types. The following sections describe the techniques in basic principles and where there are differences in methods used for different cell types goes on to provide details of some of the adaptations made and employed by the UKNCC member collections.

4.2 Serial sub-culture

Serial sub-culture is widely used and is perhaps the simplest and most cost effective method for a small laboratory, especially if cultures are required frequently and quickly. Most laboratories will retain some cultures by this method, commonly maintained on agar slopes rather than on Petri dishes and stored under controlled temperature depending on the genus. Refrigeration below room temperature is often used as it extends the subculture interval thereby reducing the number of transfers required as a result of the suppression of the metabolic rate. Limiting nutrients can also have the effect of reducing growth rate and extending periods between transfers. When maintaining large numbers of organisms or those of greater hazard it is advisable to work in an appropriate microbiological safety cabinet to protect the worker and strict aseptic technique must be observed to protect the organism (Smith & Onions, 1994).

4.2.1 Adaptation for filamentous fungi

Fungal transfer by sub-culture can be potentially disadvantageous, as frequent sub-culturing could result in contamination from other micro-organisms such as bacteria or air-borne spores of other fungal species. The choice of medium is an important factor, as some fungi are notoriously difficult to culture (e.g., many mycorrhizal fungi). Most fungi will survive on Malt Agar (MA) or Potato Carrot Agar (PCA) (Smith & Onions, 1994), but others require more specialised media. Dermatophytes, for example, may grow better on a substrate of hair (Al-Doory, 1968). Additives such as growth factors may be added to the growth medium for specific fungi (Smith, 1993). Variation of the nutrient source may prevent the permanent adaptation and modification of the strain to a specific medium. Media should not encourage excessive sporulation or fructification as meiotic or mitotic crossing may promote the formation of recombinants that may differ from the parental genotypes (Smith & Onions, 1994). When inoculating fresh plates, it is recommended to subculture from the periphery of the fungal colony, i.e. the region of actively growing mycelium The precautions mentioned above should ensure that, as far as possible, fungi maintain the characteristics exhibited upon isolation from nature and do not mutate or show selection. It is a feature of the opportunistic nature of fungi, to easily adapt to the environment. However, despite the best management, this will inevitably happen if isolates are maintained in culture for long periods. Asexual processes such as conidiogenesis and sexual processes that result in genetic recombination enhance the likelihood of selection and mutation (Burdsall, 1994). Characteristics may unintentionally be “selected out” from fungal cultures if workers sub-culture from atypical sectors on a plate (Smith & Allsopp, 1992). The use of cold storage can slow the rate of metabolism and thus increase the intervals between subculture. Storage at 4-7°C in a refrigerator, or cold room, can extend the transfer interval to 4-6 months from the average period of 2-4 months. Storage in a deep freeze (-7 to -24°C) will allow many fungi to survive 4-5 years between transfers,

though freezing damage may occur in some. Furthermore, mites do not invade cultures at these lower temperatures.

During storage, cultures should be routinely sealed with air-permeable tape to prevent invasion from mites such as *Tyrophagus* and *Tarsonemus.* Mites not only damage cultures by utilising the fungus as a food source but carry contaminants such as bacteria or fungal spores from plate to plate, thus rendering cultures unusable (see section 3.2.9). A mite infestation can be extremely costly, as all cultures may need to be destroyed. Important cultures can be recovered using a combination of antibiotics, freezing and careful sub-culturing (Smith & Onions, 1994). However, irreversible damage could be caused to the fungus due to the pressures exerted by the restorative methods. Frequent cleaning of laboratory work surfaces with bleach or acaricides can deter mites.

4.2.2 Adaptation for yeasts

Subculturing is a technique that has been used for many years and continues to be useful, particularly in the short term. However, as with fungi, it is recognised that substantial variation may occur in strains maintained over long periods (see Section 3.5.8).

There are two ways in which subculturing can be carried out. Subculturing in broth is commonly used and involves the transfer of inoculum from old stock to fresh bottles of liquid media. The NCYC includes yeasts that have been maintained by this method for periods of up to 60 years. Subculturing from solid depleted media to fresh media is the other method used and has been used at the NCYC in the past. Although this method has not been used for many years, all yeasts maintained in this way in the NCYC showed high levels of recovery. Non-fermentative strains often survive better on agar slants than in broth.

4.2.3 Adaptation for algae

The algae are also frequently maintained on agar slants but most often in liquid media. Transfer by sub-culture can be potentially disadvantageous, as frequent sub-culturing can result in contamination from micro-organisms such as bacteria. Environmental parameters such as light and temperature are also critical (see Section 3.1). The choice of medium is an important factor and is tailored to the requirements of the individual genera. When established, cultures proceed to logarithmic growth before stationary phase is achieved. Eventual exhaustion of the nutrient supply and reduction in the availability of dissolved gases associated with an accumulation of waste products will cause deterioration and subsequent loss of the culture. Transfer to fresh growth medium of viable material in late exponential or early stationary phase is advised (McLellan *et al*., 1991)

4.2.4 Adaptation for bacteria

Bacteria are frequently maintained by continual sub-culture, storing cultures in a refrigerator or freezer can extend intervals between sub-culture. Although nutrient agar is widely used to maintain bacteria, many isolates require specialist media. A list of media recipes commonly used for bacteria by the

NCIMB is included in Appendix B**.** As with all sub-culture regimes, long-term culture carries risks of unintentional selection and contamination. Therefore, continuous culture techniques should not be used for bacterial cultures unless absolutely necessary in the short-term.

4.3 Storage under mineral oil

This method is generally only used for yeasts and filamentous fungi but can be applied successfully to bacteria. It involves covering cultures with mineral oil to prevent dehydration and to slow down the metabolic activity and growth through reduced oxygen tension. The method was first extensively used by Buell and Weston (1947), and subsequent reports have indicated its wide application and success (Dade, 1960; Fennell, 1960; Little & Gordon, 1967; Smith *et al.,* 1970; Onions, 1971, 1977; Smith & Onions, 1994). Mature healthy cultures on agar slants (30° to the horizontal) in 30ml universal bottles) are covered by 10mm of sterile (achieved by autoclaving twice at 121°C for 15min) mineral oil (liquid paraffin or medicinal paraffin specific gravity 0.830-0.890). If the oil is deeper than 10mm the fungus may not receive sufficient oxygen and may die, while if the depth is less, exposed mycelium or agar on the sides of the container may allow moisture to evaporate and the culture to dry out. At CABI Bioscience, Universal bottles are stored with their caps loose in aluminium segmented racks (Denley Ltd.) in a temperature controlled (15-18°C) room. Retrieval is relatively easy and involves the removal of a small section of the colony with a mounted needle. Excess oil is drained away and the inoculum streaked onto a suitable agar medium (see protocol section 4.13.1). More than one subculture may be necessary after retrieval as the growth rate may be reduced because of adhering oil. The fungal mycelium can normally recover when it is re-isolated from the edge of the colony on the first agar plate and transferred to fresh media. Inoculating an agar slope centrally sometimes has better results as excess oil can drain down the slope allowing the fungus to grow more typically towards its top. There is an added risk of personal contamination because of the spattering of oil containing the fungus when inoculation needles are sterilised in a Bunsen flame (Fennell, 1960). The shelf life of yeast cultures preserved in oil is around 2 to 3 years. The NCYC has no direct experience of this method, but acknowledges that it has been used by a large number of laboratories over the years. There is little documentation regarding the yeast species that have been maintained successfully.

The disadvantages of oil include the possibility of contamination by air-borne spores, retarded growth on retrieval and continuous growth under adverse conditions that could lead to selection. However, preservation under oil is recommended for storage of organisms in laboratories with limited resources and facilities. The advantages of oil storage are long viability of some specimens and survival of species that do not survive other preservation regimes; expensive equipment and consumables are not required. Additionally, mites do not cause infestations in oil cultures as they are unable to escape once they have entered the culture bottle. A wide range of fungi survive this method, *Saprolegniaceae* and other water moulds survive 12-30 months (Reischer, 1949). Species of *Aspergillus* and *Penicillium* have remained viable for 40 years and some strains of *Phytophthora* and *Pythium* species have also survived 40 years at CABI Bioscience. Many cultures have shown that they deteriorate under mineral oil and must be transferred regularly to reduce this effect. However, organisms that are sensitive to

other techniques can be stored successfully in oil, for example *Cercospora*, *Arthrobotrys*, *Colletotrichum*, *Conidiobolus*, *Corticium*, *Nodulisporium* and mycelial *Basidiomycetes* should be transferred every two years.

4.4 Water storage

Immersion in sterile water can be used to extend the life of an agar culture (Burdsall, 1994). The method is generally applied to fungi and can be achieved in many different ways. One simple way is to grow the fungus on an agar slope in a Universal bottle and then cover the agar surface with water. An alternative method involves the transfer of mycelial plugs or blocks cut from cultures grown on agar in Petri dishes to Universal bottles containing 10ml of sterile deionised water (Boeswinkel, 1976). The protocol used at CABI is outlined below (section 4.13.2). To reduce the storage space occupied, cryovials may be used in a similar way (Burdsall 1994). The "shelf-life" of fungi in water is variable but Figueredo and Pimental (1975) successfully stored examples of phytopathogenic fungi for ten years by this means. Onions and Smith (1984) stored strains of *Pythium* and *Phytophthora* in water for five years but only 58% of these remained viable. Qiangqiang *et al.* (1998) preserved 78 isolates, belonging to seven genera, in water for 12 years, on resuscitation, 89.7 % of isolates were viable. Burdsall (1994) reported that water storage did not significantly affect growth rate, viability or genetic stability in 155 isolates of Basidiomycota stored for 7 years. As with all methods, some fungi are better suited to individual protocols, and notably ectomycorrhizal fungi have been successfully stored by this method (Marx & Daniel, 1976). However, the storage of ectomycorrhizal basidiospore slurries in water was not successful (Torres & Honrubia, 1994). The advantages of storage in water are the low cost and easy application. However, the length of storage is often limited and some fungi will not survive even short periods submerged. As with all methods that allow growth or metabolism during storage there are better methods and it is considered only to be useful for short-term preservation (2-5 years) and should be backed up by longer-term storage methods (Smith & Onions, 1994).

4.5 Silica gel storage

The silica gel method has been applied to fungi at CABI Bioscience and proved to be very successful. Sporulating fungi have been stored for 7-18 years in silica gel and appear to remain morphologically stable after resuscitation (Smith & Onions, 1994). The technique is relatively simple and involves the inoculation of a suspension of fungal propagules onto cold silica gel. The culture will then dehydrate to enable storage without growth or metabolism (see protocol section 4.13.3 for the method used at CABI and section 14.13.4 for that used at NCYC for yeasts).

Silica gel storage has a number of advantages, it is cheap, simple and does not require expensive apparatus. Cultures are relatively stable, allowing a wide range of sporulating fungi (including representatives of the *Basidiomycota*) to be successfully preserved. Penetration by mites is unlikely, as they cannot survive the dry conditions encountered. Repeated inocula can be removed from a single bottle. However, it is recommended that a stock bottle is prepared, to be used in case of contamination during retrieval. There are some disadvantages of silica gel storage. It is limited to sporulating fungi

and is unsuitable for *Pythium*, *Phytophthora* and other *Oomycota*, mycelial fungi or fungi with delicate or complex spores. This limitation doesn't apply to yeast cultures. There is a possibility of introducing contaminants by repeated retrievals.

This method is no longer used routinely for yeasts at NCYC, but has been used successfully by C.F.Roberts of the Department of Genetics at Leicester University. Fungi have been stored for in excess of 25 years using the method described by Perkins (1962) Furthermore, all strains assessed to date, remained genetically stable.

4.6 Soil storage

This technique can be applied to a range of micro-organisms that can withstand a degree of desiccation for example the spores and resting stages of filamentous fungi, bacteria such as *Bacillus* spp. and some microalgae such as *Haematococcus pluvialis*. At CABI Bioscience the method involves inoculation of double autoclaved soil (121°C for 15 min) with 1ml of spore suspension in sterile distilled water and then incubation at 20-25°C for 5-10 days depending on the growth rate of the fungus (see protocol section 4.13.5). This initial growth period allows the fungus to utilise the available moisture, before the induction of dormancy. The bottles are stored in a refrigerator (4-7°C). This method is widely for the storage of *Fusarium* isolates and related genera. Preservation in sterile sandy loam soil may be one of the most practical and cost-efficient ways to preserve filamentous sporulating micro-organisms. Other advantages include good viability of cultures for up to 10 years, a reduced chance of mite infection and the option of obtaining repeated inocula from the same source. This method of storage is very successful with *Fusarium* species (Gordon, 1952; Booth, 1971). Atkinson (1953) obtained good recovery of *Rhizopus, Alternaria, Aspergillus, Circinella* and *Penicillium*. No loss in pathogenicity of *Septoria* species isolated from cereals was observed after 20 months in soil (Shearer *et al.,* 1974), or of *Pseudocercosporella* spp. for 1 year in soil (Reinecke & Fokkema, 1979). However, examination of Gordon's collection showed that 76% of *Fusarium equiseti*, 75% of *F. semitectum* and 50% of *F. acuminatum* isolates had been outgrown by mutant strains (Booth, 1971). Despite this, soil storage should be used in preference to oil storage for the preservation of *Fusarium* species and other fungi that show variation under oil. There are few disadvantages but the method is not suitable for many fungi and variation may occur after storage.

4.7 Freeze-drying (lyophilisation)

Freeze-drying (lyophilisation) is a highly successful method for preserving bacteria, yeasts and the spores of filamentous fungi. During the freeze-drying process water is removed directly from frozen material by sublimation under vacuum. If carried out correctly, freeze-drying will prevent shrinkage, structural change and help retain viability. There is a vast array of freeze-drying equipment available, ranging from laboratory bench models through to pilot scale and huge industrial installations. Freeze-drying should be optimised for different organisms and cell types. If this is done it should be successful for the majority of bacteria, sporulating fungi, and yeasts. It is generally unsatisfactory for eukaryotic microalgae as levels of post preservation viability are unacceptably low (Day & McLellan, 1995).

More protocol development is required to achieve successful lyophilisation for algae and protozoa, although cyanobacteria are more likely to survive (Day & Smith, in prep).

Lyoinjury can occur during the cooling and/or drying stages (Tan, 1997). The phase changes encountered during the drying process can cause the liquid crystalline structure of the cell membranes to degenerate to the gel phase, which disrupts the fluid-mosaic structure of the membrane (Tan, 1997). This causes leakage of the membrane, which may culminate in cell damage. Optimal survival can be improved with the use of a suitable suspension medium. It should be readily available, easy to prepare and provide protection during the freeze-drying process (i.e. to protect the spores/cells from ice damage during cooling and storage problems such as oxidation). Skimmed milk is a suitable protectant for fungi and is sometimes used in combination with inositol. Saccharides such as trehalose (Tan *et al.,* 1995; Tan, 1997) protect membranes by attaching to the phospholipids, replacing water and lowering the transition temperature. Other suspending media can be used when preserving bacteria and yeasts with many collections using their preferred preservation base. For example Tan *et al.,* (1995) suggest that a mix of dextran and trehalose improves the viability of cultures.

The recommended final moisture content following drying is between 1 and 2%(w/v). To monitor freeze-drying a means of measuring vacuum both in the chamber and close to the vacuum pump is required. Comparing the measurements will allow the determination of the end point of the drying process. When the values are equal, water has ceased to evaporate from the material being dried and drying is probably complete. This is confirmed by determining the residual water content. This can be done by dry weight determination or by the use of chemical methods such as the Karl Fischer technique (Baker, 1955). The freezing point of the material should be determined and the temperature monitored during freeze-drying. The sample temperature must not rise above the melting point until most of the water has been removed. To ensure that a high quality product is produced and maintained the equipment used must be reliable and conditions reproducible from batch to batch.

The technique of centrifugal freeze-drying, which relies on evaporative cooling, can be used successfully for the storage of many sporulating fungi (Smith, 1983a), as well as bacteria and yeasts. However, this is not a method that can be adapted and changed easily, as it is dependent upon the scope of the equipment. Optimisation of cooling rate to suit the organism being freeze-dried can be applied using a shelf freeze-drier. The sealing of the ampoules or vials is most important and heat sealed glass is preferred to butyl rubber bungs in glass vials as these may leak over long-term storage and allow deterioration of the freeze-dried organism. There are many advantages of freeze-drying over other methods, including the total sealing of the specimen and protection from infection and infestation. Cultures generally have good viability/stability and can be stored for many years. Ampoules take up little space and can be easily stored. In addition, cultures do not have to be revived before postal distribution. However, there are disadvantages, some isolates fail to survive the process and others have reduced viability and genetic change may occur (Ashwood-Smith & Grant, 1976; Ryan, 1999) though unless high viability is retained it is difficult to differentiate between this and selection of spontaneous

mutants by freeze-drying (Heckly, 1978). Ampoules of freeze-dried organisms must be stored out of direct sunlight and chilled storage will reduce the rate of deterioration and should extend shelf-life. However, the process of lyophilisation is relatively complex, can be time-consuming and may be expensive.

4.7.1 Adaptation for filamentous fungi

At CABI Bioscience a two-stage centrifugal freeze-drying processes has been used since 1966 (see protocol section 4.13.6) and an optimisable shelf freeze-drying protocol was introduced in 1982 (see protocol, section 4.13.10). Freeze drying of sporulating fungi such as the Ascomycota and mitosporic fungi is routinely undertaken, but is not so suitable for the Oomycota and other non-sporulating cultures. Although it is only spores and conidia that are routinely freeze dried, research has been carried out to establish whether lyophilised hyphae can be revitalised successfully after preservation. In most cases this has met with little success, but hyphae from *Claviceps* spp. (Pertot *et al.,* 1977), a limited range of basidiomycetes (Bazzigher, 1962) and some arbuscular mycorrhizal fungi (Tommerup, 1979) have been revitalised successfully. Investigations by Tan *et al.,* (1991a, b) gave mixed results. Some cultures did not survive at all and others showed only limited viability. Success with freeze-drying varies between isolates of the same species. In general those fungi that grow and sporulate well in culture survive the process, while weak or deteriorated isolates tend to fail. It may therefore be misleading to state categorically that one particular species will not survive freeze-drying. In general the young vegetative hyphae of fungi do not survive freeze-drying. At CABI Bioscience it has been found that sterile ascomata, chlamydospores, sclerotia and in some few cases stroma and resting mycelium have survived. However, in general it is only the spores (e.g., conidia, ascospores, and basidiospores) that survive.

4.7.2 Adaptation for yeasts

Yeast cultures are held in long-term storage in the National Collection of Yeast Cultures (NCYC: Norwich, UK) freeze-dried in glass ampoules (see protocol section 4.13.9). Freeze-drying is a generally accepted method for yeast storage, although viability is generally low, typically between 1 and 30%, as compared to >30% for those of yeast preserved frozen in liquid nitrogen. Some strains have been stored for 30 years at the NCYC and for longer periods by other laboratories. There are several yeast genera, including *Lipomyces*, *Leucosporidium*, *Brettanomyces*, *Dekkera*, *Bullera*, *Sporobolomyces* and *Rhodosporidium* that have particularly low survival levels and frequently cannot be successfully freeze-dried by the standard method. However, some improvements have been made recently using trehalose as a protectant (Berny & Hennebert, 1991; Roser, 1991). Survival of yeasts following freeze-drying is remarkably strain specific and generalisations regarding survival levels should be viewed with caution. Nevertheless, all cultures maintained at NCYC, and covering nearly all yeast genera, have been recovered successfully, although the percentage survival of the population is generally low.

4.7.3 Adaptation for bacteria

Freeze-drying is the universal method employed for the preservation of bacteria although there are a number of modifications to the basic freeze-drying procedure that can be used. The UKNCC bacteria collections use modified techniques; some of the main protocols used by NCTC (section 14.13.7) and NCIMB (section 14.13.8) are described below. The majority of bacteria survive freeze-drying well, but a few species can sometimes give disappointing results. This may be due in some cases to difficulties in obtaining adequate pre-drying growth. Cultures which often prove more difficult than others include *Aquaspirillum serpens*, *Clostridium botulinum*, *C. chauvoei*, *C. novyi*, *C. putrificum*, *C. scatologenes*, *Helibacter pylori* and *Peptococcus heliotrinreducans*. Additionally, some lesser problems may be encountered with *Bacteroides melaninogenicus*, *Haemophilus canis*, *H. suis*, *Leptotrichia buccalis*, *Mycobacterium microti* and *Neisseria gonorrhoeae*.

4.8 L-drying

Liquid drying (L-drying as first described by Annear (1958) is a useful alternative method of vacuum drying for the preservation of bacteria that are particularly sensitive to the initial freezing stage of the normal lyophilisation process. The intrinsic feature of this process is that cultures are prevented from freezing; drying occurs direct from the liquid phase. In the NCIMB bacteria such as *Spirilla* and *Azomonas insignis* have been preserved by L-drying (see protocol section 14.13.11). These organisms are particularly sensitive to freeze-drying, but L-dried cultures have survived with good recovery levels for up to fifteen years. L-drying can, therefore, be considered as a suitable alternative to freeze-drying for bacteria that are susceptible to damage by freeze-drying.

4.9 Microdrying

Microdrying is a modification of the freeze-drying method used in the NCIMB for preserving bacteria (see protocol section 14.13.12). It has several advantages over centrifugal freeze-drying because fewer manipulations are involved. Resuscitation requires less skill and there is less risk of contamination as the resuspending stage is omitted. The method is very similar to freeze-drying, all the cell suspension is absorbed into a strip of thick filter paper and freeze-dried, the filter strip enables the product to be tipped out easily into a resuspending or growth medium. Microdrying has been used in the NCIMB for about 12 years, further details can be obtained from NCIMB via the UKNCC web site (http://www.ukncc.co.uk). Ampoules of various Gram-negative and Gram-positive bacteria prepared in 1978 have recently been opened to test the survival of the cultures. The results obtained indicate that, in general, the long-term survival and recovery of microdried cultures are comparable to those obtained with conventionally freeze-dried cultures.

4.10 Maintenance of bacteria in gelatine discs

Preservation of bacteria in the form of gelatine discs was first described by Stamp (1947). A harvest of bacterial growth is suspended in melted nutrient gelatine, drops of which are allowed to solidify in Petri dishes. The drops are freeze-dried, or dried over a desiccant, and the resultant flat discs are stored over silica gel. When required, a single disc is placed in warmed broth and the resulting suspension plated onto a suitable growth medium (for the protocol used at NCTC see section 4.13.13). The method is not particularly suitable for storage of numerous strains over long-periods. However, it is invaluable for storage of a limited number of frequently used strains, such as those used for quality control of media or reagents. The method therefore has advantages over both active subculture on slopes and freeze-drying in ampoules. A number of organisations (American Type Culture Collection, Difco Laboratories, Remel) provide standard strains of micro-organisms in this form at a cheaper rate than freeze-dried cultures. In addition, the Czechoslovak Culture Collection (CCM) makes available 20 different strains for control purposes or for use in diagnostic laboratories. It also prepares discs as a service to customers. Advantages of storage using gelatine discs include their ease of use and storage (30 or 40 discs can be kept in a 14mm screw-capped vial). As the discs are kept dry there is no opportunity for growth of any contaminants introduced during sampling so they remain free from contamination. Characters remain relatively stable, because the bacteria are not growing there is no opportunity for mutation and selection. Various species of Enterobacteriaceae and Staphylococci, and strains of *Pseudomonas aeruginosa* and *Corynebacterium diphtheriae* have been successfully preserved for at least 4 years although this method has not been successful with more delicate species such as *Neisseria* or *Haemophilus*. However, Obara *et al.* (1981) using a method described by Yamai *et al.* (1979) have reported successful preservation of *Neisseria*, *Haemophilus* and *Bacteroides*, using a gelatin-disc method based on a different suspending medium.

4.11 Cryopreservation

The ability of living organisms to survive freezing and thawing was first realised in 1663 when Henry Power successfully froze and revived nematodes (Morris, 1981). Polge *et al.* (1949) became the first "modern day" scientists to report the freezing of living organisms when they successfully froze and thawed avian spermatozoa. Liquid nitrogen is the preferred cooling agent for cryopreservation, although liquid air or carbon dioxide can be used. Lowering the temperature of biological material reduces the rate of metabolism until, when all internal water is frozen, no further biochemical reactions occur and metabolism is suspended (Franks, 1981). Although little metabolic activity takes place below -70°C, recrystallisation of ice can occur at temperatures above -139°C (Morris, 1981) and this can cause structural damage during storage. Consequently, the storage of micro-organisms at the ultra-low temperature of (-190°C to 196°C) in or above liquid nitrogen is the preferred preservation method of many scientists (Hubalek, 1996; Smith & Thomas, 1998). Provided adequate care is taken during freezing and thawing, the culture will not undergo change, either phenotypically or genotypically.

Choice of cryoprotectant is a matter of experience and varies according to the organism. Cryoprotection is achieved by:

i. Non-critical volume loss by the reduction of ice formation.

ii. An increase in viscosity, which slows down, ice crystal growth and formation and solute effects.

iii. Reduction of the rate of diffusion of water caused by the increase of solutes.

Glycerol 10%(v/v) gives very satisfactory results but requires time to penetrate the organism; some fungi are damaged by this delay. Dimethyl sulfoxide (DMSO) penetrates rapidly and is often more satisfactory (Hwang & Howells, 1968; Hwang *et al.,* 1976). Sugars and large molecular substances, such as polyvinyl pyrrolidine (PVP) (Ashwood-Smith & Warby, 1971) have been used but in general have been less successful (Smith, 1983b). Trehalose may be better but is expensive. Establishing the optimum cooling rate has been the subject of much research (Smith, 1993; Hwang, 1960; Morris *et al.,* 1988). Slow cooling at 1°C min^{-1} over the critical phase has proved most successful (Hwang, 1966, 1968), but some less sensitive isolates respond well to rapid cooling, preferably without protectant. Slow warming may cause damage owing to the recrystallisation of ice, therefore rapid thawing is recommended. Slow freezing and rapid thawing generally give high recoveries for fungi (Heckly, 1978).

As with other methods of preservation liquid nitrogen cryopreservation has advantages and disadvantages. Advantages include the length of storage, which is considered to be effectively limitless if storage temperature is kept below -139°C. The majority of organisms survive well, giving the method a greater range of successful application. Organisms remain free of contamination when stored in sealed ampoules. Disadvantages of liquid nitrogen storage include the high cost of apparatus such as refrigerators and a continual supply of liquid nitrogen. A regular supply cannot be obtained in some parts of the world and therefore the technique cannot be used. If the supply of nitrogen fails (or the double-jacketed, vacuum-sealed storage vessels corrode and rupture) then the whole collection can be lost. There are also safety considerations to be made, the storage vessels must be kept in a well-ventilated room, as the constant evaporation of the nitrogen gas could displace the air and suffocate workers.

4.11.1 Adaptation for filamentous fungi

Cryopreservation has been used for the preservation of fungi at CABI Bioscience since 1968 (Onions, 1971, 1977), although early work involved a very simple procedure. Storage at –196°C in liquid nitrogen or at slightly higher temperatures in the vapour phase is employed. Generally a cooling rate of -1°C min^{-1} with 10% (v/v) glycerol as a cryoprotectant is applied and to date, over 4 000 species belonging to over 700 genera have been successfully frozen (see protocol section 4.13.14). No morphological or physiological change has been observed in the 9 000 fungal isolates stored. However, some members of the Oomycota and Basidiomycota survive cryopreservation less well than sporulating fungi and it is anticipated that by employing species-specific cooling rates may provide improved viability.

4.11.2 Adaptation for yeast

There are two methods used for the cryopreservation of Yeasts at the NCYC. These ultimately achieve the same thing, but using slightly different storage techniques. As with the cryopreservation of fungi, yeast suspensions can be preserved in glass ampoules in the liquid phase of the nitrogen. However, an alternative method that saves space and provides additional security against leakage of liquid nitrogen into the ampoules, is the method of storing the yeast suspensions in heat-sealed straws in the vapour phase of the liquid nitrogen frozen (see protocol section 4.13.15). This enables a considerable reduction in storage space and extra protection against contamination by liquid nitrogen leakage or the safety implication of glass vials contaminated by liquid nitrogen leakage rupturing on rewarming. Storage in straws was first described by Gilmour *et al.* (1978) using artificial insemination straws. Variations on the original method are now in use around the world. Work on refining methods of storage in liquid nitrogen is continuing, as is research into the effects of the freezing process on the cells.

4.11.3 Adaptation for algae

Compared to other groups of micro-organisms, relatively little research has been carried out on the development of long-term preservation methods for the microalgae and cyanobacteria. Cryopreservation has been successfully employed to maintain algae (Day, 1998; Morris, 1976; Morris, 1978; Day *et al*., 1997). An overwhelming advantage of cryopreservation, is that once organisms are cryopreserved by a proven method giving high cell recovery, viability is independent of storage time. Direct immersion in liquid nitrogen, with or without the addition of cryoprotectants, has been successfully employed to preserve a few unicellular Chlorococcales and cyanobacteria (Holm-Hansen, 1963; Box, 1988). However, most protocols employ a simple two-step system with the controlled/semi-controlled cooling from room temperature to a subzero holding temperature prior to plunging into liquid nitrogen (Day, 1998). The majority of protocols in current use have been developed empirically with the pre-freezing culture regime, cryoprotectant choice (usually DMSO or methanol) and concentration, cooling rate, and thawing regime being manipulated to minimise the amount of damage to the algal cells and hence maximise post-thaw viability levels. The protocol used at CCAP Windermere is presented below frozen (see section 4.13.16).

4.11.4 Adaptation for bacteria

The UKNCC collections do not routinely cryopreserve bacteria (other than as back-up or seed stocks). However, at the NCIMB (and NCTC) this method is used for bacteria, which do not survive freeze-drying or L-drying, for patent deposits, sensitive mutants, genetically manipulated strains and all bacteriophages (see protocol section 4.13.17). The latter are preserved by this method, not because they cannot be freeze-dried or L-dried, but because of the risk of contaminating equipment and consequently other cultures with phage particles. The glass beads method has been used in the NCIMB for approximately 10 years. To date, no problems have been encountered with most genera, although certain obligate methylotrophic bacteria have been found to lose viability on storage over liquid nitrogen. Survival and recovery rates are comparable with conventional freeze-drying. The method is

also used as alternative to freeze-drying for susceptible bacteria, for example plasmid-containing strains. Most coliphages, after an initial loss of titre of approximately one order of magnitude due to the freezing process, will survive for at least 10 years without further significant loss of titre. An exception is coliphage MS2, which does not survive for more than 1.5 years. *Pseudomonas aeruginosa* phages have been shown to survive for at least 10 years and *Staphylococcus aureus* phages for 9 years. Marine phages tend to have shorter shelf lives. For example, *Pseudomonas* PM2 phage does not survive for more than 2-3 years.

4.12 Preservation of animal and human cell lines

The emergence of mammalian cell culture has its origin in the first attempts to culture tissue explants in vitro at the turn of the 20th century. The subsequent development of complex culture medium formulations in the 1950s rapidly established a wide range of cell lines and provided a valuable research tool for the study of growth mechanisms and disease. Today, the availability of thousands of animal cell lines offers a reproducible source of material for all aspects of medical and agricultural research. Maintenance of cell lines in continuous culture by subculture would therefore be impractical because of the high cost of serum containing media, risk of exposure to microbial contaminants, and the possibilities of culture cross-contamination and genetic drift. Therefore, it becomes necessary to store cell stocks for future use. Concomitant research into subzero storage methods at the time when cell culture methodology was being developed led to the discovery that addition of glycerol to fowl semen enhanced survival of spermatozoa after storage at -79°C. The use of gaseous (>-130°C) and liquid nitrogen (-196°C) now allows indefinite storage of most mammalian cell lines after cryopreservation. Techniques have advanced to permit almost unlimited storage of cell lines at liquid nitrogen temperatures (-196°C) if correct cryopreservation techniques are followed (Doyle *et al.,* 1989a). Studying the effects of freezing and thawing at various rates from which a hypothesis of freezing injury to cells was proposed have established the precise mechanism for optimal cryopreservation. A typical cryopreservation protocol followed by ECACC is described below see protocol section 4.13.18.

The majority of cell lines must be cooled down slowly (-1 to -3°C min^{-1}) and thawed rapidly to achieve maximum viability. The rate of cooling is optimised to allow time for intracellular water to escape, and subsequently reduce the amount of intracellular ice formed. The presence of intracellular ice during thawing may cause lethal damage to intracellular membranes. However, addition of cryoprotectants such as dimethylsulphoxide (DMSO) to the cells, depresses the temperature at which intracellular ice is formed and allows cooling rates to be reduced for more efficient water loss. The development of program-controlled freezers now allows individual-cooling profiles to be designed that give maximum cell viability. Within culture collections such as the European Collection of Animal Cell Cultures (ECACC, Salisbury, UK), many thousands of cell lines are now currently available to which new cell lines are added annually. A systematic approach to the quality control of cell lines and their cell banks is therefore essential to ensure future supplies of authentic material.

4.13 Preservation regimes: standard protocols

The following standard protocols provide details of procedures used in the UKNCC member collections.

All of the following protocols should be carried out in appropriate microbiological safety cabinets, using aseptic techniques and observing good laboratory practice at all times. Sterile laboratory equipment should be used at all times. Additional information can be found in Kirsop & Doyle (1991) and Smith & Onions (1994).

4.13.1 Oil storage

FOR FILAMENTOUS FUNGI AT CABI BIOSCIENCE

Equipment and reagents

- Medicinal quality liquid paraffin, specific gravity 0.830-0.890 (autoclaved twice at 121°C for 20min on consecutive days).
- Sterile solid growth medium in 30ml universal bottles: set at 30° slope with an appropriate growth medium.
- Metal segmented trays (375x175mm divided into 25x25mm squares to take 60 x 30ml Universal bottles (Denley).
- Inoculating needle or loop.

Method

1. Inoculate at least two Universal bottles for each strain to be maintained.

2. Label one culture as reserve stock, the other(s) as working stock.

3. Incubate at optimum growth temperature until the organism has reached maturity.

4. Add 8-10ml of sterile liquid paraffin to cover the slope to a maximum depth of 10mm over its highest point.

5. Store the oiled cultures, with the screw caps loose, in metal divided racks at 15-20°C.

Recovery

1. Remove a portion of the working stock culture using a sterile needle or loop.

2. Drain as much oil as possible from the inoculum.

3. Inoculate fresh growth medium (it is often best to inoculate a slope so that the adhering oil can drain and the organism can grow up the slope away from the oil at the point of inoculation).

4. The reserve stock culture is used only when re-preservation becomes necessary when all the inoculum has been removed, when it is contaminated or when the shelf life expiry date* set for the organism has been reached.

*Although several fungi have survived for 44 years at CAB Bioscience UK Centre (Egham) it is advisable to set a re-preservation date of between 2 and 10 years.

At the National Collection of Yeast Cultures (NCYC) BP medicinal oil (BDH Chemicals Ltd.) is used and the cultures stored with the screw caps loose, at 4°C.

4.13.2 Water storage

FOR FILAMENTOUS FUNGI AT CABI BIOSCIENCE

Equipment and reagents

- Sterile distilled water (10ml in 30ml Universal bottles, at least two per culture).
- Mature cultures on agar media in Petri dishes.
- Metal segmented trays (375x175mm divided into 25x25mm squares to take 60 x 30ml Universal bottles (Denley).
- Inoculating needle or loop.

Method

1. Cut (generally from the growing edge) agar blocks ($6mm^3$) through the colony*.

2. Transfer 20-30 agar blocks to 10ml of sterile distilled water in two or more 30ml Universal bottles.

3. Label one bottle as reserve stock and the other(s) as working stock.

4. Screw the caps of the universal bottles tightly and store between 20-25°C.

Recovery

Remove an agar block from the working stock and inoculate (organism face down) on a suitable growth medium and incubate under optimum growth conditions. Use the reserve stock when re-preservation is necessary.

*Alternatively sporulating or non-filamentous organisms can be harvested without the agar and simply suspended in water. Recover the organism by placing a small amount of the suspension on to suitable growth medium.

4.13.3 Silica gel storage for filamentous fungi

FOR FILAMENTOUS FUNGI AT CABI BIOSCIENCE

Equipment and reagents

- Coarse non-indicator silica gel.
- Sterile 5% (w/v) solution of non-fat skimmed milk cooled to 5°C.
- Sterile Pasteur pipettes.
- 30ml glass Universal bottles (a minimum of 2 per culture).
- Waterproof tray (100mm deep) filled to 30mm with water.
- Refrigerator.
- -20°C freezer.
- Airtight storage boxes.
- Indicator silica gel.

Method

1. One-third fill glass Universal bottles (30ml bottles are used at CABI Bioscience, but any heat resistant screw cap bottle will suffice) with medium grain plain 6-22 mesh non-indicating silica gel and sterilise by dry heat (180°C for 3h).

2. Place bottles in a tray of water up to the level of the silica gel and leave overnight in a -20°C freezer (nominal).

3. Prepare spore suspensions in cooled 5% (w/v) skimmed milk.

4. Using a Pasteur pipette add 1ml of suspension to at least two bottles of the silica gel (whilst they remain in the frozen water).

5. After 20min, remove the Universal bottles from the ice and agitate them to disperse the suspension.

6. Label one bottle as reserve stock and the other(s) as working stock.

7. Incubate the bottles at 25°C until the silica gel crystals readily separate when agitated, this may take one or two weeks.

8. Screw the bottle caps down and store in air-tight containers over indicator silica gel to absorb moisture (or include an open Universal containing indicator silica gel) at 4°C (storage between 20 and 25°C is satisfactory)

Recovery

1. Sprinkle a few crystals from the working stock on to a suitable growth medium and incubate under appropriate growth conditions.

2. If the organism fails to grow, attempt again, this time streaking a silica gel crystal over the agar to dislodge the cells and discarding the silica gel crystal before incubation.

4.13.4 Silica gel storage for yeasts

FOR YEASTS AT THE NATIONAL COLLECTION OF YEAST CULTURES (NCYC)

Equipment and reagents

- Purified silica gel (BDH Chemicals Ltd.), mesh 6-22
- McCartney bottles
- 10ml 5% (w/v) skimmed milk solution (Unipath Ltd.) in McCartney bottles (autoclaved at 116°C for 10min)
- Refrigerator
- Ice tray
- Pasteur pipettes
- Airtight plastic storage box
- Indicator silica gel (BDH Chemicals Ltd.)

Method

1. Fill glass McCartney bottles (any heat resistant caps will suit) to a depth of 1cm with medium grain plain 6-22 mesh non-indicating silica gel and sterilise in an oven at 180°C for 90min.
2. Place bottles in a refrigerator for 24h before use to become cold, transfer to an ice tray for inoculation.
3. Prepare cell suspensions in precooled 5% (w/v) skimmed milk solutions.
4. Add a few drops of the yeast suspension to each gel, remove the bottles from the ice tray and agitate to ensure the suspension is evenly dispersed. Return the inoculated gels to the ice tray for a further 30min.
5. Screw the caps on tightly and keep the gels at room temperature to dry (for approx. 2 weeks).
6. When cultures appear dry and the gel crystals separate readily, transfer the bottles to an airtight container with a layer of indicator silica gel in the bottom (BDH Chemicals Ltd.). Ensure the containers are airtight and store at 4°C.
7. Check the indicator gel occasionally and replenish or re-dry by heating in an oven at 180°C for 2h if necessary.

Recovery

As CABI method

4.13.5 Soil storage

FOR FILAMENTOUS FUNGI AT CABI BIOSCIENCE

Equipment and reagents

- Garden soil
- Sterile distilled water
- Pasteur pipettes.
- 30ml glass Universal bottles (a minimum of 2 per culture)
- Refrigerator

Method

1. One-third fill 30ml Universal bottles with garden soil and autoclave twice on consecutive days at 121°C for 20min.

2. Prepare a mycelial/spore suspension in sterile distilled water.

3. Using a Pasteur pipette add 1ml (approx.) of suspension, to at least two bottles of sterile soil for each strain.

4. Label one bottle as reserve stock and the other(s) as working stock.

5. Incubate at 20-25°C for 5-10 days depending on the growth rate of the fungus being stored (this initial growth period allows the fungus to use the available moisture and gradually to become dormant).

6. Screw the bottle caps down tightly and store in a refrigerator (4-7°C).

Recovery

Sprinkle particles of soil from the working stock on to a suitable growth medium and incubate under appropriate growth conditions.

4.13.6 Centrifugal or spin freeze-drying (CABI)

FOR FILAMENTOUS FUNGI AT CABI BIOSCIENCE

Centrifugal freeze-drying is a two-stage process. Several models of freeze-drier are available but the principles of their operation and use are similar. At CABI Bioscience, Edwards High Vacuum (Vacuum and Industrial Products) equipment adapted to take 3 spin freeze accessories is used (Modulyo 4K and a super Modulyo12K).

Equipment and reagents

- Sterile 10% (w/v) skimmed milk + 5% (w/v) inositol (autoclaved at 114°C for 10 min), (different organisms may require different protectants).
- Freeze-drier (Edwards High Vacuum International) with spin-freeze and manifold accessories (Modulyo 4k or Super Modulyo 12K).
- 0.5ml (nominal capacity) neutral glass ampoules (Anchor Glass Co. Ltd.) heat sterilised (180°C for 2-3h) and labeled with strain number of the organism to be freeze-dried, batch date - date of preservation.
- Lint caps (heat sterilised at 180°C for 2-3h) fitted to the 0.5ml ampoules either individually or in batches of 15 ampoules of the same strain.
- Pasteur pipettes
- Sterile non-absorbent cotton wool.
- Air/gas glass blowers torch (Vacuum Industrial Products Ltd).
- Phosphorus pentoxide.
- Twin flame air/gas sealing torch (Vacuum Industrial Products Ltd.).
- Glass cutter in support handle.
- Sterile distilled water.
- Heat-resistant mat.
- High frequency spark vacuum tester (Edwards High Vacuum)

Method

1. Prepare a spore suspension in a 10% (w/v) skimmed milk and 5% (w/v) inositol mixture

2. With a Pasteur pipette add 0.2ml (approx) of suspension to each sterile ampoule, ensuring that the suspension does not run down the inside of the ampoule.

3. Cover each ampoule with a sterile lint cap or in batches of 15.

4. Load the ampoules into a spin freeze accessory and place this in the chamber of the drier.

5. Spin the ampoules for 30min and cool to -40°C.

6. Evacuate the chamber and continue to spin for 30min. (The spore suspension will have frozen into a wedge tapering from the base of the ampoule. This gives a greater surface area for evaporation of the liquid).

7. Leave the ampoules in the chamber and evacuate for a further 3h (at this point the moisture content of the material will be approximately 5%).

8. Admit air into the freeze-drier chamber and remove the ampoules.

9. Remove the lint caps and plug the ampoules with sterile cotton wool compressed to 10mm in depth, 10mm (approx.) above the top of the freeze-dried material in a microbiological safety cabinet.

10. Constrict the plugged ampoule 10mm above the cotton plug using the air/gas torch. The bore of the constriction should remain greater than 1mm, the outer diameter approximately 2.5mm. [This stage where the freeze-dried material is exposed to atmospheric oxygen and moisture must be kept as short as possible as the exposure of the partially dried material can cause deterioration (Rey, 1977)].

11. Place the constricted ampoules on the secondary-drying accessory of the freeze-drier and evacuate over phosphorus pentoxide desiccant. The ampoules are sealed at the point of constriction after a 17h drying process using a cross-fire burner under a vacuum (Vacuum and Industrial Products). At this point the moisture content should be 1-2% by dry weight. This drying period is selected for the convenience of working practices in the laboratory. However, it is possible to reduce the water content of the samples to that required with a 3-6h second stage. The presence of sugars in the suspending media reduces the risk of over-drying.

12. Test the sealed tubes with a high voltage spark tester to ensure the seal is intact. (A purple to blue illumination appearing inside the ampoule indicates low pressure and an intact seal).

Recovery

1. Score an ampoule at the centre of the cotton wool plug using a glass-cutter.

2. Heat the tip of a glass rod in a Bunsen burner until red-hot and apply firmly to the score. The heat should crack the tube around the score line.

3. Snap-open the ampoule and remove the cotton plug.

4. With a Pasteur pipette add 2-4 drops of sterile distilled water and replace the cotton plug, leave for 30min to rehydrate the suspension.

5. Inoculate onto a suitable growth medium and incubate under appropriate conditions (record any differences in growth and test viability).

4.13.7 Centrifugal or spin freeze-drying (NCTC)

FOR MEDICAL BACTERIA AT THE NATIONAL COLLECTION OF TYPE CULTURES (NCTC)

Equipment and reagents

- Filter sterilised 5% inositol serum (*meso*-inositol 5.0g and horse serum to 100ml, 5ml aliquots in Bijoux bottles) used for all bacteria except enterobacteria where 5% inositol broth is used (2.5g Oxoid nutrient broth powder No. 2, 5.0g *meso*-inositol and distilled water to 100ml, autoclaved 121°C for 15min).
- Freeze-drier (Modulyo, Edwards High Vacuum International) with spin-freeze and manifold accessories).
- Labeled cotton wool plugged 7.0-7.5mm neutral glass freeze-drying ampoules (Edwards High Vacuum International), sterilised by acid-wash (2% hydrochloric acid overnight, rinsed in tapwater, followed by distilled water) before autoclaving at 121°C for 15min. Prior to autoclaving the ampoules are labeled (information is stamped or typed onto blotting paper (5x30mm) strip and placed in each ampoule).
- Pasteur pipettes
- Sterile non-absorbent cotton wool plugs, autoclaved *in situ* in empty ampoules or tubes.
- Sterile caps of gauze or cotton
- Air/gas glass blowers torch ('Flair' handtorch, Jencons Scientific Ltd. or fishtail burner or semi-automatic ampoule constrictor (Edwards High Vacuum International)
- Phosphorus pentoxide
- Crossfire burner (Edwards High Vacuum International)
- High frequency spark tester (Edwards High Vacuum International)
- Glass cutter in support handle or a diamond
- Fine glass rod
- Nutrient broth

Extra equipment and reagents for Category 3 organisms, as listed in the UK by the Advisory Committee on Dangerous Pathogens (1996):

- Solid CO_2
- 0.2 μm 'Mini Capsule' pleated filter (Gelman Sciences Ltd.)

Method

1. Prepare a suspension of bacterial cells in sterile 5% (w/v) inositol serum or 5% (w/v) inositol broth.

2. Add 1-2ml of suspending fluid to each slope of culture and gently rub off the growth with a Pasteur pipette or sterile inoculating loop, then emulsify into a uniform suspension.

3. As for centrifugal freeze-drying for filamentous fungi.

Recovery

As method for filamentous fungi except:

The culture is rehydrated with approx. 0.5ml nutrient broth. It is beneficial, with cultures known to give poor recoveries after-drying, to leave to rehydrate for a few minutes before transferring the suspension. Streak the contents onto an appropriate growth medium on an agar plate to facilitate detection of contaminants introduced during opening, incubate under suitable conditions (record differences in growth) to test viability.

4.13.8 Centrifugal or spin freeze-drying (NCIMB)

FOR BACTERIA AT THE NATIONAL COLLECTIONS OF INDUSTRIAL FOOD AND MARINE BACTERIA (NCIMB)

The principal method in the NCIMB for the maintenance of the majority of bacterial species is a standard freeze-drying procedure. There are minor variations in procedures. The first stage freeze-drying is carried out using an Edwards EFC or Supermodulyo centrifugal freeze-drier. Second stage drying is usually carried out over phosphorus pentoxide, overnight for convenience or for a period of at least 3 hours. Additionally, ampoules are prepared containing a filter paper slip and lint caps are used in place of cotton wool plugs. The latter are inserted just prior to constriction of the ampoules. In addition, the ampoules are placed in aluminium racks, and wrapped in greaseproof paper before sterilisation.

Additional equipment and reagents

- Sterile *Mist desiccans* which contains 100ml horse serum; 33ml Oxoid(Unipath) CM1; 10g glucose.
- Glucose is added to the broth serum mix and not vice versa. Once the glucose has dissolved the mixture is sterilised by pressure filtration (either membrane or Seitz) and is dispensed aseptically in 5ml aliquots.
- Labeled and autoclaved 7.0 –7.5mm neutral glass freeze-drying ampoules with lint caps. A filter paper strip (5 x 30mm) labeled with the NCIMB strain number and date of freeze-drying is placed in each ampoule prior to autoclaving.
- Sterile non-absorbant cotton wool plugs are placed in empty ampoules and sterilised in an autoclave
- Semi-automatic ampoule constrictor (Edwards High Vacuum International)
- Metal plunger for insertion of cotton plugs
- Phosphorus pentoxide
- High frequency spark tester (Edwards High Vacuum International)

Method

1. Late log, early stationary phase cells are washed from slopes using 2-4ml of *Mist desiccans.*
2. With a sterile Pasteur pipette aseptically dispense 2 to 4 drops of suspension into the bottom of each sterile ampoule, ensuring that the suspension does not run down the inside of the ampoule.
3. Replace the lint caps and load the ampoules onto the centrifuge plate of the freeze-drier ensuring the plate is balanced.
4. Place centrifuge plate in the chamber of the drier and switch on the centrifuge, evacuate the chamber of the freeze drier. After 20min switch off the centrifuge and dry for at least 3 hours.
5. Admit air into the freeze-drier chamber and remove the ampoules.
6. Aseptically remove the lint caps and plug the ampoules with sterile cotton wool (10-15mm plugs pushed *c.* 15mm into the ampoule. The plug is pushed to it final depth just above the filter paper strip using a metal plunger.

7. Constrict the plugged ampoule 15mm above the cotton plug using a constrictor (commercial apparatus is available). Alternatively, an air/gas glass blower torch (Vacuum Industrial Products Ltd.) can be used to manually constrict the ampoules.
8. Place the constricted ampoules on the secondary-drying apparatus, which consists of a vacuum system and a phosphorus pentoxide trap. Dry for at least 3 hours and seal each ampoule at the point of constriction.
9. Test the sealed tubes with a high voltage spark tester to ensure the vacuum is adequate (a purple discharge should be evident within the ampoule when viewed in a darkened room).

Recovery

1. Score an ampoule at the centre of the cotton wool plug using a glass-cutter.
2. Using a heated wire or glass rod apply firmly to the score to crack the ampoule.
3. Snap-open the ampoule using an ampoule snapper or thick wadding to protect from cuts.
4. Rehydrate the contents of the ampoule with 3-4 drops of sterile saline or growth medium from a Pasteur pipette.
5. Inoculate onto a suitable growth medium and incubate under appropriate conditions.

4.13.9 Centrifugal or spin freeze-drying (NCYC)

FOR YEASTS AT NATIONAL COLLECTION OF YEAST CULTURES (NCYC)

Equipment and reagents

- Glass ampoules (FBG-Trident Ltd.) that have been washed in detergent and rinsed in demineralised water
- Labels for ampoules (Fig 4.1) (folded in half lengthways and placed in the ampoule so that the writing faces outwards)
- Cotton wool plug
- Whatman's No. 1 filter paper (Scientific Supplies Co. Ltd.)
- Pencil or alternatively, a stamp with ENM quick-drying, non-toxic ink (Rexel, obtainable form stationers)
- Non-absorbent cotton wool
- Tins to hold freeze-drying ampoules
- YM broth (Difco Laboratories, 0711-01)
- Suspending media (see Table 4.1 below)
- 30-dropper Pasteur pipettes
- Freeze-drier (Edwards Model 2A/110 or Edwards High Vacuum International EF03)
- Phosphorus pentoxide
- Ampoule constrictor (Edwards High Vacuum International) or hand held air/gas torch
- Glass rod
- File for opening glass ampoules

Fig 4.1: Printed label for insertion in ampoule:

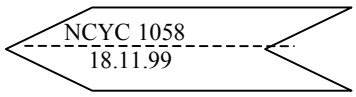

Table 4.1 Suspension media used by the NCYC

Glucose (7.5%) dissolved in inactivated horse serum No. 5 (Wellcome Reagents Ltd). (Filtration sterilised and stored in McCartney bottles at 4 °C. Glucose can be substituted with sucrose or inositol.
Skimmed milk (20%) and sodium glutamate (10%)
Honey (5%) with skimmed milk (10%) and dextran (10%) (Berny & Hennebert, 1989)
Honey (5%) with skimmed milk (10%) and glutamate (5%) (Berny & Hennebert, 1989)

Method

1. Grow the culture without aeration in YM broth (Difco Laboratories, 0711-01) at 25°C for 72h.

2. Mix equal amounts of the inoculum and suspending medium in a sterile bottle. (The suspension should contain at least 10^6 cells ml^{-1}).

3. Remove the cotton wool plugs and add 0.2ml (approx. six drops) of the cell suspension to each ampoule with a 30-dropper Pasteur pipette. Ensure the suspension does not run down the inside of the ampoule.

4. Replace the cotton wool plug, ensuring that a small part of the plug protrudes from the end of the ampoule.

5. Place ampoules into the centrifuge head of the freeze-drier (ensure the labels face towards the centre of the centrifuge head so that the writing is not obscured by the dried yeast).

6. Add phosphorus pentoxide or other desiccant to the trays provided and place in the freeze-drier, spin the ampoules.

7. Evacuate the chamber and continue primary drying for a further three hours. The cell suspension will have frozen into a wedge tapering from the base of the ampoule, which provides a greater surface area for evaporation of the liquid. Samples should appear completely dry.

8. Admit air into the freeze-drier chamber and remove the ampoules.

9. Trim the projecting ends of the cotton wool plugs and push the remainder of them halfway down the ampoule with a glass rod.

10. Constrict the ampoules above the level of the plug either by hand or using an ampoule constrictor (Edwards High Vacuum International).

11. Replace the phosphorus pentoxide in the freeze-drier for the secondary drying.

12. Place the constricted ampoules on the secondary-drying accessory of the freeze-drier, evacuate and leave overnight. (This process may be shortened after 2h, allowing process to be completed in one day).

13. Seal ampoules whilst under vacuum using a torch (Dragons Microflame).

14. Test the sealed tubes with a high voltage spark tester (Edwards High Vacuum International) to ensure the seal is intact. A purple/blue illumination inside the ampoule indicates low pressure and an intact seal. A spark to earth indicates a poor vacuum.

Recovery

As for filamentous fungi except:

An aliquot of YM broth is added using a Pasteur pipette from a Bijoux bottle containing 1.0ml of sterile broth, the yeast is thoroughly suspended and the suspension returned to the remaining YM broth in the Bijoux bottle. Using sterile forceps, remove the label from the ampoule and place it in the Bijoux. Take a further few drops of YM broth from the bijoux and use this to wash the ampoule out again, before returning this to the Bijoux. Steak the contents onto an appropriate growth medium

4.13.10 Shelf freeze-drying

FOR FILAMENTOUS FUNGI AT CABI BIOSCIENCE

*(*A similar technique is used for bacteria at NCIMB)

Controlled freezing at 1°C min^{-1} has been found to enable the freeze-drying of cultures that do not normally survive the above method (Smith, 1986). Using such a pre-freezing procedure some delicate or non-sporulating fungi can be preserved on the shelf freeze-drier.

Equipment and reagents

- Sterile 10% (w/v) skimmed milk + 5% (w/v) inositol (autoclaved at 114°C for 10min) or other protectant
- Shelf freeze-drier, Minifast 3400 (Edwards High Vacuum International) with T-matic shelf temperature control
- 2.0ml (nominal capacity) pre-constricted long-necked vials with butyl rubber bungs (Adelphi Tubes Manufacturing Ltd.), (heat sterilised at 180°C for 2-3h: bungs autoclaved 121°C for 15min) and labeled with the strain number of the organism to be freeze-dried and batch date, date of the freeze-drying
- Pasteur pipettes
- Sterile non-absorbent cotton plugs to fit the neck of the ampoules
- Air/gas glass blowers torch (Vacuum Industrial Products Ltd.)
- Phosphorus pentoxide
- Glass cutter in support handle
- Sterile distilled water

Method

1. Prepare a spore suspension in the 10% (w/v) skimmed milk and 5% (w/v) inositol mixture.

2. With a Pasteur pipette, aliquot 0.5ml into the 2ml flat bottom vials.

3. Insert the butyl rubber bungs into the necks of the vials, so that the premoulded ring sits on the lip of the neck of the vials (vials are partially closed).

4. Place the vials on the pre-cooled shelf (-35°C) of the Minifast 3400 freeze-drier (Edwards High Vacuum Ltd.).

5. Place a sample temperature probe into a control vial containing sterile 10% (w/v) skimmed milk and 5% (w/v) inositol mixture only. Close the chamber door. When the temperature reaches -20°C evacuate the chamber.

6. Using the T-matic temperature controller, hold the shelf temperature at -35°C for 3h and then raise the shelf temperature 0.08°C min^{-1} to +10°C (the temperature of the sample suspension reduces to -145°C as the latent heat of evaporation is removed).

7. When the pressure in the chamber is close or equal to the pressure measured just above the vacuum pump, seal the ampoules by lowering the stoppering device to push in the bungs (normally 24h drying from the time the temperature of the sample reached -45°C).

8. Return the chamber to atmospheric pressure, remove the vials and heat seal at the pre-constriction ready for storage. A final vacuum of approximately 4 x 10^{-2} mbar is retained.

9. Test the sealed tubes with a high voltage spark tester. A purple to blue illumination will indicate low pressure and an intact seal.

10. Store the ampoules at around 18°C.

Recovery

The glass vial is fitted with a butyl rubber bung held in place by a soft metal cap.

1. Carefully remove the metal cap.

2. In a microbiological Class I or Class II safety cabinet, loosen the bung to allow a slow influx of air.

3. Remove the bung and add 1ml of sterile distilled water, replace the bung or plug with a sterile cotton plug and leave for 30min.

4. Streak the contents of the vial onto a suitable agar medium ensuring that all the spores adhering to the vial walls are removed and incubate at an appropriate growth temperature.

4.13.11 L-drying

FOR BACTERIA AND BACTERIOPHAGES AT THE NATIONAL COLLECTIONS OF INDUSTRIAL FOOD AND MARINE BACTERIA (NCIMB)

Equipment and reagents

- Sterile *'mist. desiccans'* (Horse serum 100ml (Life Technologies Ltd., Product No. 034-6050H) nutrient broth 33ml (Unipath Ltd., CM1). Mix together in a 250ml conical flask and agitate and slowly add 10g glucose. Once the glucose has dissolved, sterilise by *pressure* filtration (membrane or Seitz filter). Dispense 5ml aliquots in sterile screw-cap Universal bottles and incubate at 30°C for 2-3 days as a sterility check. Store the *'mist. dessicans'* at 20°C until required.
- Glass tank.
- Rotary pump manifold (with valves) attached.
- Ampoules prepared as for freeze drying of bacteria at NCIMB

NB: No commercial L-driers are available

Method

1. Prepare a suspension of the bacterium in sterile *'mist. dessicans'*. Use 5ml of suspending fluid to harvest the growth from three universals containing slopes of culture by gently rubbing off the growth with a Pasteur pipette or sterile inoculating loop, then emulsifying into a uniform suspension.

2. Add approximately 0.1ml (3 drops) of the bacterial suspension to each sterile ampoule taking care not to contaminate the sides or top.

3. Replace the sterile cotton wool plugs, trim and push down the ampoule using a ramrod.

4.Attach the ampoules vertically to the underside of a horizontal manifold. Clamp above a glass tank containing water at 20°C ensuring that the ampoules can be immersed in the water to a depth of 40-50mm.

5. Connect the manifold, via a diaphragm valve and phosphorus pentoxide trap, to a rotary pump.

6. Close the valve and switch the pump on.

7.Open the valve widely for about 0.5s and quickly close it again.

8. Next, open the valve very gradually until the ampoule contents begin to degas (i.e. bubble). To prevent violent bubbling in the ampoules care must be taken to carefully control the rate of degassing by careful manipulation of the valve. If this does occur, close the valve quickly, allow the bubbling to subside and then reopen the valve cautiously.

9. When degassing is complete (approx. 5min.) open the valve fully.

10. After a further 30min the contents of the ampoules will appear dry.

11. Remove the ampoules from the manifold.

12. Constrict ampoules and follow remainder of freeze-dry protocol for fungi.

Recovery

As freeze-dry protocol for bacteria at the NCIMB

4.13.12 Micro-drying

FOR BACTERIA AND BACTERIOPHAGES AT THE NATIONAL COLLECTIONS OF INDUSTRIAL, FOOD AND MARINE BACTERIA (NCIMB)

The procedure for preserving cultures by microdrying is a modification of the freeze-drying method used in the NCIMB (see Section 4.13.8 Centrifugal freeze-drying at the NCIMB).

Ampoules are prepared exactly as for freeze-drying except that the filter strip used is a thick grade (Genzyme Biochemicals Ltd., No. 17). Bacterial suspensions, which should be highly concentrated, are prepared in *'mist. dessicans'*. Three drops (*c*. 0.1ml) are dispensed on the filter paper (the ampoules and the pipette should be held almost horizontally to ensure that the suspension falls only onto the filter paper and avoids the wall of the ampoule). All the suspension should be absorbed by the filter paper (it should appear damp, leaving no excess liquid in the ampoule). Cultures are then freeze-dried in the usual way except that centrifugation and the replacement of cotton wool plugs by lint caps are not required.

Micro-drying can be used as a convenient method of ensuring that known numbers of cells are placed in each ampoule. In this instance, sterile filter paper strips are placed aseptically in a sterile Petri dish, and an aliquot (usually 0.1ml) of a suspension (of known concentration) is carefully added to each. After all the suspension has soaked into the filter paper strips, they are transferred to freeze-dry ampoules, where a cotton wool plug can be inserted (optional). Freeze-drying is carried out as previously described. After drying a further viable count is performed. The filter paper strips can then be used as standard inocula. Resuscitation of micro-dried cultures is straightforward and does not require any manipulation with pipettes and resuspending medium. The ampoule is simply shaken or tapped to ensure that the filter paper strip is loose, the ampoule is opened, the cotton plug removed and the filter paper strip tipped aseptically into a broth or onto a slope of an appropriate medium.

4.13.13 Maintenance in gelatin discs

FOR BACTERIA AT THE NATIONAL COLLECTION OF TYPE CULTURES

Equipment and reagents

- Vented 9cm Petri dishes
- Freeze-drier (Edwards EF03 freeze-drier)
- Sterile Pasture pipettes
- Gelatin suspending medium (10.0g gelatin powder (Unipath Ltd.), 2.5g nutrient broth powder No.2 (Unipath Ltd.), 5.0g *meso*-inositol (Koch-Light Laboratories), deionised water to100ml. Dissolve by gentle heating and adjust to pH 7.2, aliquot 3ml to screw-capped 6ml Bijoux bottles and autoclave at 121°C for 15min).
- Dropping pipette capable of delivering 0.02m
- Freezer (to at least -40°C)
- Phosphorus pentoxide
- Coarse, self-indicating silica gel (BDH Chemicals Ltd.)
- 14x45mm screw-necked vials and caps (FBG Trident Ltd.)
- Non-absorbent cotton wool
- Fine-nosed forceps
- Nutrient broth
- Inoculating loop

Method

1. Prepare a bacterial suspension in a minimal volume (about 0.5ml) of nutrient broth and add this to 3ml of the gelatin suspending medium (previously melted and held at 37°C).

2. With a Pipette, dispense dropwise 0.02ml of suspension to the base of a vented plastic Petri dish. With care, approximately 80 drops can be accommodated in the base.

3. Place Petri dishes in a freezer at –20 to -40°C (care!) until the drops freeze (freezing is indicated by a change in appearance from transparent to opaque, allow up to 2h).

4. Quickly transfer the Petri dishes to the freeze-drier (which must be loaded with phosphorus pentoxide).

5. Switch the freeze-drier on and dry cultures overnight. With large numbers of discs in a batch it may be necessary to replace the phosphorus pentoxide after 2-4h (this is achieved by isolating the drying chamber, switching off the machine and venting the trap before replacing the P_2O_5).

6. Into each screw-necked vial, add self-indicating silica gel (to a depth of approx. 10mm) and pack down tightly with cotton wool. Sterilise (covered) in a hot-air oven at 160°C for1h (the caps can be sterilised in an oven at 60-80°C for 4h before placing on the bottles).

7.When the freeze-drying process has finished, transfer the discs to the vials.

8. Replace the caps of the vials and tighten.

Recovery

Using fine-nosed forceps, remove gelatin disc and inoculate into 1ml of nutrient broth. Incubate at 37°C until dissolved. Remove a loop of suspension and streak onto a suitable solid medium and incubate under appropriate conditions.

Note

Bacteria preserved by this method must be checked for viability and for retention of the particular characteristics for which they have been preserved. As this method is unlikely to be used as a sole means of preserving important cultures, it is probably not necessary to perform viable counts on the discs as simple plating will give a good indication of the level of viability. As with any method of preservation, it is essential to characterise the strain after drying to ensure that the correct strain has been preserved and has retained its important characteristics. After 4 years' experience in the use of this method, the NCTC has found little change in the phenotypic characters of strains used for quality control or in identification kits.

4.13.14 Cryopreservation

FOR FILAMENTOUS FUNGI AT CABI BIOSCIENCE

There are many protocols suitable for the cryopreservation of microbes and animal cell lines. Because, minor adaptation in methodology can affect viability, all of the main protocols used in UKNCC collections have been included.

Safety considerations

It is essential that protective clothing is worn and strict safety procedures are followed in all activities involving the use of liquid nitrogen. The major risks are the intense cold, which causes injury similar to burns, and the issuing gas, which is an asphyxiant. Areas where liquid nitrogen is used or stored must be well ventilated and oxygen content of the air monitored.

Equipment and reagents

- Sterile 10% (v/v) glycerol (other cryoprotectants can be used (Smith & Onions, 1994)
- 2.0ml sterile graduated cryotubes (LabM) or 2.0ml borosilicate cryotubes (LabM) (ampoules should be labeled with strain number and batch date for storage in the vapour phase)
- Pasteur pipettes
- Liquid nitrogen
- KRYO 10/16 series II controlled rate freezer (Planer Products Ltd.)
- Liquid nitrogen freezer with metal drawer rack inventory control system (Statebourne Cryogenics).
- Safety equipment (to include cryogloves, face shield, forceps, and oxygen monitor)
- 1% (w/v) solution of erythrosin B

- Optional: The Prolab microbank system can be used where the cryotubes are pre-filled with cryoprotectant and glass beads. Excess suspension must be removed; this may be transferred to a cryotube and frozen as a back-up. Recovery of the strain can be achieved by chipping off a bead and inoculating it on to an appropriate growth medium.

Method

1. Prepare fungal suspensions* in sterile 10% (v/v) glycerol (take care to avoid mechanical damage to the fungus) and dispense 0.5ml aliquots into labeled cryovials. *For non-sporulating fungi, plugs of agar/mycelium can be cut using a cork-borer and placed in the cryotubes with 5ml glycerol.

2. Plastic cryotubes are secured by tightly screwing down the lids. Glass cryotubes are heated sealed using an air-gas torch and placed in an erythrocin B dye bath (at 4-7°C) to check for leakage.

3. Cultures are left for approx. 1h to allow cells to equilibrate in the glycerol.

4. Samples are cooled using a KRYO 10/16 series II programmable cooler (Planer Products Ltd). The cooling rate employed depends upon the organism (a rate of -1°C min^{-1} is suitable for many fungi)* and is controlled over the critical period from +5°C to -50°C (the initial cooling rate to 5°C is not

critical and this is normally at -10°C min^{-1}). *It has been found that many strains have optimum cooling rates in the range of –0.5 to -200°C min^{-1}, to achieve optimum survival these rates of cooling should be employed (Smith & Thomas, 1998).

5. When the frozen suspensions reach -50°C, transfer to a 320l liquid nitrogen storage vessel (Statebourne Cryogenics) where cooling down to the final storage temperature is completed in the liquid (glass cryotubes) or vapour phase (plastic cryotubes) of the liquid nitrogen.

6. Record the location of each culture in the inventory control system.

Recovery

1. Thaw vial in a circulatory water bath at +37°C or the KRYO 10/16 cooler, using a suitable thawing programme. In both instances remove the ampoules when the last ice has melted (do not allow the suspensions to reach the temperature of the water bath or a high chamber temperature in the KRYO 10/16).

2. Unscrew the cryotube lid or open the ampoule by scoring the pre-constriction with a glass file and snap it open in a microbiological safety cabinet. Inoculate onto a suitable growth medium (if agar blocks have been preserved, remove these with an inoculating loop/needle leaving behind the cryoprotectant solution, and place mycelium side down onto a suitable medium).

Similar methods have been in use in other collections for example in the American Type Culture Collection (ATCC) where cryopreservation in liquid nitrogen has been used since 1965 and has given very good results (Hwang, 1966, 1968; Butterfield *et al.,* 1974; Hwang *et al.,* 1976; Alexander *et al.,* 1980).

4.13.15 Cryopreservation – straw method

FOR YEAST CULTURES AT THE NATIONAL COLLECTION OF YEAST CULTURES (NCYC)

Equipment and reagents

- Difco yeast malt (YM) broth (21gl^{-1} Difco dehydrated YM broth (Ref. No. 0711-01, Difco Inc., Detroit, MI) or YM medium (3g yeast extract, 3g malt extract, 5g peptone, 1g glucose, distilled water to 1l. Mix and adjust to pH 5/6. Dispense 10ml aliquots into suitable bottles. Sterilise by autoclaving for 15min at 121°C).
- YM agar (add 2% (w/v) agar to Difco YM broth or YM media before sterilisation. Mix and adjust to pH 5/6 Sterilise by autoclaving for 15min at 121°C. Dispense 20ml aliquots into sterile Petri dishes and leave to cool).
- 10% (w/v) glycerol cryoprotectant solution [filter sterilised (0.22µm filter) and dispensed in 15ml aliquots into suitable bottles and stored at room temperature].
- Polypropylene drinking straws 4mm diameter, (Key Catering Ltd., London, UK or Sweetheart International Ltd.). Cut into 2.5cm lengths, seal one end of each straw by holding firmly with non-ridged forceps 2mm from the end and bring the projecting end to 10mm from the flame of a fishtail Bunsen burner. Place the straws in a glass Petri dish and sterilise by autoclaving at 121°C for 15min. For ease of handling, the straws should be evenly spaced around the edge of the long dish with all the open ends pointing in the same direction. Two long unsealed straws should also be prepared for use as rests for straws awaiting final sealing. These should also be placed in glass Petri dishes and sterilised. Ensure that the straws are dry before use, using a moderate temperature (40-60°C) drying cabinet if necessary.
- Nunc cryotubes (plastic screwcap 1.8-mL ampoules are available sterilised from the manufacturer: Life Technologies Ltd., Inchinnan, UK)
- Refrigerated methanol bath precooled to -30°C
- Liquid nitrogen containers: Cryogenic storage containers with liquid phase storage racks and dividers to store 2ml cryotubes (Jencons Scientific Ltd., Leighton, Buzzard, UK)
- Safety equipment (cryogloves, goggles etc.)

Method

1. Grow the culture in 10ml of YM broth for 72h at 25°C on a reciprocal shaker.

2. Mix equal amounts of the inoculum and glycerol cryoprotectant in a sterile bottle.

3. Remove a single straw from the Petri dish. Gently grip the straw about halfway along its length to allow the insertion of the end of a Pasteur pipette containing the inoculum.

4. Insert the pipette until the pipette tip is at the sealed end of the straw and then withdraw it as the inoculum fills the straw. Fill to approx. two-thirds of its capacity (within 3mm of the open end). On withdrawing the pipette from the straw, any excess inoculum can be sucked back into the pipette.

5. The open end of the straw is sealed as described previously (see equipment and reagents).

6. Test the straws for leaks by holding with forceps above a suitable disinfectant in a high-sided beaker and gently squeeze. Any liquid forced out of the seals will be safely contained within the beaker. Discard damaged straws.

7. Place six straws in each 1.8ml cryotube. (If post-thaw cell viability counts are required, a single straw may be placed in a separate ampoule for ease of recovery.) Mark each straw and cryotube with the relevant strain designation and date of freezing using a black permanent marker pen.

8.Primary freezing: Place the filled cryotubes in the methanol bath, which has been precooled to -30°C (alternatively cryotubes can be frozen by placing in a -30°C room). The cooling rate is not critical, but is probably in the region of -5°C per minute. Leave for 2h to allow the cells to dehydrate.

9. Secondary freezing: Transfer the cooled cryotubes to the liquid nitrogen containers and place in the racking (inventory) system. Note the position of the cryotubes. Remove excess methanol from the outside of the cryotubes to prevent it from freezing the tubes to the racking system while immersed in the liquid nitrogen.

Recovery

1. Remove cryotube from storage and check the strain number and date of freezing. Remove a single straw and replace the cryotube in the racking system. Rapidly transfer the straw to a water bath and thaw at 35°C for 3s, agitate to ensure rapid and even thawing.
2. Remove the straw from the water bath and dry. Grip one end of the straw and sterilise the other end by wiping with 95% alcohol. Prior to opening the straws, resuspend the cells by squeezing the straws several times. Cut off the sterile end with scissors that have been flamed with alcohol, mix and remove the contents using a Pasteur pipette. Transfer to an appropriate growth media.

4.13.16 Cryopreservation (CCAP)

FOR ALGAE AT THE CULTURE COLLECTION OF ALGAE AND PROTOZOA (CCAP)

Equipment and reagents

- Late log or early stationary phase cultures
- Medium dependent on the nutritional requirements of the alga (see below)
- Cryoprotectant [10% (v/v) dimethyl sulfoxide (DMSO) in the appropriate medium (DMSO is cytotoxic and care should be taken when handling), alternative cryoprotectants including methanol 5-10% (v/v) or glycerol 10% (v/v) are also regularly employed for some strains]
- Cryovials (presterilised plastic screwcap 1.8ml cryovials (Costar, Cambridge MA)
- Refrigeration system (Refrigerated methanol bath precooled to -30°C. Note: Methanol is toxic and flammable. Alternatively, controlled rate coolers such as a Planer KRYO 10 may also be used)
- Liquid nitrogen dewar (small 1-2 litre wide-neck dewar)
- Safety equipment (long forceps, cryogloves, cryoapron, goggles)
- Storage system (cryogenic storage containers, with appropriate storage racks and inventory system suitable for holding cryovials)
- Fluorescein diacetate (FDA) stain stock solution (25mg fluorescein diacetate in 24ml of acetone)

Media

BG 11 medium adjusted to pH 7.8, used for freshwater cyanobacteria and nonaxenic algae (Day & McLellan, 1995). EG:JM or PP medium used for axenic freshwater Chlorococcales and euglenoids (Day & McLellan, 1995). Guillard's (f/2) medium used for marine algae (Day & McLellan, 1995).

Method

1. Grow cultures in the appropriate medium under controlled environmental conditions. Flasks (50ml) containing 30ml of medium should be incubated static at 15°C under a photo fluence rate of 25-100 μmol m^2s^{-1}. Light should be provided on a light/dark cycle, 16:8h is generally regarded as optimal. Incubate cultures until they reach stationary phase. Thirty days is used as a standard culture interval at CCAP.

2. Transfer sedimented cells with 15ml of medium into a pre-sterilised beaker. Alternatively, for uniform cell suspensions, centrifuge at 500*g* for 10min, decant supernatant and resuspend algae in 15ml of fresh sterile medium. If desired a 5ml aliquot can be removed for use as a control for viability assays.

3. Add 10ml of medium containing 10% (v/v) DMSO to the remaining 10ml of dense culture and mix thoroughly to give a final concentration of 5% (v/v) DMSO.

4. Dispense 1ml aliquots into sterile prelabeled cryovials. Seal the vials and incubate at room temperature for 5min.

5. Transfer the cryovials to a precooled refrigerated bath (-30°C) and incubate for 15min.

6. Using forceps, rapidly transfer the vials to a wide-necked dewar containing liquid nitrogen and transport to the storage system.

7. Using long forceps transfer to the storage/racking system. Storage is generally in the liquid phase of the liquid nitrogen.

8. A full inventory/stock list, including the locations of vials within the storage system, should be maintained. This can most easily be retained either on a computer database, as card indices, or any other appropriate systems.

Recovery

1. Transfer stored cryovials to a dewar containing liquid nitrogen for transport and temporary storage. When required, deposit the vials in a preheated water bath (40°C) and agitate until the last ice crystal has melted. On completion of the thawing remove immediately and transfer to a safety cabinet.

2. Wipe the cryovial with 70% (v/v) ethanol and transfer the contents to 30ml of appropriate sterile medium and incubate as in step one of the above method section.

4.13.17 Cryopreservation (NCIMB)

FOR BACTERIA AND BACTERIOPHAGES AT THE NATIONAL COLLECTIONS OF INDUSTRIAL, FOOD AND MARINE BACTERIA (NCIMB)

Equipment and reagents

- Polypropylene cryotubes (2ml, Life Technologies Ltd.), containing 25-30 3mm glass beads (available from craft shops) wrapped in greaseproof paper and then autoclaved at 121°C for 15min and dried at 50°C
- Sterile 2ml polypropylene cryotubes (Life Technologies Ltd.) autoclaved at 121°C for 15min
- 10%(v/v) sterile glycerol
- LR40 liquid nitrogen refrigerators (Union Carbide)
- Liquid nitrogen
- Forceps
- Centrifuge equipment
- Membrane filters (0.45µm pore size)
- Pasteur pipettes

Method (for bacteria)

1. Prepare a concentrated suspension of bacteria in an appropriate medium containing 10% (v/v) sterile glycerol.

2. To each cryotube add a sufficient quantity of the bacterial suspension to immerse the glass beads.

3. Gently agitate the beads to ensure a thorough coating of bacteria.

4. Remove excess suspension (i.e. liquid remaining above the top level of the glass beads).

5. Place the cryotubes in aluminium racks and store in the LR40 liquid nitrogen refrigerator.

6. Ensure that all tubes remain in the vapour and not the liquid phase of the nitrogen. This prevents seepage of the liquid nitrogen into the tubes and reduces the chance of contamination.

Method (for bacteriophages)

1. Prepare high-titre lysates using media and methods appropriate to the phage being preserved.

2. Remove host cells and debris by low-speed centrifugation followed by membrane filtration (0.45µm pore size).

3. Dispense 1ml aliquots of cell free lysates into 2ml sterile cryotubes.

4. Store over liquid nitrogen as described above for bacteria.

Recovery

1. Remove the required cryotube from the refrigeration system. The cryotubes should be kept frozen in a dewar flask and only removed for a few seconds to facilitate removal of a bead.

2. Remove one glass bead using sterile forceps and drop onto a suitable medium and incubate under appropriate conditions.

4.13.18 Cryopreservation (ECACC)

ANIMAL AND HUMAN CELL LINES: THE EUROPEAN COLLECTION OF CELL CULTURES (ECACC)

Equipment and reagents

- An appropriate safety cabinet (class II or III depending on the cells that are being handled).
- Cell cultures (These should be in active log phase, usually 2-4d after subculture, cells that have entered stationary phase are not suitable).
- Freeze medium (either growth medium supplemented with 20-25% (v/v) serum and 10% (v/v) cryoprotectant or whole serum and 7-10% (v/v) cryoprotectant). Selection of cryoprotectant is dependent on cell type, but for the majority of cell lines dimethyl sulfoxide (DMSO) or glycerol can be used. Occasionally polyvinyl pyrrolidine, a high molecular weight polymer can be used.
- 1.0 or 1.8ml presterilised cryovials or ampoules (Nunc Roskilde, Denmark / Becton-Dickenson (Bedford, MA) / Corning (Corning, NY). Some manufacturers supply special racks for holding the vials during filling. Sterile glass ampoules (capacity-1.5-2.0ml, Wheaton) can also be used.
- Freezing system (programmable freezer, e.g., Planer Products UK or Sy-Lab Austria)
- Storage system: (liquid nitrogen storage vessels with inventory system suitable for cryovials that can be arranged to store vials in gas, liquid, or a combination of both. Automatic filling (top-up) and alarm systems are advisable to prevent accidental loss of stored material)
- Improved Neubauer heamocytometer and 0.4% (w/v) trypan blue in phosphate-buffered saline (PBS) for calculating both total and viable cell numbers
- Small liquid nitrogen vessel for transporting ampoules
- Sterile pipettes or automatic dispensing apparatus
- Rack to hold ampoules (Nunc)
- Safety equipment (protective full-face mask, cryogenic gloves, waterproof apron, long forceps, and clamping scissors)

Method

1. Ensure that specific cell lines are handled in the appropriate laboratory conditions. Only one cell line should be handled at any one time to avoid cross-contamination.

2. Microscopically examine cell lines for morphology, density and any microbial contaminants using a good quality inverted phase microscope (capable of 100 and 200x magnification). Cell density should not exceed 85% of its maximum growth density and they should have been passaged at least twice in the absence of all antibiotics prior to freezing. Suspect cultures should be rejected.

3. Count the cells to estimate the percentage of viable cells in the culture. Suspension cells can be counted directly by diluting 100µl between 2-fold (1:1) and 10-fold (1:9) with trypan blue. Adherent cells will require a proteolytic enzyme (e.g., trypsin or trypsin and ethylenediaminetetraacetic acid (EDTA) to disrupt cell sheet). Cells should be prepared as for routine subculture (remembering to neutralise the enzyme by addition of serum containing medium or soya bean inhibitor in the case of serum free cultures). Dilute an aliquot of cells in trypan blue.

4. Load a prepared haemocytometer with the diluted cells using a file tip Pasteur or micropipette. Allow the mixture to be drawn under the coverslip by capillary, rather than active pipetting. Fill the chamber completely. Using a phase microscope, count the cells over one of the nine $1mm^2$ squares (bright, retractile cells are viable and dark blue cells are dead). Repeat the process over three more squares (corner squares are normally used). For statistically accurate counts, a range of 30-100 cells

mm^{-2} should be counted. Prepare another sample if the counts are outside this range. Estimate the total and viable cell count as follows:

$$Cells/ml^{-1} = (No.\ of\ cells\ counted/No.\ of\ 1mm^2\ squares\ counted) \times dilution \times 10^4$$

$$Percentage\ of\ viable\ cells = [total\ viable\ cells/total\ cell\ count\ (viable+dead)] \times 100$$

5. Healthy cultures should exceed 90% viability. Low viability or the presence of large quantities of cell debris is an indication of sub-optimal culture conditions or exhaustion of the nutrient supply. Calculate the volume of cells required to fill the ampoules, e.g., 10 ampoules at $5x10^6$ cells per ampoule = $50x10^6$ cells. A recommended number of cells/ampoule is between $4\text{-}10x10^6$ cells (maximum number should not exceed $2x10^7$ cells per ampoule).

6. Centrifuge the cells using the minimum g force necessary to sediment them, e.g., 100 x *g* for 5 min.

7. Decant the medium and resuspend cells in the freeze medium to the required cell density. To aid resuspension, gently vibrate the after decanting the medium.

8. Dispense 1ml aliquots of cells into premarked ampoules (marked with cell designation, passage number, freeze batch number, and date of freezing).

9. Keep the ampoules vertical to avoid spillage into the cap and transfer to the freezer.

10. When frozen, transfer to nitrogen storage (face mask and full protective clothing should be worn). Record the ampoule location. Graphical databases specifically designed for the use with cryogenic systems are available form I/O Systems Ltd. (Ashford, UK).

11. Check at least one ampoule from each batch for viability and growth potential. Always allow 24h storage before undertaking quality control tests.

Recovery

1. Transfer an ampoule to the top of the storage vessel using long forceps or clamping scissors, placing it in an aluminium screwtop canister. Transport ampoule to a water bath (after temperature equilibration in a small dewar with nitrogen or preferably in dry ice). It is not essential to fully re-immerse ampoules in liquid nitrogen. Therefore, if a dewar is used, place ampoules through holes in a piece of polystyrene that will float on the liquid surface, keeping the screwthread above liquid.

2. Thaw ampoules in a water bath set at the cells normal growth temperature, 37°C for mammalian cells, 25°C for amphibian cells (float ampoules in racks or polystyrene, do not submerge). Rapid and complete thawing is vital to retain viability.

2. Wipe the ampoule surface with 70% (v/v) ethanol. Using a sterile Pasteur, or 1ml pipette, transfer contents to a 15ml-screwcap centrifuge tube. Add 2ml of antibiotic-free growth medium dropwise and mix gently by swirling. Add another 2ml of medium. Remove 100µl for total and viable cell counts. Establish new cultures at between 30 and 50% of their maximum cell density. Maintain the culture for at least 5d and monitor cell growth, check for microbial contamination. Master cell banks should be quality controlled to ensure their authenticity.

4.14 Cryogenic light microscopy

4.14.1 Introduction

There is an increasing need to ensure that the genetic and physiological characters of micro-organisms are retained during preservation and storage. It is not sufficient just to keep them "viable". Not only must they retain the property in which you are interested but they must retain their full suite of biochemical abilities in case they are required in future research programmes. This requires the employment of suitable preservation techniques and this essentially means the use of storage at ultra-low temperatures in or above liquid nitrogen. However, one freezing protocol will not result in the optimum recovery of every organism and therefore techniques must be optimised for groups of organisms or even for individual strains (Smith, 1992). In the past, research was directed towards finding better cryoprotectants, but this did not always result in successful cryogenic storage.

Cryogenic light microscopy allows observation at the cellular level of the response of micro-organisms to freezing and thawing. At CABI Bioscience the CM-3 cryomicroscope system (Planer Products Ltd) is used for the optimisation of cryopreservation techniques (Smith, 1992). The conduction stage is mounted on a Zeiss Axioskop H-DIC microscope fitted with Plan-Neofluar 40/0.75 (PH2) objectives. The temperature of the stage is computer controlled with dedicated software. Through an interface, the computer controls a stage heater, cooling of the stage is achieved using cold nitrogen gas (c. -170°C) flowing through the hollow stage. A cooling protocol is entered and the computer compares the temperature of the stage immediately below the sample with that required, by switching off the heater as the stage cools. The samples are cooled at different rates and the response recorded on video (Panasonic AG6200 recorder; JVC TK870E camera) for further analysis. Using a video character generator (Planer Products CM3200-00) the temperature of the stage is superimposed onto the video recording.

Intracellular ice can be observed which may be damaging or in a lot of cases lethal. Shrinkage can also cause injury and this can be seen and measured. The cooling rates that avoid these stresses can be employed as part of the preservation protocol (Tables 4.2 and 4.3). The best cryopreservatives for each particular group of micro-organisms can also be established.

4.14.2 Means of achieving reproducible cooling rates

Once an optimum cryopreservation protocol has been developed there are several ways of achieving the cooling rates required.

Controlled rate freezer

The simplest way (for slow cooling -0.1 to -30°C min^{-1}) is to use a programmable cooler. There are several available, at CABI Bioscience a KRYO 10/16 controlled rate freezer (Planer Products Ltd.) is

used. The cooling programme controls the chamber temperature so that any programme will have to be adjusted to achieve the desired cooling of the sample.

Table 4.2 Quantitative cryogenic light microscopy of fungal hyphae) after Morris *et al.* (1988)

Fungus	Critical cooling rate (°C min^{-1})*	Ice nucleation temperature (°C)
OOMYCOTA		
Achlya ambisexualis	6	-6 to -18
Phytophthora humicola	53	-8 to -17
P. nicotianae	>120	No ice seen
Pythium aphanidermatum	16	-6 to -14
Saprolegnia parasitica	4	-2 to -7
ZYGOMYCOTA		
Mortierella elongata +	18.5	-9
Mucor racemosus	10	-11 to -14
ASCOMYCOTA		
Sordaria fimicola	4	-8.5
BASIDIOMYCOTA		
Lentinus edodes	>100	No ice seen
Schizophyllum commune	9	-25 to -30
Serpula lacrymans		
Hyphae from 7-21d cultures	15.5	-10
Hyphae from 28d cultures		
<4.45 µm in diameter	>100	No ice seen
>4.45 µm in diameter	1	-15.5
Sporobolomyces roseus	>100	No ice seen
Volvariella volvacea	>100	No ice seen
MITOTIC FUNGI**		
Alternaria alternata	12.5	-14.5
Aschersonia alleyrodis	>100	No ice seen
Aureobasidium sp.	>100	No ice seen
Penicillium expansum	18	-14
Trichoderma viride	5	-5 to -11
Trichophyton rubrum	18	-18
Wallemia sebi	9	-18

*, The rate of cooling at which intracellular ice formed in 50% of the hyphae; +, the results presented here are those after growth in liquid medium; **, fungi not linked to perfect state.

Vapour phase cooling

The cryotubes can be suspended in a container (metal basket) in the neck of the liquid nitrogen storage tank at a vapour temperature of -35°C. Using this system a suspension of fungi in glycerol cools at an average of -1°C min^{-1}. The rate of cooling is faster to start with slowing to -1°C min^{-1} from -2°C to -20°C and slowing further as the suspension approaches the temperature of the vapour. By lowering the basket, greater cooling rates can be obtained; suspending immediately above the liquid level will give a rate of cooling of between -40 and -50°C min^{-1} for 0.5ml of suspension in glycerol. Faster cooling can be achieved by placing ampoules in a metal drawer rack system. A range of cooling rates can be obtained by placing the ampoules/vials at different levels in the vapour phase. Glass ampoules tend to cool slightly faster then plastic cryotubes. This method was employed at CABI Bioscience UK Centre (Egham) from 1968 to 1988, but has now been replaced by the controlled rate freezer method above.

Table 4.3 The optimum cooling rate and recovery of 20 species of fungi suspended in either growth medium or glycerol 10% (v/v) after Morris *et al.* (1988)

Fungus	Cooled in growth medium		Cooled in Glycerol	
	Optimum cooling rate (°C min^{-1})	Recovery (%)	Optimum cooling rate (°C min^{-1})	Recovery (%)
OOMYCOTA				
Achlya ambisexualis	0	0	9	88
Phytophthora humicola	0	0	3	69
P. nicotianae	0	0	0.5-11	72-91
Pythium aphanidermatum	0	0	8-29	52-53
Saprolegnia parasitica	0	0	10	32.5
ZYGOMYCOTA				
Mortierella elongata *	10-200	95	1-200	100
Mucor racemosus	25	36	5	31
ASCOMYCOTA				
Sordaria fimicola	1-200	c.100	1-200	c.100
BASIDIOMYCOTA				
Lentinus edodes	1	27	1-3.5	100
Schizophyllum commune	1	92	23	92
Serpula lacrymans				
Hyphae from 7-21d cultures	0	0	0.5	99
Hyphae from 28d cultures				
<4.45 μm in diameter	0	0	13	20
>4.45 μm in diameter	0	0	13	20
Sporobolomyces roseus	4.5	c.70	1	100
Volvariella volvacea	0	0	1	38
MITOTIC FUNGI**				
Alternaria alternata	21.5	88	20	97
Aschersonia alleyrodis	10.5	86	1	100
Aureobasidium sp.	10.2	83	200	91
Penicillium expansum	0.5-200	85-100	1-12	75-90
Trichoderma viride	3.8	c.100	3.8 to 10	c.100
Trichophyton rubrum	4	70.5	9	86
Wallemia sebi	83	c.100	77	c.100

*, The results presented here are those after growth in liquid medium; **, fungi not linked to a perfect state.

Cooling baths

Cooling baths are available from many scientific equipment distributors. Recirculating baths are more suitable for achieving more linear cooling than the non-circulatory baths. Alcohol is usually the cooling medium and the final temperature that can be achieved is dictated by the freezing point of the alcohol. Morris & Farrant (1972) describe several ways of controlling cooling using metal containers or dewars containing alcohol, suspending them in liquid nitrogen or the vapour above it. The methods of Morris & Farrant (1972) may present a more than acceptable risk to personnel.

Freezers

Placing ampoules/vials/cryotubes containing the suspension of cells in cryoprotectant directly onto the shelves or racking system in a freezer can give reproducible cooling rates. Freezers are now available

that achieve temperatures from -20 to -150°C. The lower the temperature of the freezer the faster the cooling rate achieved when cultures are placed directly in it. Despite the freezer being kept at a single temperature the use of insulating materials will enable slower cooling rates to be obtained. Placing ampoules in a polystyrene box, insulating using a polystyrene tile on a freezer shelf, using different thickness or quality materials will all give different cooling rates.

References

Al-Doory, Y. (1968) Survival of dermatophyte cultures maintained on hair. *Mycologia* **60**, 720-723.

Alexander, M., Daggett, P.M., Gherna, R., Jong, S.C., Simione, F. & Hatt, H. (1980). *American Type Culture Collection Methods I. Laboratory Manual on Preservation Freezing and Freeze Drying*. Rockville, Maryland: American Type Culture Collection.

Annear, D.I. (1958). *Australian Journal of Experimental Medical Science* **36**, 211-221.

Ashwood-Smith, M.J. & Grant, E. (1976) Mutation induction in bacteria by freeze drying. *Cryobiology* **13**, 206-213.

Ashwood-Smith, M.J. & Warby, C. (1971). Studies on the molecular weight and cryoprotective properties of PVP and dextran with bacteria and erythrocytes. *Cryobiology* **8**, 453-464.

Atkinson, R.G. (1953). Survival and pathogenicity of *Alternaria raphani* after 5 years in dried soil cultures. *Canadian Journal of Botany* **31**, 542-547.

Baker, P.R.W. (1955). The micro-determination of residual moisture in freeze-dried biological materials. *Journal of Hygiene* **53**, 426-435.

Bassal, J, Contopoulou, R., Mortimer, R. & Fogel, S. (1977). *UK Federation for Culture Collections Newsletter* No. 4, 7.

Bazzigher, G. (1962). Ein vereinfachtes gefriertrocknungverfarhen zur konservierung von Pilzkulturen. Phytopathol. Z. **45**: 53-56

Berny, J.F. & Hennebert, G.L. (1989). Abstracts, Meeting of Contractors, Biotechnology Action Programme of the Commission of the European Community, Biotechnology Division.

Berny, J.F. & Hennebert, G.L. (1991). Viability and stability of yeast cells and filamentous fungus spores during freeze-drying-effects of protectants and cooling rates**.** *Mycologia* **83,** 805-815.

Boeswinkel, H.J. (1976) Storage of fungal cultures in water. *Transactions of the British Mycological Society* **66**, 183-185.

Booth, C. (1971). *The genus Fusarium*. Kew: Commonwealth Mycological Institute.

Box, J.D. (1988). Cryopreservation of the blue-green alga *Microcystis aeruginosa*. *British Phycology Journal* **23**, 385-386.

Buell, C.B. & Weston, W.H. (1947). Application of the mineral oil conservation method to maintaining collections of fungus cultures. *American Journal of Botany* **34**, 555-561.

Burdsall H.H., & Dorworth E.B. (1994). Preserving cultures of wood decaying Basdiomycotina using sterile distilled water in cryovials. *Myxologia* **86**, 275-280.

Butterfield, W., Jong, S.C. & Alexander, M.J. (1974). Preservation of living fungi pathogenic for man and animals. *Canadian Journal of Microbiology* **20**, 1665-1673.

Dade, H.A. (1960). Laboratory methods in use in the culture collection, IMI. In: *Herb IMI Handbook*. Kew: Commonwealth Mycological Institute.

Day J.G. (1998) Cryo-conservation of microalgae and cyanobacteria. Cryo-Letters Supplement **1**, 7-14.

Day J.G., Fleck RA. & Benson E.E (2000) Cryopreservation-recalcitrance in microalgae: novel approaches to identify and avoid cryo-injury. *Journal of Applied Phycology* **12**, 369-377.

Day J.G. & McLellan M.R, (1995). Cryopreservation and freeze-drying protocols. *Methods in Molecular Biology,* Vol. 38, Totowa, New Jersey, Humana Press Inc.

Day J.G. , Priestley I.M. & Codd G.A. (1987). Storage reconstitution and photosynthetic activities of immobilised algae. *In*: Plant and Animal Cells, Process Possibilities. C. Webb and F. Mavituna (Eds.). Ellis Harwood Ltd., Chichester. pp 257-261.

Day J.G., Watanabe M.M., Morris G.J., Fleck R.A. & McLellan M.R. (1997). Long-term viability of preserved eukaryotic algae. *Journal of Applied Phycology* **9**, 121-127.

Doyle, A., Morris, C.B. & Armitage, W.J. (1989). *In*: Advances in Biotechnological Processes Volume 7 (A. Mizrahi, ed). New York: Alan R. Liss Inc.

Fennell, D.I. (1960) Conservation of fungus cultures. *Botanical Review* **26**, 79-141.

Figueiredo, M.B. & Pimentel, C.P.V. (1975). Metodos utilizados para conservacao de fungos na micoteca de Secao de Micologia Fitopatologica de Instituto Biologico. *Summa Phytopathologica* **1**, 299-302.

Franks, F. (1981). Biophysics and biochemistry of low temperatures and freezing. *In*: Effects of Low Temperature of biological Membranes. (edited by Morris, G.J & Clarke, A.). pp 3-19. London: Academic Press.

Gilmour, M.N., Turner, G., Berman, R.G. & Kreuzer, A.K. (1978). Compact liqiid nitrogen storage system yielding high recoveries of gram negative anaerobes. Applied and Environmental *Microbiology* **35**, 84-88.

Gordon, W.L. (1952). The occurrence of *Fusarium* species in Canada. *Canadian Journal of Botany* **30**, 209-251.

Heckly, R.J. (1978). Preservation of Micro-organisms. *Advances in Applied Microbiology* **24**, 1-53.

Holm-Hansen, O. (1963). Viability of blue-green algae after freezing. *Physiologia planta* **16,** 530-539.

Holm-Hansen, O. (1967). Factors affecting the viability of lyophilised algae. *Cryobiology* **4,** 17-23.

Holm-Hansen, O. (1973). Preservation by freezing and freeze drying In, *Handbook of Phycological Methods (*ed Stein, J.R.) pp 195-206, Cambridge University Press, UK.

Hubalek, Z. & Kochova-Kratochvilova, A. (1978). Antonie van Leewenhoek **44**, 229-241.

Hubalek, Z. (1996). Cryopreservation of micro-organisms at ultra-low temperatures. Prague: Academy of Sciences of the Czech Republic, pp 286. ISBN80-200-0557-9.

Hwang, S.-W. (1960). Effects of ultralow preservation of fungus cultures with liquid nitrogen refrigeration. *Mycologia* **52**, 527-529.

Hwang, S.-W. (1966). Long term preservation of fungus cultures with liquid nitrogen refrigeration. *Applied Microbiology* **14**, 784-788.

Hwang, S.-W. (1968). Investigation of ultra-low temperature for fungal cultures. I. An evaluation of liquid nitrogen storage for preservation of selected fungal cultures. *Mycologia* **60**, 613-621.

Hwang, S. -W. & Howells, A. (1968) Investigation of ultra-low temperature of fungal cultures II. Cryoprotection afforded by glycerol and dimethyl sulphoxide to eight selected fungal cultures. *Mycologia* **60**, 622-626.

Hwang, S. -W., Kwolek, W.F. & Haynes, W.C. (1976). Investigation of ultra low temperature for fungal cultures III. Viability and growth rate of mycelial cultures cryogenic storage. *Mycologia* **68**, 377-387.

Kirsop, B. (1974). The stability of biochemical, morphological and brewing properties of yeast cultures maintained by subculturing and freeze-drying. *Journal of the Institute of Brewing* **80**, 565-570.

Kirsop, B. (1978). *In*: Abstracts of the XII International congress of Microbiology, 1978, p39. Munchen.

Kirsop, B. & Doyle, A. (eds) (1991). *Maintenace of micro-organisms and cultured cells*, pp 308. New York: Academic Press.

Little, G.N. & Gordon, M.A. (1967). Survival of fungus cultures maintained under mineral oil for twelve years. *Mycologia* **59**, 733-736.

Marx, D.H. & Daniel, W.J. (1976). Maintaining cultures of ectomycorrhizal and plant pathogenic fungi in sterile water cold storage. *Canadian Journal of Microbiology* **22**, 338-341.

McGrath, M. S., Daggett, P. M. & Dilworth, S. (1978). Freeze-drying of algae: Chlorophyta and Chrysophyta. *J Phycol* **14**, 521-525.

McLellan M.R., Cowling A.J., Turner M.F. & Day J.G. (1991). Maintenance of algae and protozoa. In: Maintenance of Micro-organisms. B. Kirsop & A. Doyle (eds.). Academic Press Ltd., London. pp 183-208.

Morris, G.J. (1976). The cryopreservation of *Chlorella* 2. Effect of growth temperature on freezing tolerance. *Arch. Microbiol* **107**, 309-312.

Morris, G.J. (1978). Cryopreservation of 250 strains of Chlorococcales by the method of two step cooling. *British Phycology Journal* **13**, 15-24.

Morris, G.J. (1981). Cryopreservation: An introduction to cryopreservation in culture collections. Cambridge: Culture Centre of Algae and Protozoa.

Morris, G.J. & Farrant, J. (1972). Interactions of cooling rate and protective additive on the survival of washed human erythrocytes. *Cryobiology* **9**, 173-181.

Morris, G.J., Smith, D. & Coulson, G.E. (1988). A comparative study of the changes in the morphology of hyphae during freezing and viability upon thawing for twenty species of fungi. *Journal of General Microbiology* **134**, 2897-2906.

Obara, Y., Yamai, S., Nikkawa, T., Shimoda, Y. & Miyamoto, Y. (1981). **TITLE** *Journal of Clinical Microbiology* **14,** 61-66.

Onions, A.H.S. (1971). Preservation of fungi. In: *Methods in Microbiology* **4** (edited by C. Booth) pp. 113-151. London and New York: Academic Press.

Onions, A.H.S. (1977). Storage of fungi by mineral oil and silica gel for use in the collection with limited resources. In: *Proceedings of the Second International Conference on Culture Collections*. Brisbane: World Federation for Culture Collections.

Onions, A.H.S. & Smith, D. (1984). Current status of culture preservation and technology. In: *Critical problems of Culture Collections* (edited by L.R. Batra & T. Iigima). Osaka: Institute of Fermentation Osaka.

Pearson, B.M., Jackman, P.J.H., Painting, K.A. & Morris, G.J. (1990). The effect of freezing and thawing on plasmid expression in *Saccharomyces cerevisiae*. *Cryoletters* **11**, 205-210.

Perkins, D.D. (1962). Preservation of *Neurospora* stock cultures in anhydrous silica gel. *Canadian Journal of Microbiology* **8**, 591-594.

Pertot, E., Puc, A. & Kresmer, M. (1977). Lyophilisation of non-sporulating strains of the fungus *Claviceps*. *European Journal of Applied Microbiology* **4**, 289-294.

Polge, C., Smith, A.U. & Parkes, S. (1949). Revival of spermatozoa after dehydration at low temperatures. *Nature* **164**, 666.

Qiangqiang, Z., Jiajun, W. & Li, L (1998). Storage of fungi using distilled water or lyophilisation: comparison after 12 years. *Mycoses* **41**, 255-257.

Raper, K.B. & Alexander, D.F. (1945) Preservation of molds by lyophil process. *Mycologia* **37**, 499-525.

Reinecke, P. & Fokkema, N.J. (1979). *Pseudocercosporella herpotrichoides:* Storage and mass production of conidia. *Transactions of the British Mycological Society* **72**, 329-331.

Reischer, H.S. (1949). Preservation of Saprolegniaceae by the mineral oil method. *Mycologia* **41**, 177-179.

Rey, L.R. (1977). Glimpses into the fundamental aspects of freeze drying. In: *Development in Biological Standardisation* (edited by V.J. Cabasso & R.H. Regamey). Basel: S. Krager.

Ryan, M.J., Smith, D. & Jeffries, P. (2000). A key to distinguish the most appropriate protocol for the preservation of fungi. *World Journal of Microbiology and Biotechnology (In Press).*

Ryan, M.J. (1999). *The Effect of Preservation Regime on the Physiology and Genetic Stability of Economically Important Fungi*. Ph.D. Thesis, University of Kent, U.K.

Shearer, B.L., Zeyen, R.J. & Ooka, J.J. (1974). Storage and behaviour in soil of *Septoria* species isolated from cereals. *Phytopathology* **64**, 163-167.

Smith, D. (1983a). A two stage centrifugal freeze-drying method for the preservation of fungi. *Transactions of the British Mycological Society* **80**, 333-337.

Smith, D. (1983b). Cryoprotectants and the cryopreservation of fungi. *Transactions of the British Mycological Society* **80**, 360-363.

Smith, D. (1986). *The evaluation and development of techniques for the preservation of living filamentous fungi*. PhD thesis, London University.

Smith, D. (1992). Optimizing preservation. *Laboratory Practice* **41**, 25-28.

Smith, D. (1993). Tolerance to freezing and thawing. In: *Stress Tolerance in Fungi* (edited by D.H. Jennings) pp. 145-171. New York: Marcel Dekker Inc.

Smith, D. & Allsopp, D. (1992). Preservation and maintenance of biotechnological important micro-organisms. In: *Industrial Biotechnology International 1993*, pp 103-107. Edited by R.N. Greenshields. UK: Sterling Press.

Smith, D. & Onions, A.H.S. (1983). A comparison of some preservation techniques for fungi. *Transactions of the British Mycological Society* **81**, 535-540.

Smith, D. & Onions A.H.S. (1994). The Preservation and Maintenance of Living Fungi. Second edition. IMI Technical Handbooks No. 2, pp 122. Wallingford, UK: CAB INTERNATIONAL.

Smith, D.H., Lewis, F.H. & Fergus, C.L. (1970) Long term preservation of *Botryosphaeria ribis* and *Dibotryon morbosum*. *Plant Disease Reporter* **54**, 217-218.

Smith, D. & Thomas, V.E. (1998). Cryogenic light microscopy and the development of cooling protocols for the cryopreservation of filamentous fungi. *World Journal of Microbiology & Biotechnology* **14**, 49-57.

Souzu. H. (1973). The phospholipid degradation and cellular death caused by freeze-thawing or freeze-drying of yeast. *Cryobiology* **10,** 427-431

Stamp, L. (1947). The preservation of bacteria by drying. *Journal of General Microbiology* **1,** 251-265

Tan, C.S. (1997). Preservation of fungi. *Cryptogamie Mycol*. **18**, 157-163.

Tan, C.S., Stalpers, J.A. & van Ingen, C.W. (1991a). Freeze-drying of fungal hyphae. *Mycologia* **83**, 654-657.

Tan, C.S., Ingen, C.W. van. & Stalpers, J.A. (1991b). Freeze-drying of fungal hyphae and stability of the product. *Genetics and breeding of Agaricus. Proceedings of the first International Seminar on Mushroom Science, Mushroom Experimental Station, Horst, Netherlands, 14-17 May 1991* (ed Griensven, L. J.D. van). Wageningen, Netherlands: Pudoc.

Tan, C.S., Ingen, C.W. van, Talsma, H., Miltenburg, J.C. van, Steffensen, C.L., Vlug, I.A. & Stalpers, J.A. (1995). Freeze-drying of fungi: influence of composition and glass transition temperature of the protectant. *Cryobiology* **32,** 60-67.

Tommerup, I.C. & Kidby, D.K. (1979). Preservation of spores of vesicular-arbuscular endophytes by L-drying. *Applied and Environmental Microbiology* **37**, 831-835.

Torres, P. & Honrubia, M. (1994). Basidiospore viability in stored slurries. *Mycological Research* **98:** 527-530.

Wellman, A.M. & Stewart, G.G. (1973) .*Applied Microbiology* **26,** 577-583.

Woods, R. (1976). *UK Federation for Culture Collections Newsletter* **2,** p5.

Yamai, S. Obara, Y., Nikkawa, T., Shimoda, Y. & Miyamoto, Y. (1979). *British Journal of Venerial* Disease, **55,** 90-93.

Chapter 5

Microbial properties

Matthew Ryan

The member collections of the UKNCC hold a large and diverse number of strains and cell lines. Of these, a significant number have important properties that can be utilised by the wider scientific community. This chapter discusses some of these properties and should be used in conjunction with Appendix (a) where an extensive list of microbial properties is given citing examples of the organisms and the strains that exhibit them. The information on strain properties often originates from the depositor and on occasion has been confirmed by the collection. In the process of collection activities and research or that of its parental organisation the data may have been generated internally. CABI data is diferentiated marking depositor data with 'D' and inhouse generated data 'I'. Occasionally strains may cease to exhibit properties during storage but more often organisms fail to exhibit the property because they are not grown under the prescribed conditions. It is essential that appropriate growth conditions are provided, information that is included in chapter 2 and generally available in the published literature or directly from the collection itself. Where a collection is not able to confirm a particular property it cannot guarantee that it will be present when supplied, for example if an organism is cited as a tree pathogen rarely has this been confirmed by tests following preservation.

Many cultures are representative of their species and are deposited as "ex type strains" while others may exhibit a particular morphological, physiological or anatomical property that that can used for research or teaching purposes. The commercial exploitation of microbes, cell lines and their properties has been associated with substantial research on a large number of economically important organisms. This chapter (in association with Appendix A, which is divided into sections that correspond with those listed below) is designed to help scientists locate cultures with specific chemical or functional properties and Chapter 6 provides an insight to the methodology used to determine some of these properties.

5.13 Tolerant, resistant or sensitive strains

5.14 Test strains

5.15 Assay strains

5.16 Special properties: morphological and physiological

5.17 Special properties: chemical (bioconversion/biotransformation etc)

5.18 Genetic strains (phages, transposons, vectors and genetically modified organisms).

*For **ANIMAL CELL LINES** the European Collection of Cell Cultures (ECCAC) should be contacted directly. Alternatively, the UKNCC web site contains information on ECCAC's unique and diverse collections of animal and human cell lines including the:*

- *General animal cell collection*
- *Hybridoma collection (monoclonal antibody-secreting hybridomas)*
- *HLA-defined human B-lymphoblastoid cell lines*
- *DNA probe collection*
- *Human genetic cell line collection.*

http://www.camr.org.uk/ecacc.htm

5.1 Type or ex-type strains

Type strains are living cultures of fully described, scientifically validated and named cultures where in the case of fungal strains the holotype is deposited as a dried specimen or physiologically inactive strain in a reference collection (herbaria) (Hawksworth *et al.,* 1995). Type strains (bacteria) or ex-type strains (fungi) are representative examples of their species, and as such, can be used for morphological, anatomical, physiological and genetic comparisons with newly isolated strains. These strains are widely used by taxonomists and are becoming increasingly important as reference strains particularly for patent deposits and isolates that are release into the environment. Representatives of type and ex-type strains can be located at http://www.ukncc.co.uk/html/Databases/Search.asp.

5.2 Enzyme producing strains

Micro-organisms produce a novel and diverse array of extra and intra-cellular enzymes from urease synthesised by strains of the bacterium *Bacillus fastidiosus* to cellulase and lipase synthesised by strains of the fungus *Trichoderma viride*. Over 200 enzymes and the strains that produce them (c. 500) are listed in Appendix A. The UKNCC member collections attempt to characterise their holdings but inevitably they hold many untested representatives, the potential of the uncharacterised strains is enormous. Some methods used to detect enzyme activity are discussed in chapter 6. Saprophytic organisms (e.g., many fungi) excrete extracellular enzymes into the substratum and these can be easily extracted and purified. Other organisms produce intracellular enzymes that are more difficult to extract. Many enzymes are produced on an industrial scale (e.g., amylase from strains of *Aspergillus niger*, *A. oryzae, Bacillus polymyxa, B. subtilis,* and *Trichoderma viride*). Enzymes can also be used to help taxonomically characterise organisms, while some enzymes and combinations of enzymes are distinct for specific micro-organisms (e.g., inulinase synthesis by *Sphingobacterium multivorum*). Other

enzymes are representative of an organisms mode of life (e.g., insect cuticle degrading enzymes synthesised by the entomopathogenic fungus *Metarhizium anisopliae*). Enzymes and their genes are studied widely within the fields of biochemistry and molecular biology, and the UKNCC holds accessions for genetically modified enzyme producers (e.g., arginase from a modified strain of *Zygosaccharomyces pombe*).

5.3 Metabolite producing strains

The UKNCC holds a unique and diverse array of metabolite producing strains, over 750 compounds are listed in Appendix A, produced by over 2000 organisms, the numbers of which are increasing all the time. Both primary and secondary metabolite producers are included. However, many metabolites are only produced under pre-specified experimental conditions. Some methods used to detect the ability of an organism to produce metabolites are discussed in Chapter 6. Microbial metabolites are of enormous economic importance in today's society and many are extracted for use in the food and chemical industries and are produced on an industrial scale (e.g., alcohol's by *Rhizopus oryzae* and organic acids by *Aspergillus niger*). Examples of primary metabolites produced by UKNCC strains include carbohydrates (e.g., cellulose by *Acetobacter aceti*, exopolysaccharide by *Alteromonas atlantica* and mannocarlose by *Penicillium fellutanum*), fats (e.g., produced by *Penicillium indonesiae*), amino acids (e.g., tryptophan and lysine produced by strains of *Escherichia coli*) and organic acids which are widely produced by both bacteria and fungi (e.g., glutamic acid by *Corneybacterium* sp. and mycophenolic acid by *Penicillium brevicompactum*). Some micro-organisms produce vitamins (e.g., vitamin B_{12} synthesis by the bacterium *Propionibacterium freudenreichii*), secondary carotenoids (e.g., asfaxonthin synthesised by Haematococcus pluvialis) and hormones (e.g., absisic acid synthesised by strains of *Cercospora rosicola*; antheridiol synthesised by strains of *Achyla bisexualis*; gibberellin synthesised by strains of *Fusarium moniliforme*).

Secondary metabolites are of huge economical importance, many are of fungal origin (Turner & Aldridge, 1983). Of these, the antibiotics are of particular economic significance (see Section 5.4). Some secondary metabolites are highly toxic (e.g., the carcinogen patulin produced by *Penicillium expansum*, aflatoxin produced by *Aspergillus flavus*; anatoxin-a produced by the cyanobacterium *Anabaena flors aquae* and exotoxin produced by the bacterium *Pseudomonas aeruginosa*) while others are beneficial and utilised by food and industry (e.g., a blue pigment produced by *Pseudomonas fluorescens* and a fluorescent red diffusible pigment produced by *Arthrobacter* sp.). Some secondary metabolites are specific to an organism's mode of life, for example the destruxins produced by *Metarhizium anisopliae* which are essential for the induction and maintenance of pathogenesis in the insect host. Some workers have used metabolite profiles to classify fungi. For example, Svendsen & Frisvad (1994) demonstrated that chemosystematics of secondary metabolite profiles could be applied to separate 279 terverticillate *Penicillium* spp. using cluster analysis.

5.4 Antibiotic producing strains

Since the discovery of penicillin production by *Penicillium notatum* by Alexander Flemming in 1930's, the range and effectiveness of antibiotics has been steadily improved and increased. An antibiotic is a microbial product (typically a secondary metabolite) that in low concentration can suspend or extinguish microbial growth. There are in excess of 240 such compounds listed in Appendix A produced by over 420 UKNCC strains which are effective against a broad spectrum of microbes with many different modes of action. Antibiotics are mainly beneficial to man, although in excess, antibiotics can be toxic to eukaryotic organisms (e.g., griseofulvin). The majority of antibiotics producing strains are antibacterial, but antifungal and antiprotozooal producing strains are also held. The number of antibiotic producing strains is likely to be much greater, as thousands of UKNCC strains have not been screened for antibiotic activity.

5.5 Food producing strains

Some algae and fungi have been directly utilised as food for many centuries. Macro-algae (seaweeds, e.g., *Porphyra* spp.) are commonly consumed in Wales (laverbread) and Japan (nori) and the cyanobacterium *Spirulina* has been consumed as a food supplement for thousands of years in Central America and sub Saharan Africa. Furthermore, fungal fruiting bodies "mushrooms" are eaten world-wide. Some mushrooms are commercially produced, e.g., the edible mushroom *Agaricus bisporus,* the oyster mushroom *Pleurotus ostreatus*, the shiitake mushroom *Lentinus edodes* and the straw mushroom *Volvariella* spp. (Chang *et al.,* 1993) while others are strictly the domain of the specialist field mycologist (e.g., the Dotted-stem Bolete**,** *Boletus luridiformis*). More recently, biotechnological development has allowed the mass production of single celled proteins (e.g., from strains of *Saccharomyces cerevisiae*, *Kluyveromyces fragilis marxianus* and *Candida utilis* (Arora *et al.,* 1991) and the bacterium *Methylophilus methylovorus* which produces single cell protein from methanol. and processed mycelium (Quorn™ mycoprotein) production by *Fusarium* sp. (Trinci, 1992). Algae are also used as health supplements (*Chlorella* and *Spirulina*) and as a food source for juvenile fish in the aquaculture industry (e.g., *Chaetoceros calcitrans* and *Skeletonema costatum*).

Many microbial products are used as ingredients in food production (e.g., alginates produced by *Pseudomonas mendocina*), while others are vital components of food manufacturing. For example, bacteria and fungi are used in the production of fermented food, baking and cheese production. Strains of the yeast *Saccharomyces cer*evisiae are used in the brewing of beers, fermentation of wines and distilling of spirits and the baking of bread. Many foods are fermented including Indonesian Tempe from Soybean using *Rhizopus* sp. (Onions *et al.,* 1981) or *Lactobacillus* spp. Other examples include acid fermented vegetables e.g., sauerkraut and pickles using *Aspergillus oryzae*, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Pediococcus cerevisiae* and *Lactobacillus brevis*; Soy sauce using *Aspergillus oryzae* and Japanese Miso Soybean using *Saccharomyces rouxii (*Steinkraus, 1983). Mould ripened cheeses require cultures of *Penicillium camembertii*, *Penicillium roquefortii* (Beuchat, 1987) and yoghurts require bacterial starter cultures

(e.g., *Lactobacillus* spp.). There is also a keen interest in recent years in the use of organisms, such as *Lactobacillus,* in probiotics.

5.6 Pathogens

Pathogens that are deposited in culture collections to provide valuable reference resources to plant and animal pathologists and the wider scientific community. Many pathogens have been developed for use as biological control agents (see Section 5.7), while others exhibit physiological and biochemical properties, which allow scientists to utilise them in the fight against virulent environmental strains. Additionally, suspensions of killed or live attenuated bacteria can be used as vaccines (see Appendix A: vaccine producers). There are thousands of pathogens and parasites deposited in the UKNCC member collections. Most animal and human pathogens are held by the National Collection of Type Cultures (NCTC) and the National Collection of Pathogenic Fungi (NCPF) whereas plant pathogenic fungi and bacteria are held at CABI and the National Collection of Plant Pathogenic Bacteria (NCPPB) respectively. Appendix A, microbial properties, lists only examples of mycoparasites and those pathogens employed as biological control agents for insect pests and plant diseases. It is not practical to include all parasite and opportunistic pathogens which would total over 15 000 strains. Users can access the UKNCC holdings (http://www.ukncc.co.uk/html/Databases/Search.asp.) of pathogens using organism or additional host specific search criteria or view the UKNCC integrated catalogues as hard copy or on CD-ROM. As mentioned elsewhere in this manual, licenses are often required before pathogens are dispatched and certain pathogens will not be released to unknown or unauthorised parties.

There are many and varied microbial plant pathogens that are pathogenic to nearly every identified plant species. Common fungal plant pathogens include *Fusarium oxysporum* (which has over 100 special forms), other *Fusarium* spp., *Elsinoe* spp., *Alternaria* spp., *Aphanomyces* spp., *Ganoderma* spp., *Pleurotus* spp., *Nectria* spp., *Ventura* spp., *Ascochyta* spp., *Gibberella* spp., *Amylostereum* spp. Fungal pathogens of humans include the causative agents of athlete's foot (*Epidermophyton floccosum* and *Trichophyton* spp.) alimentary toxic aleulia mycotoxicosis (*Fusarium tricinctum*) and farmer's lung aspergillosis (*Aspergillus fumigatus*). There are hundreds of pathogenic human bacteria, the most severe include *Shigella dysenteriae* causative agent of dysentery, *Vibrio choleriae* causative agent of cholera, *Clostridium tetani* causative agent of tetanus.

Other animal pathogens include tic-borne disease of domesticated animals (e.g., *Babesia bigmenia* and *Babesia bovis*) and systemic infection in vertebrate animals (e.g., *Babesia equi* and *Babesia micrptii*). There are many pathogens of lower animals for example insects (e.g., *Malphigamoeba* spp. in bees, *Endamoeba* spp. in cockroach's), nematodes (*Cordyceps gracilis* and *Harposporium arthrosporum*) and endoparasites of aquatic annelids, molluscs and amphibians. (e.g., *Anoplophrya*, *Cepedietta*, *Jirovecella* and *Radiophyra* spp.). Microbial pathogens are of particular interest and many have potential for development as biological control agents for fungal parasites of crops (see Section 5.7 and Appendix A: Biological Control Agents. Mycoparasites include *Pitocephalis* spp., *Syncephalis* spp. and

Verticillium spp. Examples of fish pathogens are *Vibrio anguillarum*, *Aeromonas salmonicida* and *Aerococcus viridans*.

5.7 Biological control strains

Concern about the use of chemical agents against animal and crop diseases and to control insect pests and weeds has led to the development of biological control agents (BCA's), many of which are microbial. BCA's should not damage the natural biota of the target environment and generally have no adverse physical effects, making them preferable over potentially harmful chemical agents, which may not be specific to the target organism and may pollute and damage the ecosystem. Many BCA's have been developed from cultures directly isolated from the target organisms or from isolates showing an *in vitro* chemical or antagonistic property against the target organism. Indeed, non-pathogenic forms are being used to control pathogenic forms of the same species. BCA's are used in integrated pest management and crop protection. Some of the strains listed as pathogens may have the potential for development as biological control agents. The entomopathogenic fungus *Metarhizium anisopliae* has been used to control a wide-range of insect pests including: locusts (*Schistocerca gregaria*) (Prior *et al.,* 1992), spittle bugs (*Manhanarva posticata*) (Risco, 1980), German cockroach's (*Blatella germanica*) (McCammon & Rath, 1994), the Australian pasture pests (*Adoryphorus couloni)* (Rath *et al.,* 1995) and Tsetse flies (*Glosina* spp.) (Kaaya & Munyini, 1995). Plant weeds have been controlled with *Phragmidium* spp. and *Puccinia* spp., (Julien, 1992), soil borne plant pathogens have been controlled with *Trichoderma* spp. and *Fusarium* spp. (Hornby, 1990) and plant parasitic nematodes controlled with *Arthrobotrys* spp. and *Dactylella* spp. (Stirling, 1991). Other fungal biocontrol strains include *Cordyceps* spp. *Ascheronia* spp. *Verticillium* spp. and *Beauveria* spp (see Appendix A: Biological control agents). Bacterial biocontrol agents are also found in a large range of taxa including actinomycetes, *Bacillus* spp and fluorescent pseudomonads.

5.8 Strains used in horticulture

There are many applications of microbes in horticulture. *Agrobacterium* spp. have been used in plant breeding applications (mediated gene transfer) to improve herbicide tolerance, insect resistance, virus resistance and male sterility. *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* are used as vectors for the production of transgenic plants because the organism is capable of transferring its plasmid DNA directly into plant chromosomes (Evans, 1993).

The bacteria *Rhizobium*, *Bradyrhizobium* and *Azorhizobium* are leguminosae plant symbionts, strains that may be host specific, e.g., *Rhizobium leguminosarum* biovar. *phaseoli* to bean plants. Rhizobia make nitrogen available to the plant host and harvest yields may be increased if *Rhizobium* spp. are inoculated directly into plant rhizosphere (Jones & Lewis, 1993). A further symbiotic association that makes atmospheric nitrogen available to plants (especially to rice in paddy fields) is that between the cyanobacterium (*Anabaena* spp.) and *Azolla* (water-fern). Many "free living" cyanobacteria (e.g., *Calothrix* spp. *Gloeotrichia* spp.) also make nitrogen available to plants.

Similarly, fungal mycorrhizal plant symbionts can be beneficial to the horticulturist. Mycorrhiza aid the establishment, growth and development of plants by improving mineral nutrition, increasing drought tolerance, supplying growth-regulating substances and vitamins and protecting against pollutants and soil-borne pathogens (Mitchell, 1993). Inoculating agricultural soils with mycorrhizae may therefore improve harvest yield. There are a number of types of mycorrhizal association: Ectomycorrhizal (e.g., *Cantharellus cibarius*, *Boletus edulis*), arbuscular mycorrhizal (e.g., *Glomus*, *Gigaspora*), ericoid mycorrhizal (e.g., *Hymenoscyphus ericae*) and orchidaceous mycorrhizal (e.g., *Armillaria* spp. *Thanatephorus* spp.). Mycorrhizal technology also has applications in forestry, dessertified ecosystems, bioremediation, biodegredation, recovery of sterilised soils and environmental recovery programmes.

5.9 Environmental strains

Micro-organisms isolated from extreme and interesting environments often exhibit unique properties, many of which are biologically important and can be utilised in research projects. For example, strains isolated from hot springs (e.g., *Naegleria clarki* and *Sulfolobus acidocaldarius*) may withstand high temperatures (i.e. are thermophilic). Isolates isolated from the Antarctic (e.g., *Heteromita globosa* and *Heterothrix antartica*) may withstand low temperatures (i.e. are psychrophilic). Likewise organisms isolated from diverse chemical environments can withstand extreme pH and chemical conditions (e.g., the halophile *Marinococcus halophilus*, the alkalophile *Natronobacterium gregoryi* and the acidophile *Lactobacillus acetotolerans*). Other examples are a strain of *Radiospheara negevensis* isolated from the desert that may be able to survive dehydration, a strain of *Anabaena variabilis* isolated from the deep-sea (70m), which can survive high pressure and *Deinococus radiodurans* which can survive high levels of gamma radiaion. An isolate of *Hemichloris antartica* isolated from a rock in the Antarctic may be able to withstand low temperature, osmotic and chemical extremes. Similarly, organisms that are isolated from mutualistic associations (symbionts) often exhibit unique or unusual chemical properties (e.g., the lichen symbionts *Coccomyxa pringsheimii* and *Trebouxia crenulata*). Other micro-organisms isolated from interesting environments that may have unique properties include the copriphilous fungi (e.g., *Utharomyces* and *Piptocephalis* spp.), river foam isolates (e.g., *Triposermum campelopardus* and *Varicosporium helicosporum*), aquatic protistan bloom isolates (e.g., *Stentor* sp. and *Chrysochromulina breviturrita*) and thermostable protease producing *Bacillus* spp. which are used in biological washing powder.

5.10 Biodeteriogens

(also see Section 5.11 food spoilage strains and section 5.12 utlisers / biodegraders and bioremediators)

Biodeterioration, any undesirable change in the properties of a material caused by the vital activities of organisms, (Hawksworth *et al.*, 1995) of synthetic and natural products by micro-organisms costs £millions each year. Many biodeteriogens may have important physiological and chemical properties (e.g., enzymes capable of breaking down complex organic or synthetic substrates) that can be utilised by the wider scientific community or in the development of biodegraders or bioremediators. While many others can be used in research aimed at establishing the mechanisms involved in substrate

breakdown, the information from which can be used to improve the resistance of materials to microbial attack.

The UKNCC holds accessions to many diverse and interesting biodeteriogens including those isolated from: aviation fuel (*Amorpotheca resinae*, *Aspergillus fumigatus*), a binocular lens (*Aspergillus tonophilus*), cellophane paper (*Aspergillus versicolor*), a concrete wall (*Arthrobotrys javinica*), cotton yarn (*Aspergillus tamarii*) creosoted wood (*Amorpotheca resinae*), diesel fuel (*Trichoderma harzianum*), kerosene fuel filter (*Pestalotiopsis aquatica*), metal working fluid (*Scopulariopsis brevicaulis*), paint (*Cladosporium sphaerospermum*), plastics (*Penicillium pinophilum*), polurethane foam (*Penicillium chrysogenum*), a radio set (*Aspergillus ustus*), vinyl wall covering (*Penicillium pupurogenum*) and wall paper (*Aspergillus tamarii*), PVC (*Pseudomonas* sp), rubber (*Nocardia* sp), cider (*Zygomonas mobilis* ss *pomacii*), steel and cutting fluids (*Shewanella putrefaciens*), and concrete (*Thiobacillus concretovorans*).

5.11 Food spoilage strains

Microbial spoilage of foodstuffs costs the food industry £millions world-wide every year. Added to this is the significant risk to public health that arises after the consumption of spoiled food, either due to the direct pathogenesis by food-borne bacteria (see Section 5.6), or the toxins released by the micro-organisms during and after substrate breakdown. Many food biodeteriogens are tolerant to added preservatives or have survived sterilisation procedures and are therefore useful to scientists whose research is aimed at improving food preservation. Examples of food products from which strains have been isolated include: gelatine (*Geomyces pannorum*), lime flavour carton drink (*Trichoderma viride*), table jelly (*Xeromyces bisporus*), jam (*Wallemia sebi*), canned blueberries (*Penicillium lapidosum*), cheese (*Lactobacillus brevis*), fish (*Shewanella putrefaciens*), milk (*Enterobacter aerogenes*) and meat (*Pseudomonas* sp).

5.12 Utlisers / biodegraders and bioremediators

Degraders and utilisers are a distinct and economically important group of micro-organisms. Often these microbes possess physiological and biochemical properties that can be utilised by the wider economic community. Substrates degraded include alcohol (*Rhodococcus* sp.), aldehydes (*Amycolata hydrocarbonoxydans*), benzaldehyde (*Pseudomonas* sp.), benzene (*Acinetobacter* sp.), crude oil (*Pseudomonas* sp., *Rhodococcus rhodochrous*), detergent (*Rhizobiaceae* sp.) industrial waste (*Chaetomium jodhpurense*), lignin (*Phanerochaete chrysosporium*), methane (*Methylococcus capsulatus*) methanol (*Altermonas* sp.), nitriles (*Rhodococcus* sp.), paper (*Phoma violacea*), phenol (*Pseudomonas putida*), rubber (*Nocardia* sp.), salicylic acid (*Acinetobacter* sp.), xylan (*Bacillus subtilis*), cyanide (*Pseudomonas fluorescens*) and cresols (*Pseudomonas putida*).

Strains used in the biodegradation of wastes include *Acetobacterium woodii, Methanosarcina barkeri* and *Lactobacillus casei* which are used specifically for the anaerobic digestion of food processing wastes (Greenshields, 1989). Agricultural wastes are degraded using *Zooglea ramigera* (Hawker &

Linton, 1960). A controlled form of biodegradation is bioremediation, which can be achieved using bioreactors, solid phase treatments, composting, land farming or *in situ* treatment (Balba, 1993). For example: the recovery of heavy metals from aqueous effluents: copper using *Penicillium spinulosum* and *Trichoderma viride* (Greenshields, 1989), gold using *Chlorella vulgaris* and uranium using *Chlorella regularis* and *Aspergillus niger*, or the degredation of the phased out herbicide 2,4,5-T [(2,4,5-trichlorophenoxy) acetic acid-triethylammonium] with bacteria.

5.13 Tolerant/ resistant/ sensitive strains

Tolerant strains are often isolated from extreme environments (see Section 5.9), their unique biochemistry makes them useful for research in a variety of fields. Strains held by the UKNCC include those tolerant to alcohol (*Saccharomyces cerevisiae*), benzimidazole (*Venturia inaequalis*), benzoic acid 300ppm (*Zygosaccharomyces bailii*), copper (*Scytalidium acidophilum*), creosote (*Vibrio alginolyticus*) 60% glucose (*Candida magnoliae*), 20% sodium chloride (*Zygosaccharomyces rouxi*i), silver (*Stenotrophomas maltophila*) and temperature (*Alicyclobacillus acidorterrestris*).

Sensitive strains are useful in research, for example in the understanding of the mechanisms of strain sensitivity to specific chemicals or physical actions. It may be possible for the genes that encode sensitivity to be cloned into resistant organisms. Strains held by the UKNCC include those sensitive to benzimidazole (*Venturia inaequalis*), 4-quinulone antibiotics (*Yersinia ruckeri*), gentamycin (*Pseudomonas aeruginosa*), metronidazole (*Clostridium perfringens*) serum (*Escherichia coli*) and temperature (*Escherichia coli*).

Resistant strains are useful in research aimed at determining the mechanisms of resistance (i.e. to antibiotics or radiation) and for researching new mechanisms to counter the resistance. Antibiotic resistance is an increasing problem and the UKNCC holds accessions to a large number of antibiotic resistant strains including those resistant to: erythromycin (*Micrococcus luteus*), aminoglycosides (*Escherichia coli*), ampicillin (*Escherichia coli* resistant phage) benomyl fungicide (*Penicillium hirsutum*), gentamycin (*Escherichia coli*), methicillin (*Staphylococcus aureus*), penicillin (*Staphylococcus aureus*), streptomycin (*Micrococcus luteus*), tetracycline (*Staphylococcus aureus*). Comparing such organisms with those deposited in the collection prior to use of antibiotics can generate useful information in the combat of resistant diseases. Chemical and physical resistant strains include those resistant to UV (*Deinococcus radiodurans*), formaldehyde (*Pseudomonas putida*) gamma ray (*Rubrobacter radiotolerans*), germicides (*Pseudomonas aeruginosa*), heavy metal ions (*Stenotrophomonas maltophilia, Thermus flavus, Zooglena ramigira*), chlorine (*Flavobacterium* sp.) radiation (*Deinococcus radiodurans*), and phenol mercury 2.5ppm (*Penicillium crustosum*).

5.14 Test strains

There are 376 bacterial, fungal and algal test strains held by the member collections of the UKNCC. There are many general test strains e.g., fungus resistance test (*Paecilomyces variotii*), general inhibitor test, detection of biological residues (*Bacillus subtilis*), sterility test (*Staphylococcus aureus*), Camp

test (*Rhodococcus equii*), mutagenicity test (*Saccharomyces cerevisiae*), Eijkman test-control (*Escherichia coli*), freshwater ecotoxicity test (*Scenedesmus subspicatus*) and Ames test (*Salmonella typhimurium*). Many micro-organisms are used to assess the effectiveness of antimicrobials, e.g., antiseptics (*Mycobacterium hirae*), antibiotic susceptibility (*Pseudomonas aeruginosa*) and disinfectants (*Bacillus cereus, Mycobacterium fortuitum, Staphylococcus aureus*). Other strains assess the effectiveness of materials against microbial attack. For example: emulsion paint (*Enterobacter aerogenes*), cutting fluids (*Shewanella putrefaciens*), fabrics (*Klebsiella pnumoniae* subsp. *pnumoniae*), vinyl (*Streptoverticillium reticulum*) and wall covering adhesive (*Aspergillus niger*). Many strains are standards conforming to national and international specifications, for example: mould proofing (by strains of *Penicillium pinophilum* and *Rhizopus stolonifer*) wood preservatives in BS6009:1982 (*Poria placenta*). CABI Bioscience maintains mould resistance test sets for use in MIL 810D, BS2011 part 2J, DED 133 and ASTM G21 standards.

The National Collection of Type Cultures has a number of reference sets including a food and dairy products set (consisting of 14 strains, e.g., *Enterococcus faecalis* and *Proteus mirabilis*), a water set (consisting of 10 strains isolated from water sources e.g., *Aeromonas hydrophila* and *Escherichia coli*), an enterobacteriaceae set (consisting of 6 strains e.g., *Shigella sonnei*, *Serratia macerens*), a *Listeria* set (consisting of 5 strains, e.g., *Listeria innocua*, *L. ivanovii*), a spoilage set (consisting of 7 spoilage strains, e.g., *Aspergillus niger*, *Clostridium sporogenes*) and an advanced pathogens subset (consisting of 10 pathogenic strains, e.g., *Salminarium typhimurium*, *Vibrio cholerae* Non O:1). NCIMB has a wide range of strains used in sterility testing (*Bacillus stearothermophilus),* disinfectant testing *(Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa*) as well as food spoilage and starter culture organisms used in many national and international test protocols.

5.15 Assay strains

The UKNCC has strains that assay for over 850 substances for example, assay for antibiotics (e.g., chloramphenicol by a strain of *Pseudomonas aeruginosa*; amoxycillin by a strain of *Micrococcus luteus*; erythromycin by a strain of *Escherichia coli*; oxytetracycline by a strain of *Staphylococcus aureus*; penicillin by a strain of *Lactobaci1llus helveticus*; amino acids (e.g., alanine by a strain of *Pediococcus pentosaceus*, methionine by a strain of *Pedicoccus acidilactici*, proline by a strain of *Neurospora crassa* and valine by a strain of *Enterococcus hirae*). Other strains are used to assay for primary metabolites (e.g., cholesterol by a *Rhodococcus* sp., fructose by a strain of *Lactobacillus fructosus*), secondary metabolites (e.g., patulin by a strain of *Escherichia coli*, fusidic acid by a strain of *Corneybacterium xerosis*), vitamins and co-enzymes (e.g., biotin by a strain *of Lactobacillus plantarum* and vitamin B_{12} by a strain of *Lactobacillus leichmanni*).

5.16 Special properties: morphological and physiological

Although not deposited as ex-type strains, some isolates may clearly exhibit a feature representative of a taxonomic group or of particular interest. This could be an anatomical or morphological feature, a physiological feature (also see Section 5.9) or a molecular feature. Anatomical features range from

strand formation in the fungus *Serpula lacrymans* and akinete production in the alga *Anabaena variabilis* to rhapidosomes in the bacterium *Saprospira grandis*. Physiological features include adjuvant effects in the bacterium *Gordona rubropertinctus* and the study of adhesion in *Pseudomonas* sp. to the white mutant of the fungus *Neurospora crassa*. In addition CABI-IMI has 144 fungal mating strains which can be used for teaching or research. Other UKNCC member collections also list strains suitable for teaching purposes.

5.17 Special properties: chemical (bioconversion/biotransformation etc.)

Many strains carry out specific chemical processes such as bioconversion or biotransformation, for example ascorbic acid reduction by a strain of *Escherichia coli*, caproic acid oxidation by a strain of *Bacillus sphaericus*, hydrocarbon oxidation by a strain of *Corynebacterium* sp. and steroid hydroxylation by a strain of *Rhizopus arrhizus* (see Appendix A: Chemical transformation, bioconversion and bioaccumulation).

5.18 Genetic strains

The UKNCC holds a large number of phages, plasmids and genetically modified organisms that have a wide range of properties and applications including mapping strains, suppressor strains, mis-sense suppressors, producer strains, auxotrophs, sensitive mutants, deficient mutants, nuclear division arrest strains, MINI INC -GAL plasmids, plasmids used for molecular weight determinations, transformable strains, transposon donor vectors, fermentation markers, cell division cycle mutants (see Appendix A).

References

Arora, D., Mukerji, K.G.& Martin, E.H. (eds) (1991). *Handbook of Applied Mycology: Foods and Feeds* Volume 3. New York:Marcel Dekker Inc.

Balba, N.T. (1993). Micro-organisms and detoxification of industrial waste. In: *Exploitation of Micro-organisms* (ed Jones D.G.) pp 371-410. London U.K: Chapman and Hall.

Beuchat, L.R. (ed) (1987). Food and beverage mycology. Second edition. New York: Van Nostrand Reinhold Company Inc. pp 661

Rath, A.C., Worledge, D., Koen, T.B. & Rowe, B.A. (1995). Long-term field efficacy of the entomogenous fungus Metarhizium anisopliae against the subterranean scarab, Adoryphorus couloni. *Biocontrol Science and Technology* **5**, 439-451.

Chang, S.T., Miles, P.G. and Buswell, J.A. (eds) (1993). *Genetics and Breeding of Edible Mushrooms*. pp324. Philadelphia, USA: Gordon and Breach Publishers.

Evans, G.M. (1993). The use of micro-organisms in plant breeding. In: *Exploitation of Micro-organisms* (ed Jones D.G.) pp225-248. London UK: Chapman and Hall.

Greenshields, R. (ed) (1989). *Resources and Applications of Biotechnology: The new wave*. New York: Stockton Press.

Hawker, L.E.& Linton, A.H. (eds) (1971). *Micro-organisms: Function, form, and environment.* London: Edward Arnold.

Hawksworth, D.L., Kirk, P.M., Sutton, B.C. & Pegler, D.N. (1995). Ainsworth & Bisby's Dictionary of the Fungi. Eighth edition, pp616. Wallingford: CAB International.

Hornby, D. (1990). *Biological Control of Soil-borne Plant Pahtogens.* Wallingford, UK: CAB International.

Jones, D.G. & Lewis, D.M. (1993). Rhizobium inoculation of crop plants. In: *Exploitation of Micro-organisms* (ed. Jones D.G.) pp 197-224. London UK: Chapman and Hall.

Julien, M.H. (ed) (1992). *Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds*, third edition. Wallingford, UK: CAB International.

Kaaya, G.P. & Munyinyi D.M. (1995). Biocontrol potential of the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae* for Tsetse flies (*Glossina* spp.) at developmental sites. *Journal of Invertebrate Pathology* **66**, 237-241.

McCammon, S.A. & Rath, A.C. (1994). Separation of *Metarhizium anisopliae* strains by temperature dependent germination rates. *Mycological Research* **98**, 1253-1257.

Mitchell D.T. (1993). Mycorrhizal associations. In: *Exploitation of Microorganism.s* (ed Jones D.G.).pp 169-196, London UK: Chapman and Hall.

Risco, B.S.H. (1980). Biological control of the leaf froghopper, Mahanarva posticata Stal, with the fungus Metarhizium anisopliae in the state of Alagoas - Brazil. *Entomology Newsletter, International Society of Sugarcane-Technologists*. **9**.

Onions, A.H.S., Allsopp, D. & Eggins, H.O.W. (eds) (1981). *Smith's Introduction to Industrial Mycology*. Seventh edition, pp398. London: Edward Arnold.

Prior, C. (1992). Discovery and characteristics of fungal pathogens for locust and grasshopper control. In: *Biocontrol of Locusts and Grasshoppers*. (edited by C.J Lomer & C. Prior) pp. 159-180. Wallingford, UK: CAB International.

Steinkraus, K.H. (1983). Handbook of indigenous fermented foods. New York: Marcel Dekker Inc. pp672

Stirling, G.R. (1991). Biological control of plant parasitic nematodes. Wallingford: CAB INTERNATIONAL.

Svensden, A. & Frisvad, J.C. (1994). A chemotaxonomic study of the terverticillate penicillia based on high performance liquid chromatography of secondary metabolites. *Mycological Research* **98**, 1317-1328.

Trinci, A.P.J. (1992). Mycoprotein: a twenty-year overnight success story. *Mycological Research* **96**, 1 - 13.

Turner, W.B. & Aldridge, D.C. (1983). Fungal Metabolites II. London: Academic Press.

Chapter 6

Characterisation and Screening Methods

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6.1 Introduction

There are many methods available for screening anatomical, physiological and biochemical properties. Techniques have been derived from a wide range of different biological disciplines including biochemistry, bacteriology, mycology and ecology. The methods presented mainly provide diagnostic characters, but may be extended to provide general applications such as purification, identification and screening of properties of industrial importance. Many of the methods have been extensively tested at UKNCC collections and are generally applicable to a wide range of micro-organisms. The procedures given can be roughly divided into two groups, those that determine a single activity (e.g., urease activity or citric acid production) and those that can be used to investigate multiple activities (e.g., chromatography to detect a range of metabolites). Many tests are commercially available in easy to use formats (e.g., APIZYM strips) that allow standardisation between laboratories. Advances in molecular biology have resulted in a further increase in the variety of methods available for investigating the uses and properties of microbes. These techniques can also be utilised in taxonomic and evolutionary studies, industrial screening and strain improvement. Every laboratory requires a basic level of equipment, e.g., autoclave, balance, incubators, etc. Specialised procedures (e.g., for electrophoresis and chromatography), however, do require specific and often expensive apparatus and depend upon the precise methodology to be employed.

6.2 Culture based tests

6.2.1 Background

There are many culture-based test methods for the identification of specific properties and characterisation of micro-organisms. Some of the more useful tests are included in this chapter. Simple agar based plate tests can enhance the production of pigments e.g., sugar alcohol media and Czapek-ammonium media. In addition, media can be supplemented with inhibitory compounds to assess growth.

6.2.2 Assessing enzyme activity on solid media

Determination of the ability of cultures to grow on specific substrates in defined and semi-defined media provides a simple way in which a variety of bacterial and fungal enzymatic activities can be tested. For example, the ability of a species of fungus to grow on media containing sucrose and nitrate salts as sole sources of carbon and nitrogen implies invertase and nitrate reductase activities. Complex substrates in culture media, often in conjunction with indicators, can be used to screen for a wide range of enzymatic activities in a similar fashion. Table 6.1 shows several examples of media and the properties for which they may be used to screen, although many others are available (e.g. Skerman, 1969; Cowan, 1974; Klement *et al.*, 1990; Paterson & Bridge, 1994).

Table 6.1 Examples of media used to detect enzymic activity (based on Paterson & Bridge, 1994)

Medium	***Presumptive properties detected:***	***Reference***
Tween 80 medium	*Fatty acid esterase activity*	*Sierra (1957)*
Tributyrin lipase medium	*Lipase activity*	*Lima* et al*. (1991)*
Casein hydrolysis medium	*Protease activity*	*Skerman (1969)*
Gelatin hydrolysis medium	*Protease activity*	*Skerman (1969)*
Cellulose medium	*Cellulase production*	*Eggins & Pugh (1962)*
Ligninolytic medium	*Ligninolytic activity (ligninase)*	*Glenn & Gold (1983)*
Citrus pectin medium	*Pectinase activity (esterase, lyase & polygalacturonase)*	*Cruickshank & Wade (1980)*
Starch agar	*Amylase activity*	*Bridge (1985)*
Aesculin agar	*β-Glucosidase activity*	*Skerman (1969)*
Nucleic acid hydrolysis medium	*Extracellular nuclease activity*	*Gochenaur (1984)*

See Appendix B for ingredients, formulation and methodology.

6.2.3 Short-term tests for assessing mutagens

Several methods have been developed in order to detect the potential mutagenicity of an agent. These tests have involved the use of bacteria, yeasts, and animal cell cultures (WHO, 1985). The first system to be developed - the Ames test (Ames *et al*., 1973) - is still the most well-known. The ability of an agent to cause reversion to prototrophy in certain histidine mutants of *Salmonella typhimurium* is assessed in the test. The most common approach is the addition of a post-mitochondrial fraction of rat liver homogenate (the S9-mix), bacteria and the test agent to a soft agar containing a low concentration of histidine. This is poured onto an agar plate containing minimal media, which is then incubated in the dark at 37^{o}C for 48h (Ames *et al.*, 1975). This method was modified to include a co-incubation step of 30min for the S9-mix, bacteria and the suspected mutagen prior to mixing with the soft agar (Bartsch *et al.*, 1976; Yahagi *et al.*, 1977). Auxotrophic mutant cells utilise the minimal histidine, which results in a limited background of confluent light growth in the upper layer of agar. Isolated colonies of prototrophic revertants whose growth is not limited can also be seen. During scoring, the spontaneous reversion rate of the strains used should be considered (WHO, 1985).

6.2.4 Diffusion tests and susceptibility testing

The standard test for antibiotic sensitivity in bacteria involves the following method. Filter discs are impregnated with extract and allowed to dry (antibiotic-impregnated disks may be obtained from commercial suppliers such as Oxoid products from Unipath Ltd., Basingstoke, UK). These are then placed onto a lawn of the test bacterium and incubated. The diameters of the zones of inhibition or antagonistic/synergistic interaction (if different bioassays are conducted on the same plate) are recorded. This method may be used to assess general microbial resistance to relevant classes of antibiotics and antiseptics. In addition, diffusion tests have been applied to the study of translocation in fungi (e.g. Olsson & Jennings, 1991).

6.2.5 Tolerance, sensitivity and resistance tests

Compounds can be added to nutrient agar to test the tolerance and sensitivity of particular strains. For, example, varying the pH of the media helps to identify and characterise acidophilic and/or alkalophilic organisms. Similarly, growing organisms on nutrient agar that contain high salt concentrations can help screen for halotolerant and halophilic isolates. Variation of temperature, light, aeration and humidity allows assessment of the individual environmental tolerances of strains. Strain resistance to a specific chemical (e.g. heavy metal tolerance) can be tested in the same way.

6.2.6 Biodegradative ability and bioremediation

Growth on a basal medium containing polycaprolactone diol ("plastic" substrate) (Benedict *et al*., 1983; Kelley & Yaghmaie, 1988) can be used to asess the ability of a fungus to degrade plastic materials. This is important in biodeterioration studies and in the development of commercial biodegraders, such as those used in composting. Similarly, other synthetic and organic substrates can be incorporated in growth media to allow an assessment of the biodegredative ability of an isolate. Modifications to this method can allow screening of micro-organisms for their ability to remove heavy metals and/or radionuclides from solution - a process known as "biosorption" (Gadd, 1994). The scope for use of biosorption in microbial remediation of contaminated effluents and soil has been demonstrated using fungi and bacteria (McEldowney,1990; Gadd, 1993; White *et al*., 1996).

6.2.7 Interaction tests

Growing a strain on a Petri dish in the presence of another organism has been widely used in microbiology to study the nature of the interaction between them. This can allow assessment of the antagonistic or synergistic activity of particular strains. Antagonistic properties may be due to chemical or vegetative incompatability. Strains exhibiting antagonistic activity or parasitism may have potential for development as biological control agents. In mycology, vegetative compatibility group testing of isolates grown on adapted media (i.e. with nitrate) has been used to characterise many fungi including *Fusarium* spp. and *Colletotrichum* spp. (Puhalla, 1985; Leslie, 1987; Brooker *et al*., 1991). Likewise, interaction tests are useful for genetic and molecular investigations such as mating type experiments (Casselton & Olesnicky, 1998) and parasexuality studies (Hastie, 1981).

One other significant form of microbial interaction is the "killer yeast" phenomenon (mycocins). Mycocins are toxins produced by many yeasts that are lethal to other yeasts. All yeast mycocins appear to be proteinaceous and are cytoplasmically-determined. "Killer" activity is expressed predominantly at pH 3-6. Expression of, and sensitivity to, mycocins have been used in yeast systematics (Golubev, 1998).

6.2.8 Pathogenicity testing

In vitro pathogenicity tests are used in preliminary studies for the development of biological control agents. The target organisms are inoculated onto test media with the potential control strain (or

metabolite/protein extract from the control strain) and the extent of pathogenicity and mortality are recorded. Data obtained from such experiments may aid the assessment of the fitness of potential biological control strains before release into the target environment. Additionally, the data are useful in the assessment of the impact of potential biological control strains against indigenous organisms from the target environment. Pathogenicity tests can also be used to monitor the cultural and physiological stability of an organsim and to assess whether an isolate is likely to synthesise enzymes or metabolites that may be of wider importance, during pathogenesis.

6.2.9 Carbon utilisation studies

A very important tool in bacterial identification is the ability of organisms to utilise a wide range of organic molecules as the sole source of carbon and energy, such as carbohydrates, amino acids, carboxylic acids, aromatic compounds and others. Indeed, this has been exploited commercially by the makers of several widely-available kits, including the API system (Biomerieux, S.A. France) and Biolog plates (Biolog Inc, USA). In some genera of bacteria (e.g., *Methylobacterium*) carbon utilisation spectra are the only way of distinguishing between species phenotypically.

There are a variety of commercially- available kits for testing carbon source utilisation (e.g., Biolog™ plates and API™ tests). Biomerieux produce a range of specific bacterial and yeast kits with specific carbon sources, e.g., for the *Enterobacteriaceae*, and the genera *Bacillus*, *Campylobacter, Candida, Listeria*, *Staphylococcus* and *Streptococcus*. Tests involve either a simple colour reaction that indicates carbon source utlisation (caused by the reaction of a dye with free naphthyl compounds), or simply opacity of the growth medium caused by growth of the organism.

Biolog tests (Biolog INC USA) consist of a series of 96-well microtitre plates containing 95 available substrates each designed for specific groups of bacteria (i.e. Biolog GN for gram negative and Biolog GP for gram positive bacteria). Incubation of the plates with a live suspension allows the characterisation and assessment of bacteria depending on the substrates that are and that are not utilised. Assessment of a positive reaction is facilitated by the presence of a tetrazolium dye that gives a purple coloration. Wildman (1994) extended the use of this technique to fungi, where each well of a Biolog GN plate is inoculated with fungus, after an incubation period, utilisation is assessed by observation of growth in each well. Subsequently, Biolog now market a test plate for filamentous fungi (without the tetrazolium dye incorporated in the bacterial plates).

6.2.10 Nitrogen-source screening

Yeasts are known to be able to utilise a wide range of nitrogen sources. Growth on particular nitrogen sources has been used to help identify yeasts at the spcific and sub-specific levels. For example, the genus *Pichia* is distinguished from its former genus *Hansenula* by its inability to grow utilising nitrate as its sole nitrogen source (Yarrow, 1998). The use of nitrogen sources is also important in the discovery of fungal mutants for vegetative compatibility screening (see section 6.3.6).

6.2.11 Fermentation studies

One of the most commonly-used classes of test for the identification of yeasts is the fermentation of carbohydrates as measured by the production of CO_2 in liquid culture. The basic method employs Durham tubes containing 2% solutions of the appropriate sugar (van der Walt, 1970). The yeast is then inoculated into the broth and fermentation is visualised by the presence of a bubble (of CO_2) in the small inverted test tube. Genera such as *Rhodosporidium* and *Sterigmatomyces* cannot ferment glucose, whilst others such as *Kluyveromyces*, *Saccharomyces* and *Zygosaccharomyces* are able to do so (Yarrow, 1998).

6.3 Enzymatic activity of extracts and broths

6.3.1 Background

The enzymatic activities of microbial cultures may be screened directly by testing for a range of intra- and extracellular enzymes. Such methods were used initially in studies of bacteria and yeasts (e.g. Cowan, 1974; Barnett *et al.,* 1983) but were later adapted for analysis of fungi where extracellular enzymes have been screened in conidial suspensions and spent culture fluid (Bridge & Hawksworth, 1985; Mugnai *et al.,* 1989). The methods can also be used with diluted intracellular extracts. Intracellular enzymes are retained inside the cells and are detected following the breaking open of the cells and testing the crude or purified extracts.

6.3.2 Chromogenic and fluorogenic substrates

Specific enzyme activity can be demonstrated using a wide variety of chromophore substituted compounds that are available. Perhaps the most widely used, are the large range of o- and p-nitrophenyl substituted compounds, where cleavage of the substrate liberates free nitrophenol which is a yellow/brown colour. For example, *o*-nitrophenyl β-D-galactopyranoside has been used as a substrate for β-galactosidase (Cowan, 1974). The enzyme activity cleaves the galactopyranoside leaving free nitrophenol, which is yellow. Fluorogenic substrates such as the 4-methylumbelliferyl (4-MU) substituted compounds also have been used on a wide range of micro-organisms (e.g. Grange & Clark, 1977; Bradbury, 1977; Slifkin & Gil, 1983; O'Brien & Colwell, 1987; Claeyssens, 1989; Barth & Bridge, 1989). The basic principle is the same as for the chromogenic substrates except that the free methylumbelliferone is fluorescent, appearing blue/white under long wave ultra violet light. There are a large number of commercially-available 4-MU substrates that allow screening of a diverse range of enzymes (e.g., β-glucosidase, trypsin, and arabinofuranosidase; Sigma, Poole, UK). Methylumbelliferyl-substituted compounds have been used, also, in environmental investigations, for example to measure microbial activity in soil (Miller *et al.,* 1998). One of the main advantages of using chromophores and/or fluorophores in analyses, is that they may provide quantitative data.

There are several test kits available commercially for testing enzymatic activity. One of the best-known is the APIZYM system (Biomeriuex, S.A. France), which has been used in a number of studies with bacteria (e.g. Ling et al., 1994), cell lines (e.g. Nardon *et al.,* 1976), and filamentous fungi (Bridge &

Hawksworth, 1984; St Leger *et al.,* 1986), and is used to characterise and test for bacteria from crude extracts (e.g. Kilian, 1978). A simple colour reaction indicates enzyme activity due to the reaction of a dye with free naphthyl compounds.

6.3.3 Production of organic acids

The production of organic acids by fungi has been studied for many years. Extracellular citric acid production by fungi such as *Aspergillus* spp. can be screened in 10-14 day old liquid cultures with Altman reagent (Gaffney *et al.,* 1954; Smith, 1969). Presumptive positive culture fluids should be confirmed by thin layer chromatography in butanol :water [120:30:50 (v/v/v)]. Altman reagent will give a pink colour with citric acid, but may also react with ketoglutarate, oxaloacetate and glutaconic acid. Additional investigations have elicited methodology for screening for malic acid production by bacteria and fungi (Chibata *et al.*, 1983; Campbell *et al*., 1987); and oxalic acid production by fungi (Takao, 1965).

6.4 Analysis of proteins

6.4.1 Protein electrophoresis

Electrophoresis can be used to characterise intracellular proteins from a wide range of organisms. The technique involves the separation of proteins down a supporting gel (historically starch but now predominantly polyacrylamide). After separation, the proteins can be identified with specific stains. Some enzymes are present in more than one form (isoenzymes or isozymes) and profiles of these can be used to differentiate genera, species and populations of microbes.

6.4.2 Cell disruption

In order to study microbial enzyme activities great care must be taken to optimise growth conditions to suit the enzyme system of interest. Microbial cells must be disrupted to allow extraction of the cytosol. The degree of breakage required - and the subsequent methodology - is dependent upon the nature of the organism being studied plus the properties of the protein involved. The physical methods used to break cells include sonification (of bacteria, yeasts, unicellular algae), manual disruption (of lyophilised or frozen fungal mycelia and algae with carborundum powder using a pestle and mortar) and band/bead beating (e.g., with a Mickle cell disintegrator) but chemical and enzymatic means of lysis are also employed (Doonan, 1996). Crude protein extracts are obtained by centrifugation of the biomass following its homogenisation with sterile buffer. Total protein concentrations may be obtained (e.g. Lowry *et al.*, 1951) and may be used for standardisation of sample loading. Further purification is not necessary prior to electrophoresis but protease inhibitors may need to be added to the samples to prevent degeneration. Most extracts can be stored, successfully, at -20°C for at least 6 months although there are cases where the enzymes must be analysed immediately after extraction. Repeated freezing and thawing will result in a loss of activity and is not recommended.

The methodology has been extended to the study of extracellular enzymes that may be produced in sufficient quantities to be detected by gel electrophoresis. Isolates are incubated in culture fluids that contain relevant substrates to induce production of particular enzymes (e.g., wheat grains may be added to media to induce amylase production or pectin broth used for pectinase studies - see Paterson & Bridge, 1994).

6.4.3 Electrophoresis methods

Electrophoresis on polyacrylamide gels with a discontinuous buffer system is used, normally, with varying conditions, dependant upon the type of enzyme or protein system being examined. There are two main classes of protein electrophoresis:

1. Denaturing gels most commonly incorporate the detergent sodium dodecyl sulphate (SDS) and a thiol reagent. In general, polypeptides bind SDS in a constant weight ratio allowing separation on the polyacrylamide gel on the basis of polypeptide size. Employing a set of standard polypeptide markers of known size allows calculation of the sample polypeptides to be made (Hames, 1981).
2. The second major class of protein electrophoresis is native gel electrophoresis. This form of electrophoresis does not denature the proteins and separation is due, therefore, to both the size and charge of the polypeptide at a given pH.

There are a wide variety of enzyme systems that are of interest for a number of different reasons. For example, fungal dehydrogenase enzyme systems appear to be generally conserved and are therefore useful for studying relationships between species or species complexes. Other general cytoplasmic enzymes may be extremely variable and may have use in discriminating species and populations. Enzyme systems studied include esterases, acid and alkaline phosphatases and catalases (Woodbury *et al.,* 1971; Shaw & Prasad, 1970; Gabriel & Gersten, 1992). These have been found to be useful for characterisation studies on a range of filamentous fungi (May & Royse, 1982; Micales *et al*., 1986; Bonde *et al*., 1991), yeasts (Lambert & Garcia, 1990) and bacteria (Gardes *et al.*, 1987; Kubicek *et al.*, 1989). Specific enzymes can be purified and their characters and structures determined using a variety of techniques including diode array high performance liquid chromatography, spectrophotometry techniques, mass spectrometry and crystallography (see Section 6.7).

6.5 Immunological methods

Currently, the most commonly used immunological technique is the enzyme-linked immunosorbent assay (ELISA). Many modifications have been made to the method but the most straightforward approach is the addition of a specific antigen to a microtitre plate. The antigen (usually protein) adheres to the wall of the microtitre plate. Unbound antigen is washed off before the bound antigen is dried. Independently, an antibody-enzyme conjugate is prepared and is diluted in buffer containing inert proteins (e.g. 5% skimmed milk powder in phosphate-buffered saline). The diluted conjugate is added to the antigen in the wells of the microtitre plate and the wells are washed out to remove unbound components. On addition of a particular substrate that changes colour (or fluoresces, for enzyme-linked fluorescent assay) following enzyme catlysis, the plate is incubated. A quenching reagent is added to

stop the reaction and the plates are analysed by spectrophotmeter. Positive reactions are indicated by the presence of the specific colour (or fluorescence, for ELFA). This system is termed direct ELISA but modifications such as indirect, sandwich and competition ELISA have proved successful, also (Hampton *et al*.,.1990). ELISA has been applied to many situations such as confirmation of clinical disease, phytopathogenic disease, analysis of immune responses, antigenic comparison and production of monoclonal antibodies (Crowther, 1998)

Additional immunological techniques that have been useful in screening micro-organisms include the use of single and double diffusion plates, microagglutination, latex agglutination and Western Blotting (Hampton et al., 1990).

6.6 DNA molecular methods

6.6.1 Background

There are many methods available for the analysis and characterisation of nucleic acids. The techniques described have diverse applications including gene cloning, screening of gene expression, species definition, study of interactions, phylogeny, study of degradation processes, detection of metabolite producers, studies of biodiversity and identification of species in crude samples. General molecular procedures such as restriction endonuclease digestion, cloning, hybridisation and sequencing are applicable to microbial DNA. Common techniques that utilise the Polymerase Chain Reaction (PCR) include RFLPs (Restriction Fragment Length Polymorphism), RAPDs (Random Amplified Polymorphic DNA), AFLP (Arbitrary Fragment Length Polymorphism), SSCP (Simple Strand Conformation Polymorphism). Fingerprinting techniques have allowed scientists to separate taxa that are difficult to distinguish using anatomical or morphological criteria. Non-PCR techniques such as electrophoretic karyotyping of chromosome size and number may also be applied. Other techniques that amplify nucleic acids and not considered in this chapter include the Ligase Chain Reaction (LGR), Nucleic Acid Sequence-Based Amplification (NASBA), Q- beta replicase (Qβ) amplification and Strand Displacement Amplification (SDA) (Edel, 1998).

6.6.2 DNA extraction methods

Many techniques require purified DNA, which must be extracted from cells with minimal mechanical disruption to the DNA. Most common methods use a cell lysis solution followed by purification and storage in an appropriate buffer. Commercially available and simple-to-use kits are available to extract human, animal and bacterial DNA. However, nucleic acids are more difficult to obtain from plant cells, protists and fungi, primarily because of the nature of their cell walls. Cetyltrimethyl ammonium bromide (CTAB) is often used for extracting total genomic DNA from plants and filamentous fungi (Zolan & Pukilla 1986). Other methods for extracting DNA from fungi have been suggested by Raeder & Broda (1985) and Taylor & Natvig (1987). The Raeder & Broda (1985) DNA extraction method is undertaken, routinely, at CABI Bioscience and has proved successful for many fungal genera.

However, the CTAB method (Zolan & Pukilla 1986) has been employed often with fungi that produce excess extracellular polysaccharides.

6.6.3 RFLP analysis

Restriction fragment length polymorphisms (RFLP) have been used widely in microbial systematics at different taxonomic levels (Bruns *et al.,* 1991; Cooley 1992; Hibbett, 1992). Probes have been derived for use with chloroplast, mitochondrial and chromosomal DNA. There are many reported maps and sequences from fungal mitochondrial DNA. Mitochondrial genomes have two advantages over chromosomes which make them attractive to study: 1) they are generally inherited uniparentally (Fort *et al*., 1984); and 2) they are present in fungi, at least, in multiple copies (Bruns *et al*., 1991). Single-parent inheritance is important for genetic analysis as it means that mitochondrial-derived recombination products do not occur. Fungal mitochondria possess one additional advantage for study - they are particularly rich in the bases adenine (A) and thymine (T) (Hudspeth *et al*., 1980; Bruns *et al*., 1991) with guanine and cytosine levels (G+C) of 24-39 Mol % (Marriott *et al*., 1984). The result of this base imbalance is that, when total DNA extracts are digested, the chromosome is digested into many small fragments (usually below 2kb in size) whereas the mitochondrial DNA is digested less frequently. When these digests are separated on agarose gel electrophoresis, clear mitochondrial bands can be seen, which are distinctly separate from the smear of smaller chromosomal fragments. This technique allows some mitochondrial RFLPs to be visualised quickly, without the need for labelling, hybridisation, etc., and can also be used to produce well-separated mitochondrial fragments for the subsequent production of probes. Particularly useful enzymes for this procedure are *Hae*III and *Msp*I.

Currently, the most common use of the RFLP technique is in digestion of PCR generated amplification products such as the (chromosome-located) ribosomal DNA gene cluster. In particular, RFLPs of the internal transcribed spacer (ITS) regions are used for species delimitation.

6.6.4 The Polymerase Chain Reaction (PCR)

The most significant advance in molecular biology in the last 15 years or so has been the development of the Polymerase Chain Reaction (PCR), first described by Saiki *et al.* (1985) and Mullis & Faloona (1987). The idea of a chain reaction amplification of DNA had been described in the early 1970's by Kleppe and co-workers (1971) but it was not made practicable until the crucial development of the isolation of a thermostable DNA polymerase *Taq* (derived from the thermophilic bacterium *Thermus aquaticus*). This allowed repeated extension of the DNA molecule to progress without the addition of fresh enzyme at the end of each cycle - i.e. continuous cycling without the DNA polymerase being heat-inactivated by the denaturation step. The PCR technique allows the amplification of tiny amounts of DNA (in theory from one molecule of DNA) by the following steps:

1. Denaturing of the DNA by heating to c. 95 °C;
2. Annealing of the primers to complementary target DNA sequences;
3. Extension of the new copies of the DNA;
4. Repeated cycles of steps 1-3.

Certain factors must be borne in mind at each step. The whole reaction must be carried out in small (0.5 ml) microcentrifuge tubes or other specialised vials, with thin walls for easy heat transfer, within a thermal cycler, an extremely sensitive heated (and cooled) block which is capable of raising (and lowering) temperatures in seconds. There must also be a carefully defined reaction mixture in the tube as the amplification is a closed-system process (Edel, 1998). The reaction mixture should contain target DNA to be amplified [c. 10-100 ng, (Edel, 1998)]; an excess of free deoxyribonucleotides (i.e. dATP, dCTP, dGTP and dTTP); short specific sections of DNA (the primers) in order to anneal to specific sections of the target DNA; and the thermostable DNA polymerase. The DNA polymerase will not allow *de novo* DNA synthesis. Hence, the primer DNA is required to give the DNA polymerase an available site on which to hang fresh nucleotides. The major benefit of the PCR technique - the ability to amplify minute amounts of target DNA - is also, in some ways, its biggest drawback as any contaminating DNA also stands a very good chance of being replicated. Indeed, in some cases the contaminant DNA may be replicated preferentially to the target DNA. Alternatively, a contaminant may compete with the target DNA to the extent that it nullifies the reaction (in terms of it being visualised by ethidium bromide staining) or both types of DNA may get amplified together with the result that the investigator does not know whether the DNA profile obtained is reliable or not. Thus, scrupulous attention to good laboratory practice and the use of negative controls (i.e. reaction mixtures containing no DNA) in order to check integrity of the system, are an essential part of PCR work.

6.6.5 PCR fingerprinting techniques

There are a number of fingerprinting techniques that can be used to characterise genetic variations within micro-organisms. RAPDs (Random Amplified Polymorphic DNA) have been widely used. RAPDs do not require specific nucleotide sequence details for primer design (Edel, 1998), DNA fragments of undefined length can be amplified (Edel, 1998), only nanogram quantities are required (Bridge & Arora, 1998) and samples can be processed quickly (Bridge *et al.,* 1997). However, the number and nature of bands produced are often difficult to interpret (Bridge *et al.,* 1997) and results may not be reproducible because of low stringency of the primers, which emanate from the low PCR annealing temperature. A technique has been developed that employs the advantages of the RAPD method but with significantly improved reproducibility. This modified method has been referred to, variously, as variable number tandem repeat (VNTR) PCR, arbitrarily primed (AP) PCR and simple sequence repeat (SSR) PCR. Primers that had previously been used as fingerprinting probes were adapted for this RAPD-like PCR. Simple sequence repeats, which are complementary to the flanking regions or the core sequence of variable number tandem repeats of the microsatellite DNA (Welsh & McClelland, 1990) were used. Banding patterns were produced that were simple and easier to interpret than RAPD patterns, that could distinguish between strains of the same species and were more reproducible than RAPD fingerprints (Bridge *et al.,* 1997). Other primers that are commonly used to fingerprint micro-organisms include primers that correspond to enterobacterial repetitive intergenic consensus (ERIC) sequences and repetitive extragenic palindromic (REP) elements (Edel, 1998).

Amplified Fragment Length Polymorphism-PCR (Vos *et al.,* 1995) is a highly reproducible PCR fingerprinting method. Genomic DNA is digested with restriction endonuclease to produce restriction fragments which are ligated to oligonucleotide "adapters". A PCR reaction is undertaken with primers that include the adapter sequence and between one and five randomly chosen additional nucleotides. This allows selective amplification of the restriction fragments in which the nucleotides flanking the restriction site match the additional nucleotides of the primer (Edel, 1998).

6.6.6 Sequencing / SCAR analysis

Sequencing is a time-consuming, but powerful technique that allows scientists to read the nucleic acid make-up of individual genes. Sequence information is essential for gene cloning and genetic manipulations. Many laboratories such as the molecular laboratories at CABI Bioscience and NCIMB have facilities for sequencing, but there are many commercial companies who operate external sequencing services for individuals who haven't got their own facilities. Sequencing can be carried out from known or random amplified samples, this technique is known as SCAR (Sequence Characterised Amplified Region) and can be used to detect sequence variation that is useful for separating isolates at the infraspecific level. In addition, SCAR analysis allows the detection of sequence variation among genes from different organisms. Once sequence data have been obtained, sequences can be compared for similarity with the vast libraries of known sequences that are available over the internet (e.g. at the EMBL or GENBANK databases). Sequencing has been used in systematics to define groupings at the most basic level through the sequences of specific gene regions such as the subunits of the ribosomal gene clusters, allowing proper phylogenetic analyses of taxa to be undertaken.

6.6.7 DGGE / SSCP analysis

Denaturing Gradient Gel Electrophoresis (DGGE; Fischer & Lerman, 1983) and Single Strand Conformation Polymorphism (SSCP; Orita *et al*., 1989) can be used as alternatives to sequencing. These techniques are useful particularly for ecological studies of mixed populations - e.g. from soil, activated sludge, or other environmental samples - and have been used with bacteria, predominantly. Both are powerful techniques that can detect sequence variations in amplified products. DGGE is especially useful for detecting single base substitutions, it differs from SSCP because samples are not denatured prior to electrophoresis, but are run in a gradient gel under denaturing conditions. SSCP can be used to separate taxa and identify micro-organisms in crude samples but is particularly useful for mutation analysis in DNA fragments of 100-300 bases (Sambrook & Russell, 2001). Amplified PCR products are denatured and samples run in a non-denaturing polyacrylamide gel. More complex banding patterns can be obtained if restriction enzymes are used during the procedure.

6.6.8 Electrophoretic karyotyping

Large pieces of DNA, such as whole chromosomes, can be separated by methods such as Pulsed Field Gel Electrophoresis (PFGE) (Brody & Carbon, 1989). This technique allows the separation of complete chromosomes or large chromosomal elements. In PFGE, electrodes are arranged around the gel, the current is switched between different opposing pairs, this causes large pieces (Mb size) to move

through the gel, the procedure being analogous to the shaking of a sieve. Variations in the placing of the electrodes (and, subsequently, the nature of the electric field) have led to refinements such as contour-clamped homogeneous electric field (CHEF), orthogonal field-alternating gel electrophoresis (OFAGE) and rotating field gel elelectrophoresis (ROFE) (Burmeister & Ulanovsky, 1992). The "pulse" time (or switching time) and the running time both increase with increasing fragment size. Most separations of chromosomes from filamentous fungi have involved pulse times of 100-3000 seconds and electrophoresis runs of between 2 and 7 days (bacterial runs are normally about 1 day). It is of great importance to the reproducibility of results that the DNA sample is not physically disrupted and the chromosomes remain intact. To achieve this, protoplasts are prepared from fungi and lysed *in situ* in agarose blocks. The blocks are then set into the electrophoresis gel (Sambrook et al., 1989).

6.6.9 DNA-DNA homology

When describing or differentiating new species or comparing relatedness of strains within taxa at the sub-generic level, DNA-DNA homology is a useful and at times an essential tool. This involves assessing the degree of homology between complementary strands of DNA taken from different organisms. Strains which are very similar, i.e. belong to a homogeneous species, give a high (>80%) homology value. Thermal denaturation, buoyant density and spectrophotometric methods are all used in this assessment method. DNA homology has been studied by the use of gene probes. Genomic DNA is separated by electrophoresis on an agarose gel, subsequently the DNA is transferred to a nylon membrane by capillary action in the presence of a high salt concentration (a process known as Southern Blotting). The DNA is baked onto the membrane by heating for 2h at 80°C before hybridisation. A previously-prepared DNA probe is hybridised to the membrane-bound DNA in a hybridization solution. Non-bound probe DNA is washed off and the membrane is subjected to a detection procedure. The method was originally developed with radio-labelled probes that required the use of X-ray film. This has been superseded by improvements in non-radioactive labelling with compounds such as digoxigenin (Roche Diagnostics Ltd.) that can be detected by colorimetric, or chemiluminescent methods.

6.7 Chromatographic methods

6.7.1 Background

Chromatography is a method for separating metabolites and can also be used for the separation of other biomolecules and chemicals and in the characterisation of individual molecules. Techniques commonly used include thin layer (both normal and reversed phase), preparative layer (normal), liquid (normal phase), gas and high performance liquid chromatography.

6.7.2 Thin Layer Chromatography (TLC) of metabolites

This procedure requires stationary phase cells to be layered over a support plate made of glass, aluminium or plastic. Samples are suspended in a suitable solvent and loaded onto the plate, when this is dry it is placed into a sealed glass TLC tank containing a solvent. This solvent then ascends the TLC

plate by capillary action. The choice of solvent depends upon the nature of the chemical being targeted (Stahl, 1969). Thin layer chromatography is a very flexible method, and this flexibility can be increased by the technique of two-dimensional TLC. Applications of 2D TLC include confirming the identity of a metabolite (i.e. if the metabolite runs to the same position as a standard in both solvents then greater confidence in its identity is possible) and determining the purity of individual spots. The utility of this method can be demonstrated through its use in the assessment of conspecificity of fungus strains that produce pigments. *Hobsonia christiansenii* and *Illosporium corallinum* are lichenicolous species that share certain morphological similarities, one of these being the production of a pink/red pigment (Lowen *et al.,* 1986). This method can be used to see if the pigments they are producing are the same, and can be used for other fungi for which a 5 x 5cm TLC plate is used, routinely. If spots on a TLC plate are not well separated after the initial development, it is possible to redevelop the TLC plate using the same solvent, this is referred to as multidevelopment TLC. Cell wall sugars and amino acids are an important diagnostic tool for coryneform bacteria, TLC can be used to differentiate such molecules and patterns can be used to identify the bacteria. For all methods of TLC a variety of post sprays can be used to enhance the detection of specific metabolites (Stahl, 1969). For example, *p*-anisaldehyde can be used to detect sugars.

Agar plug technique

The agar plug technique (standard method) is most frequently used for secondary metabolite production of filamentous fungi and was initially developed for terverticillate penicillia (Filtenborg *et al.*, 1983; Paterson, 1986). This method is suitable for most filamentous fungi including *Chaetomium*, *Fusarium*, *Metarhizium* and *Verticillium* (Paterson & Bridge, 1994). Growth media, incubation temperatures and growth periods may be varied to optimise metabolite production but must be kept constant thereafter for comparison purposes. Extracellular metabolites can be detected by direct application of the agar side of a mycelial plug onto the TLC plate, intracellular metabolites by direct application of the colony to the TLC plate with appropriate solvent (e.g. chloroform/methanol).

Extraction from complete cultures or solid material

Solid food, feed commodities (e.g., grains, peanuts) and agar cultures of fungi, bacteria and yeasts can be screened for metabolites using this method. Metabolites are extracted using a suitable solvent (e.g., methanol), however, different solvents can be used to optimise the extraction of specific metabolites. Chemical extraction may require complex procedures but such extracts may be inoculated directly onto the TLC plates.

Analysis of liquid samples and broths

Depending on the liquid to be screened a defatting procedure may or may not be required. For substances such as milk, which contain lipid, an n-hexane extraction stage will be required. When carrying out this analysis it is important to analyse a presumptive uncontaminated control and have an indication of which microbe is suspected to have contaminated the product to support decisions about the identity of the spots on the TLC plates (Paterson & Bridge, 1994).

Multi-mycotoxin screen

Biological material suspected of being contaminated by mycotoxins or other secondary metabolites can be analysed using this procedure. A control sample of non-contaminated material should also be extracted in case non-contaminated material contains metabolites that have similar TLC characteristics to the fungal products (Paterson & Bridge, 1994).

Extraction from dried herbarium cultures

Data on secondary metabolites can be obtained from dried herbarium cultures (Paterson & Hawksworth, 1985). It is possible to obtain metabolites from cultures dried almost half a century previously. Such metabolites may be used as taxonomic characters, or for screening for particular compounds.

Metabolite identification

Colours and Rf values (of metabolite spots on TLC plates) can be used in an attempt to determine the identity of metabolites by comparing these to available information (e.g., from databases, literature or other sources - see Paterson & Bridge, 1994). If Rf values and colours are not available, pure samples of particular fungal metabolites can be obtained from chemical supply companies or from individual laboratories where metabolite research is undertaken (e.g., CABI Bioscience for fungal metabolites). If specific chemical characteristics are known the chemical of interest can be extracted from the producers, although this can be time consuming and experimentally demanding. Once characterised, metabolites can be extracted and purified.

Ubiquinones

Ubiquinones are a class of terpenoid lipids, consisting of a benzoquinone ring with a non-polar isoprenoid chain. In the electron transport chain they function as highly mobile electron carriers between the flavoproteins and the cytochromes. Ubiquinones have been used in microbial chemotaxonomy due to the inherent structural variation observed between some taxa (i.e. the length of the isoprenoid chain and its degree of saturation).

Partially saturated and unsaturated structures are found in fungi, whereas only unsaturated quinones have been observed in bacteria. Their use as taxonomic characters in yeasts, fungi and bacteria has attracted considerable interest (e.g. Yamada *et al.*, 1973; Collins, 1985; Kuraishi *et al.*, 1985; Sugiyama *et al.*, 1988). Analysis of ubiquinones has suffered in the past, however, due to the time-consuming processes involved, the specification (and expense) of equipment such as HPLC, and the use of rigorous extraction procedures, particularly saponification. A "direct" method avoids these problems as it does not require expensive equipment, it is experimentally undemanding and it gives highly reproducible results using a mild extraction procedure. This method was orginally developed for characterisation of *Legionella* spp. (Mitchell & Fallon, 1990) but was modified, subsequently, for fungal analyses (Paterson & Buddie, 1991). In the modified method all spots that appear on TLC plates

are considered to be characters, whether ubiquinones or non-ubiquinones (i.e. predominantly phospholipids).

6.7.3 Fatty acid composition by Gas Chromatography (GC)

All cellular life forms possess lipid-rich cytoplasmic membranes. The membrane lipids may be used as markers for the identification and classification of microbes owing to the diversity of their precise chemical structure. Amongst the most important microbial lipid components are amphipathic lipids (e.g. phosphoglycerides and glycolipids) that possess both polar and non-polar regions. The non-polar tails consist, generally, of long chain fatty acid esters (Hamilton & Hamilton, 1992).

Fatty acid analysis may be considered in the following steps: 1. Cells are grown under standard culture conditions; 2. Saponification of the cells liberates fatty acids from the cell surface; 3. Fatty acids are methylated for greater volatility; 4. Analysis of the fatty acids is by gas chromatography; and 5. Comparisons are made between the test fatty acid profile obtained in 1-4 and a database of profiles of known micro-organisms.

When grown and harvested under such defined conditions, many species produce their own characteristic profile. This can be exploited as a taxonomic tool (Tornabene, 1985) and commercial databases such as the MIDI are available to identify unknown isolates, CABI, NCIMB and NCPPB operate identification services based on this equipment. Whilst the technique has been applied to bacteria, predominantly, refinements have been made to allow its use with yeasts and fungi (Marumo & Aoki, 1990; Muller *et al*. 1994).

6.7.4 High Performance Liquid Chromatography (HPLC)

Extractions can be made from whole plates, culture broth or undefined sources as previously described for the TLC method. HPLC is an automated system for analysing complex mixtures that produces rapid reproducible results. Small columns with high-resolution power are used which increase the speed of elution. This is possible because the particle size of the stationary phase is very small. The small columns are eluted with a mobile phase that is pumped through in a highly controlled manner. Microlitre volumes are injected onto the column through an injection port. After the solvent has passed through the column detector systems, the solutes are monitored by chemical or physical properties of the solutes (e.g., absorbance, fluorescence, etc.). **Isocratic HPLC:** The term isocratic refers to the fact that the effluent composition remains constant throughout the separation procedure. **Gradient HPLC:** This refers to the controlled alteration of the effluent to allow improved separation, particularly of complex mixtures of solutes (Paterson & Bridge, 1994).

HPLC systems can be standardised using alkylphenone standards (Frisvad & Thrane, 1987; Paterson & Kemmelmeier, 1989). Alkylphenone standards are a homologous series of compounds, are stable, they have a wide range of polarities and are readily detectable. Retention indices from alkylphenone standards are independent of column efficiency, variations of column efficiency, column flow rate, and

temperature. Retention indices may be used between laboratories as a reliable guide to the tentative identification of mycotoxins as the use of alkylphenones reduces variability from chromatographic conditions. There are a number of ways to identify unknown peaks, either by comparing with metabolite standards (e.g., from a collection of fungal metabolites, like that held at CABI Bioscience) or by using UV/Vis spectroscopy.

6.7.5 UV/VIS Spectroscopy

Chromatographic methods are used to separate secondary metabolites, whereas UV spectroscopy is used to identify them. Spectra can be obtained from metabolites removed from thin or preparative layer chromatography plates. Fractions from preparative HPLC compounds and proteins purified from column chromatography can also be used.

The spectra obtained from spectroscopy are associated with transitions between electronic energy levels. Because the promotion of electrons from the ground state to the excited state of such systems gives rise to absorption, they are used to detect conjugated systems. Recently developed technology, such as Diode Array Detection (DAD) with HPLC, (HPLC/DAD), and the ability to take UV spectra spots on TLC plates for the identification of compounds develops the link between UV spectroscopy and chromatography (Frisvad & Thrane, 1987). Comparing spectra of metabolites in neutral solvents to those in strongly alkaline solvents and subtracting the neutral from the alkaline spectrum generates a difference spectrum. This serves to increase the utility of UV spectroscopy for certain classes of compound. Also, the UV spectra of certain metabolites with similar UV spectra in neutral solvents can sometimes be differentiated by the UV spectra in alkaline solvents and/or the difference spectra (Paterson & Bridge, 1994).

Difference curves can be used in three ways: 1) to give quantitative and qualitative information on the compounds in mixtures (but are characteristic only of the ionizable elements of the compounds); 2) to aid the differentiation of metabolites with similar chromophores by using the data and method of UV/Vis spectroscopy; 3) to permit the study of separate chromophores in complex mixtures without the need for physical separation. Difference curves have advantages over absorption curves for the analyses of ionizable chromophores when the absorbing units are present either as parts of the molecule or in mixtures of various molecules and may be useful for the rapid determination of distinctive compounds in crude extracts of fungi.

6.7.6 Mass Spectrometry

Mass spectrometry can be used to determine the chemical constituents of metabolites and compounds. Such information can be vital for determining the molecular structure of chemicals. The technique of Pyrolysis Mass Spectrometry involves samples being completely pyrolysed. The identities of the broken-down components are then determined. This technique has been used extensively at the Universities of Kent and Newcastle to separate actinomycetes and related genera.

6.7.7 Assessing biological activity

A primary screen is useful when the rapid screening of many fungal strains is required. Volumes and quantities of biomass needed for this method vary with the number of positive results required. If more positives are required then larger quantities of biomass can be used, and/or the final extract can be made more concentrated. After the primary screen it is often desirable to isolate and characterise the active components in the most active extracts discovered. Thin layer chromatography offers an excellent means of doing this and can be carried out at a useful laboratory scale. This can be done using thin and preparative layer chromatography. A further modification is the use of agar overlays (subsequently inoculated with bacteria or fungi) in order to determine zones of inhibition/lysis.

6.8 Microscopy techniques

6.8.1 Background

Many anatomical and morphological properties may be detected using microscopy (Lillie *et al.*, 1977). **Bright field microscopy** is most commonly used as samples are easy to prepare and a good microscope is not expensive. However, the technique is limited through poor resolution, requiring oil immersion lens. Some specimens are translucent, but can be viewed after staining (see Table 6.2). There are a number of adaptations to the standard microscope that aim to improve viewing. **Dark field microscopy** is used to examine specimens that are too small to view under standard bright field microscopy. **Phase contrast microscopy** is used to view specimens that do not absorb light very well and is useful for distinguishing between viable and non-viable cells that contain plastids. **Differential interference microscopy** (Nomarski) produces clearer images than the phase contrast technique and the thickness of specimens can be elucidated through colour differences within the specimen. **Cryomicroscopy** allows direct visualisation of the effects of freezing on cells and has been used for optimising preservation protocols for fungi (Morris *et al.*, 1988; Smith & Thomas, 1998) In addition, the method has been employed with other eukaryotic microbes and cells (see section 4.14 for further details).

6.8.2 Fluorescence microscopy

Fluorescence microscopy is a powerful technique that selectively stains for cellular components or whole micro-organisms. Specimens are treated with a fluorochrome and then viewed on a microscope equipped with a UV light facility. Examples of fluorochromes include acridine orange (which distinguishes between single and double stranded nucleic acids), 4',6'-diamidino-2-phenylindoledihydrochloride (DAPI; for DNA), rhodamine 123 (viable mitochondria), auramine rhodamine (acid fast strains) or fluorescein isothiocyanate (FITC; for proteins). Immunofluorescence microscopy is a very powerful technique, antibodies specific to a cellular component or biochemical are ligated with a fluorescent stain such as fluorescein. Antigens homologous to the fluorescent antibodies are readily identified by regions of fluorescence within the specimen (Singleton & Sainsbury, 1993). The Fluorescent Antibody Technique (FAT) is an immunofluorescent protocol used for detecting specific micro-organisms in liquid and environmental samples. Fluorescence *in situ*

hybridisation (FISH) is another technique which utilises fluorescence microscopy - in this case to observe *in situ* hybridisation to gene probes (Trask, 1991).

Table 6.2 Some examples of microscopical and general microbial stains (information extracted from Singleton & Sainsbury 1993).

Target	STAIN (OR PROCEDURE)
General purpose stains	Crystal violet Eosin Fuchsin Methylene blue Cotton blue in lactoglycerol
Protein	Amildo black 10B Coomassie brilliant blue
Nucleic acids	Acridines Chromomycin DAPI Methyl green-pyronin stain
Lipids	Nile blue A Sudan black B
Starch	Iodine
Actinomycetes and fungi in tissue	Methenamine-silver stain
Bacterial capsules	Capsule stain, e.g., aqueous nigrosin
Bacterial endospores	Ziehl-Neelsen strain (modified) Malachite Green Toluidine blue
Bacterial Gram type	Gram stain (procedure with stain crystal violet, mordant Lugol's iodine, a decoloriser such as alcohol and a counter stain such as Saffranin)
Blood parasites (e.g., protozoan)	Romanowsky stain Leishman stain
Cellulose and fibrillar polysaccharides	Congo red
Detection of acid fast strains	Ziehl-Neelsen strain Auramine rhodamine stain
Detection of flagella	Leifson flagella stain
Metachromatic granules	Albert's stain Laybourn's stain

6.9.4 Electron microscopy (EM)

EM has been particularly useful in taxonomic investigations and morphological analysis and characterisation. Scanning electron microscopy is ideal for looking at the surface architecture of organisms at high magnification, whereas transmission electron microscopy is suited to investigating ultrastructural features. A major disadvantage of electron microscopy is the huge financial outlay that is required to purchase microscopes and the subsequent high running costs that arise through maintenance and time consuming specimen preparation. Many organisations employ dedicated technicians to run their electron microscopes. A further disadvantage of electron microscopy is the possibility of damage to specimens that occurs during preparation. Dehydration and fixing often result in artefacts and disruption that can influence interpretation. However, techniques such that utilise cryo-stages and freeze fracture reduce the chances of damage. The technique of immuno electron microscopy can allow

the detection of specific cellular components to which antibodies have been raised and attached to an electron dense material (see Section 6.9.2 Immunofluorescence microscopy).

References

Ames, B.N., Dursten, W.E., Yamasaki, E. & Lee, F.W. (1973). Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. *Proceedings of the National Academy of Sciences, USA* **70**, 2281-2285.

Ames, B.N., McCann, J. & Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian microsome mutagenicity test. *Mutation Research* **31**, 347-364.

Barnett, J.A., Payne, R.W. & Yarrow, D. (1983). *Yeasts: Characteristics and Identification*. Cambridge University Press, Cambridge, UK.

Bartsch, H., Camus, A.-M. & Malaveille, C. (1976). Comparative mutagenicity of *N*-nitrosamines in a semi-solid and liquid incubation system in the presence of rat or human tissue fractions. *Mutation Research* **37**, 149-162.

Barth, M.G.M. & Bridge, P.D. (1989). 4-methylumbelliferyl substituted compounds as fluorogenic substrates for fungal extracellular enzymes. *Letters in Applied Microbiology* **9**, 177-179.

Benedict, C.V., Cook, W.J., Jarrett, P., Cameron, J.A., Huang, S.J. & Bell, J.P.. (1983). Fungal degradation of polycaprolactones. *Journal of Applied Polymer Science* **28**, 327-334.

Bonde, M.R., Petersen, G.L. & Maas, J.L. (1991). Isozyme comparisons for identification of *Colletotrichum* species pathogenic to strawberry. *Phytopathology* **81**, 1523-1528.

Bradbury, J.M. (1977). Rapid biochemical tests for characterisation of the *Mycoplasmatales*. *Journal of Clinical Microbiology* **5**, 531-534.

Bridge, P.D. (1985). An evaluation of some physiological and biochemical methods as an aid to the characterisation of species of *Penicillium* subsection *Fasciculata*. *Journal of General Microbiology* **131**, 1887-1895

Bridge, P.D. & Arora, D.K. (1998). Interpretation of PCR methods for species definition. In: *Applications of PCR in Mycology*, (P.D. Bridge, D.K. Arora, C.A. Reddy & R.P. Elander. eds). pp.63-84. CAB International, Wallingford, U.K..

Bridge, P.D., Pearce, D.A., Rivera, A., & Rutherford M.A. (1997). VNTR derived oligonucleotides as PCR primers for population studies in filamentous fungi. *Letters in Applied Microbiology* **24,** 426-430.

Bridge, P.D. & Hawksworth, D.L. (1984). The APIZYM testing system as an aid to the rapid identification of *Penicillium* isolates. *Microbiological sciences* **1**, 232-234.

Bridge, P.D. & Hawksworth, D.L. (1985). Biochemical tests as an aid to the identification of *Monascus* species. *Letters in Applied Microbiology* **1**, 25-29.

Brody, H. & Carbon, J. (1989). Electrophoretic karyotype of *Aspergillus nidulans*. *Proceedings of the National Academy of Sciences, USA* **86**, 6260-6263.

Brooker, N.L., Leslie, J.F. & Dickman, M.B. (1991). Nitrate non-utilizing mutants of *Colletotrichum* and their use in studies of vegetative compatibility and genetic relatedness. *Phytopathology* **81**, 672-677.

Bruns, T.D., White, T.J. & Taylor, J.W. (1991). Fungal molecular systematics. *Annual Reviews in Ecology and Systematics* **22**, 525-564.

Burmeister, M. & Ulanovsky, L. (eds.) (1992). *Methods in Molecular Biology Vol. 12: Pulsed-field Gel Electrophoresis*. Humana Press Inc., Totowa, NJ, USA.

Campbell, S.M., Todd, J.R. & Anderson, J.G. (1987). Production of *l*-malic acid by *Paecilomyces varioti*. *Biotechnology Letters* **9**, 393-398.

Casselton, L.A. & Olesnicky, N.S. (1998). Molecular Genetics of mating recognition in basidiomycete fungi. *Microbiology and Molecular Biology Reviews* **62**, 55-70.

Chibata, I., Tosa, T. & Takata, I. (1983). Continuous production of *L*-malic acid by immobilized cells. *Trends in Biotechnology* **1**, 9-11.

Claeyssens, M. (1989). Chromophoric and fluorophoric glycosides as tools in biochemical and biotechnological research. *Applied Fluorescence Technology* **1**, 11-12.

Collins, M.D. (1985). Isoprenoid quinone analysis in bacterial classification and identification. In: *Chemical Methods in Bacterial Systematics*. Society for Applied Microbiology. Technical Series 20 (ed. M Goodfellow & D.E. Minnikin) pp. 267-287. Academic Press, London, UK.

Cooley, R.N. (1992). The use of RFLP analysis, electrophoretic karyotyping and PCR in studies of plant pathogenic fungi. In: *Molecular Biology of Filamentous Fungi* (Edited by U. Stahl & P. Tudzynski) pp. 13-26. VCH: Weinheim.

Cowan, S.T. (ed.) (1974). *Cowan & Steel's Manual for the Identification of Medical Bacteria*. 2nd edition. Cambridge University Press, Cambridge, UK.

Cruickshank, R.H. & Wade, G.C. (1980) Detection of pectin enzymes in pectin-acrylamide gels. *Analystical Biochemistry* **107**, 177-181.

Doonan, S. (ed.) (1996). *Methods in Molecular Biology Vol. 59: Protein Purification Protocols.* Humana Press Inc., Totowa, NJ, USA.

Edel, V. (1998). Polymerase chain reaction in mycology: an overview. In: *Applications of PCR in Mycology*, (Bridge P.D., Arora D.K., Reddy C.A. & Elander R.P. eds) pp. 1-20. CAB International, Wallingford, U.K.

Eggins, H.O.W. & Pugh, G.J.F. (1962). Isolation of cellulose decomposing fungi from soil. *Nature* **193**, 94-95.

Filtenborg, O., Frisvad, J.C. & Svendsen. (1983). Simple screening methods for moulds producing intracellular mycotoxins in pure culture. *Applied and Environmental Microbiology* **45**, 481-585.

Fischer, S.G. & Lerman, L.S. (1983). DNA fragments differing by single base-pair substitutions are separated in denaturing gradient gels: correspondence with melting theory. *Proceedings of the National Academy of Sciences, USA* **80**, 1579-1583.

Fort, P., Bonhomme, F., Darlu, P., Piechaczyk, M., Jeanteur, P. & Thaler, L. (1984). Clonal divergence of mitochondrial DNA versus populational evolution of nuclear genome. *Evolutionary Theory* **7**, 81-90

Frisvad, J.C. (1981). Physiological criteria and myco-toxin production as aids in identification of common asymmetric penicillia. *Applied and Environmental Microbiology* **41**, 568-579.

Frisvad, J.C. &Thrane, U. (1987). Standardized high-performance liquid chromatography of 182 mycotoxins and other secondary metabolites based on alkylphenone retention indices and UV-VIS spectra (diode array detection). *Journal of Chromatography* **404**, 195-214.

Gabriel, O. & Gersten, D.M. (1992) Staining for enzymatic activity after gel electrophoresis, I. *Analytical Biochemistry* **203**, 19-21.

Gadd, G.M. (1993). Interactions of fungi with toxic metals. *New Phytologist* **124**, 1-35.

Gadd, G.M. (1994). Interactions of fungi with toxic metals. In *The Genus* Aspergillus (eds. K.A. Powell, A. Renwick & J.F. Peberdy) pp. 361-374. Plenum Press: New York, USA.

Gaffney, G.W., Schneier, K., Diferrante, N. & Altman, K.I. (1954). The quantitative determination of hippuric acid. *Journal of Biological Chemistry* **206**, 695-698.

Gardes, M., Bousquet, J. & Lalonde, M. (1987). Isozyme variation among 40 *Frankia* strains. *Applied and Environmental Microbiology* **53**, 1596-1603.

Glenn, J.K. & Gold, M.H. (1983). Decolorization of several polymeric dyes by the lignin-degrading basidiomycete *Phanerochaete chrysosporium. Applied and Environmental Microbiology* **45**, 1741-1747.

Goller, S.P., Gorfer, M., Mach, R.L., & Kubicek, C.P. (1998). Gene cloning using PCR. In: *Applications of PCR in Mycology*, (Bridge, P.D., Arora, D.K., Reddy, C.A. & Elander R.P. eds). pp. 21-46. CAB International, Wallingford, U.K.

Golubev, W.I. (1998). Mycocins (Killer Toxins). In: *The Yeasts: A Taxonomic Study* (4th edn; Kurtzman, C.P. & Fell, J.W. eds.) pp. 55-62. Elsevier, Amsterdam, Netherlands.

Grange, J.M. & Clark, K. (1977). Use of umbelliferone derivatives in the study of enzymatic activities of mycobacteria. *Journal of Clinical Pathology* **30**, 151-153.

Hamilton, R.J. & Hamilton, S. eds. (1992). *Lipid Analysis: A Practical Approach*. IRL Press, Oxford, UK.

Hampton, R., Ball, E. & De Boer, S. (eds.). (1990). *Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens: A Laboratory Manual*. APS Press, St. Paul, Minnesota, USA.

Hastie, A.C. (1981). The genetics of conidial fungi. In: Biology of Conidial Fungi Vol. 2 (eds. G.T. Cole & B. Kendrick) pp. 511-547. Academic Press, New York, USA.

Hames, B.D. (1981). An introduction to polyacrylamide gel electrophoresis. In: *Gel Electrophoresis of Proteins* (eds. B.D. Hames & D. Rickwood) pp. 1-91. IRL Press, Oxford, UK.

Hayat, M.A. (1981). *Principles and Techniques of Electron Microscopy: Biological Applications*. Edward Arnold, London, UK.

Hudspeth, M.E.S., Shumard, D.S., Tatti, K.M. & Grossman, L.I. (1980). Rapid purification of yeast mitochondrial DNA in high yield. *Biochimica et Biophysica Acta* **610**, 221-228.

Kelley, J. & Yaghmaie, P.A. (1988). Screening of fungal strains employed in the testing of plastics materials. *International Biodeterioration* **24**, 289-298.

Kilian, M. (1978). Rapid identification of *Actinomycetaceae* and related bacteria. *Journal of Clinical Microbiology* **8**, 127-133.

Klement, Z., Rudolph, K. & Sand, D.C. (eds.) (1990). *Methods in Phytobacteriology*. Akadémiai Kiadó, Budapest, Hungary.

Kubicek, Q.B., Civelero, E.L., Bonde, M.R., Hartung, J.S. & Peterson, G.L. (1989). Isozyme analysis of *Xanthomonas campestris* pv. *citri*. *Phytopathology* **79**, 297-300.

Kuraishi, H., Katayama-Fujimura, Y., Sugiyama, J. & Yokoyama, T. (1985). Ubiquinone systems in fungi. I. Distribution of ubiquinones in the major families of ascomycetes, basidiomycetes and deutoromycetes, and their taxonomic implications. *Transactions of the Mycological Society of Japan* **26**, 383-395.

Lambert, R.H. & Garcia, J.R. (1990). Evidence of morphology-specific isozymes in *Candida albicans*. *Current Microbiology* **20**, 215-221.

Leslie, J.F. (1987). A nitrate non-utlizing mutant of *Gibberella zeae*. *Journal of General Microbiology* **133**, 1279-1287.

Lillie, R. D., Stotz, Elmer Henry, Emmel, V. M. (1977). H. J. Conn's biological stains: a handbook on the nature and uses of the dyes employed in the biological laboratory. (edited by Harold Joel Conn) 9th edition. Baltimore: Williams and Wilkins.

Lima, N., Teixeira, J.A. & Mota, M. (1991) Deep agar-diffusion test for preliminary screening of lipolytic activity of fungi. *Journal of Microbiological Methods* **14**, 193-200.

Ling, W.H., Saxelin, M., Hanninen, O. and Salminen, S. (1994). Enzyme profile of *Lactobacillus* strain GG by a rapid APIZYM system: a comparison of intestinal bacterial strains. *Microbial Ecology in Health and Disease* **7**, 99-104.

Lowen, R., Brady, B.L., Hawksworth, D.L. & Paterson, R.R.M. (1986). Two new lichenicolous species of *Hobsonia*. *Mycologia* **78**, 842-846.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**, 265-275.

McEldowney, S. (1990). Microbial biosorption of radionuclides in liquid effluent treatment. *Appl. Biochem. Biotechnol.* **26**, 159-180.

Marriott, A.C., Archer, S.A. & Buck, K.W. (1984). Mitochondrial DNA in *Fusarium oxysporum* is a 46.5 kilobase pair circular molecule. *Journal of General Microbiology* **130**, 3001-3008.

Marumo, K. & Aoki, Y. (1990). Discriminant analysis of cellular fatty acids of *Candida* species, *Torulosis glabrata* and *Cryptococcus neoformans* determined by gas-liquid chromatography. *Journal of Clinical Microbiology* **28**, 1509-1513.

May, B. & Royse, D.J. (1982). Genetic variation and joint segregation of biochemical loci in the common meadow mushroom, *Agaricus campestris*. *Biochemical Genetics* **20**, 1165-1173.

Micales, J.A., Bonde, M.R. & Peterson, G.L. (1986). The use of isozyme analysis in fungal taxonomy and genetics. *Mycotaxon* **27**, 405-449.

Miller, M., **Palojarvi A., Rangger A., Reeslev M. & Kjoller A.** (1988). The use of fluorogenic substrates to measure fungal presence and activity in soil. *Applied and Environmental Microbiology* **64,** 613-617.

Mitchell, K. & Fallon, R.J. (1990). The determination of ubiquinone profiles by reversed-phase high-performance thin-layer chromatography as an aid to the speciation of *Legionellaceae*. *Journal of General Microbiology* **136**, 2035-2041.

Morris, G.J., Smith, D. & Coulson, G.E. (1988). A comparative study of the changes in the morphology of hyphae during freezing and viability upon thawing for twenty species of fungi. *Journal of General Microbiology* **134**, 2897-2906.

Mugnai, L., Bridge, P.D. & Evans, H.C. (1989). A chemotaxonomic evaluation of the genus *Beauvaria*. *Mycological Research* **92**, 199-209.

Muller, M.M., Kantola, R. & Kitunen, V. (1994). Combining sterol and fatty acid profiles for the characterization of fungi. *Mycological Research* **98**, 593-603.

Nardon P., Monget D., Didier-Fichet, M.L. & de The, G. (1976). Comparison of zymogram of three lymphoblastoid cell lines with a new microtechnique. *Biomedicine* **24,** 183-190.

O'Brien, M. & Colwell, R.R. (1987). A rapid test for chitinase activity that uses 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide. *Applied and Environmental Microbiology* **53**, 1718-1720.

Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K. & Sekiya, T. (1989). Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformational polymorphisms. *Proceedings of the National Academy of Sciences, USA* **86**, 2766-2770.

Olsson, S. & Jennings, D.H. (1991). Evidence for diffusion being the mechanism of translocation in the hyphae of three moulds. *Experimental Mycology* **15**, 302-309.

Paterson, R.R.M. (1986). Standardised one- and two-dimensional thin-layer chromatographic methods for the identification of secondary metabolites in *Penicillium* and other fungi. *Journal of Chromatography* **368,** 249-264.

Paterson, R.R.M., & Bridge, P.D. (1994). *Biochemical Techniques for Filamentous Fungi*. CAB International, Wallingford, Oxon, U.K.

Paterson, R.R.M. & Buddie, A. (1991). Rapid determination of ubiquinone profiles in *Penicillium* by reversed phase high performance thin layer chromatography. *Letters in Applied Microbiology* **13**, 133-136.

Paterson, R.R.M. & Hawksworth, D.L. (1985). Detection of secondary metabolites in dried cultures of *Penicillium* preserved in herbaria. *Transactions of the British Mycological Society* **85**, 95-100.

Paterson, R.R.M. & Kemmelmeier, C. (1989). Gradient high performance liquid chromatography using alkylphenone retention indices of insecticidal extracts of *Penicillium* strains. *Journal of Chromatography* **483**, 153-168.

Paterson, R.R.M. & Rutherford, M.A. (1991). A simplified rapid technique for fusaric acid detection in *Fusarium* strains. *Mycopathologia* **113**, 171-173.

Puhalla, J.A. (1985). Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. *Canadian Journal of Botany* **63**, 179-183.

Raeder, U. & Broda, P. (1985). Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* **1**, 17-20.

Sambrook, J. & Russell, D.W. (2001). *Molecular Cloning: A Laboratory Manual* (3rd edn) Vols. 1-3. Cold Spring Harbor Laboratory Press, New York, USA.

Shaw, C.R. & Prasad, R. (1970). Starch gel electrophoresis of enzymes – a compilation of recipes. *Biochemical Genetics* **4**, 297-320.

Sierra, G. (1957). A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. *Antonie van Leeuwenhoek* **23**, 15-22.

Singleton, P & Sainsbury, D. (1993). *Dictionary of Microbiology and Molecular Biology*, 2nd Edition. Wiley, London, U.K..

Skerman, V.B.D. (ed.) (1969). *Abstracts of Microbiological Methods*. John Wiley Interscience, London, UK.

Slifkin, M. & Gil, G.M. (1983). Rapid biochemical tests for the identification of groups A, B, C, F and G streptococci from throat cultures. *Journal of Clinical Microbiology* **18**, 29-32.

Smith, D. & Thomas, V.E. (1998). Cryogenic light microscopy and the development of cooling protocols for the preservation of filamentous fungi. *World Journal of Microbiology and Biotechnology* **14**, 49-57.

Smith, I. (1969). *Chromatographic and Electrophoretic Techniques*, Volume 1. Pitman Press: Bath, UK.

Stahl, E. (ed.) (1969).Thin-Layer Chromatography – A Laboratory Handbook, 2nd edition. Springer-Verlag, Berlin. Germany.

St Leger, R.J., Charnley, A.K. & Cooper, R.M. (1986). Enzymatic characterisation of entomopathogens with the API ZYM system. *Journal of Invertebrate Pathology* **48**, 375-376.

Sugiyama, J., Itoh, M., Katayama, Y., Yamaoka, Y., Ando, K., Kakishima, M. & Kuraishi, H. (1988). Ubiquinone systems in fungi. II. Distribution of ubiquinones in smut and rust fungi. *Mycologia* **80**, 115-120.

Takao, S. (1965). Organic acid production by basidiomycetes. *Applied Microbiology* **13**, 732-737.

Taylor, J.W. & Natvig, D.O. (1987). Isolation of fungal DNA. In: *Zoosporic Fungi in Teaching and Research* (Eds. M.S. Fuller & A. Jaworski) pp. 252-258. Southeastern Publishing Corporation Athens, USA:.

Tornabene, T.G. (1985). Lipid analysis and the relationship to chemotaxonomy. *Methods in Microbiology* **18**, 209-234.

Trask, B.J. (1991). Fluorescence in situ hybridization: Applications in cytogenetics and gene mapping. *Trends Genet.* **7**, 149-154.

van der Walt, J.P. (1970). Criteria and methods used in classification. In: *The Yeasts, A Taxonomic Study*, 2nd edn. (ed. J. Lodder). pp. 34-113. North Holland, Amsterdam, Netherlands.

Vos, P., Hogers, R., Bleeker, M., Reijans M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman. J., Kuiper, M. & Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **18,** 7213-7218.

Welsh, J. and McClelland, M. (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* **18**, 7213-7218.

White, G.F., Snape, J.R. & Nicklin, S. (1996). Biodegradation of glycerol trinitrate and pentaerythritol tetranitrate by *Agrobacterium radiobacter*. *Applied and Environmental Microbiology* **62**, 637-642.

WHO (1985). *Environmental Health Criteria 51*: *Guide to short-term tests for detecting mutagenic and carcinogenic chemicals*. Geneva, World Health Organization 208 pp.

Wildman, H.G. (1995). Influence of habitat on the physiological and metabolic diversity of fungi. *Canadian Journal of Botany* **73** (Supplement 1), S907-S916

Woodbury, W., Spencer, A.K. & Stahmann, M.A. (1971). An improved procedure using ferricyanide for detecting catalase isoenzymes. *Analytical Biochemistry* **44**, 301-305.

Yahagi, T., Nagao, M., Seino, Y., Matsushima, T., Sugimura, T. &Okada, M. (1977). Mutagenicities of *N*-nitrosamines on *Salmonella*. *Mutation Research* **48**, 121-130.

Yamada, Y. & Kondo, K. (1973). Coenzyme Q system in the classification of the yeast genera *Rhodotorula* and *Cryptococcus*, and the yeast-like genera *Sporobolomyces* and *Rhodosporidium*. *Journal of General and Applied Microbiology* **19**, 59-77.

Yarrow, D. (1998). Methods for the isolation, maintenance and identification of yeasts In: *The Yeasts: A Taxonomic Study* 4th edn (C.P. Kurtzman & J.W. Fell, eds.). pp. 77-100. Elsevier, Amsterdam, Netherlands.

Zolan, M.E. & Pukkila, P.J. (1986). Inheritance of DNA methylation in *Coprinus cinereus*. *Molecular and Cellular Biology* **6**, 195-200.

Chapter 7

Ordering, charges, payments, quarantine and safety

David Smith

It is the policy of the UKNCC to endeavour to provide its clients, on every occasion, with the products and services they require. The products and services supplied are of marketable quality and will fulfil product claims as defined in the individual member collection catalogues. At all times good techniques and procedures as defined in the individual collection's laboratory procedures manual or standard operating procedures will be in operation and regular audits carried out to ensure that these procedures are followed and are effective. The UKNCC collections either employ a recognised accreditation scheme, e.g., ISO 9000 or follow the UKNCC Quality management system (see http://www.ukncc.co.uk for details).

In addition to many specialist services (see Chapter 9) the UKNCC member collections offer a culture/cell supply service. The organisms that are supplied include actinomycetes, algae, animal cells, bacteria, bacteriophage, cyanobacteria, filamentous fungi, nematodes, protozoa, mycoplasmas and yeasts. You can select the strains you require by accessing the culture collection databases through the UKNCC web site or from catalogues. Alternatively, consult the microbial properties list in Appendix A, which lists strains according to the property exhibited.

7.1 Ordering cultures

Orders should be sent directly to the relevant UKNCC collection. Most UKNCC collections accept telephone, written company orders, fax, e-mail, internet and mail orders when accompanied by an official customer order number and/or official forms. An official department order form is preferable. Clients not providing an official order number and orders from overseas may be sent a proforma invoice requesting payment in advance. In the case of CABI a signed undertaking, declaring that the client will operate within the spirit of the Convention on Biological Diversity (CBD) (see Chapter 3) must be provided and will cover all subsequent orders unless otherwise agreed. Postal quarantine and other regulations control the distribution of strains, see Chapter 2 for details of what is required to comply.

7.1.1 Customer undertaking

The customer receiving material protected by the CBD must agree not to claim ownership over organisms provided, nor to seek intellectual property rights over them or related information. If they wish to utilise or exploit such organisms commercially, suitable and adequate recompense to the country or origin, in the spirit of the Convention of Biological Diversity must first be discussed with the appropriate authority in that country or with relevant UKNCC collection in the first instance.

7.1.2 Information required from the customer

Customers are required to provide: Strain details (name and accessions number if applicable); A customer order number; A contact name; An invoice/delivery address; Telephone/fax number; VAT registration number (if the customer is within Europe); Details of how they would like the strain delivered (i.e. as a freeze-dried ampoule, living culture, etc.). Supply and use of certain organisms require a permit or authorisation by local or international legislation, where this is required a copy must accompany the request (see Section 7.3). Details can be obtained from the relevant collection if in doubt.

7.2 Strain availability

Following the provision of the above details, the UKNCC databases will be checked to ensure strain availability. If the required culture is not available, and if the customer so desires, the database will be searched for alternatives. Staff will advise as far as possible where to obtain the strains required if they are unavailable to customers from the UKNCC. Freeze dried cultures are often sent within 24 hours, but if the customer requires a live culture, or one that is stored in oil or liquid nitrogen, then delivery time is normally in the order of two to three weeks or longer for cryopreserved slow-growing strains. If the culture cannot be supplied as a freeze-dried sample, or the customer requires the culture in another form, the customer will be contacted with an estimated supply date. If any unexpected delays arise (e.g., because of failure to recover from preservation), customers are informed immediately by telephone, fax or email to ensure new deadlines are satisfactory. Despite rigorous quality control and standard procedures being followed it may be possible that the strain may fail to grow, may be contaminated or may not have the property stipulated in the order or that is reasonably expected of the organism on receipt. It is normal policy to replace the strain free of charge with a growing culture on agar where possible. If this is not possible a refund will be given often in the form of a credit note. Cultures from cryopreservation or other methods of storage are grown on agar and viability; purity and identity are checked before dispatch, again if refunds are considered appropriate they will normally be given in the form of a credit note. If the situation changes during recovery of the strain(s) the customer will be informed.

7.3 Restrictions

Once UKNCC staff have confirmed that the strain is available, information about the containment level of the organism, the forms it is preserved in and any restrictions on the sale or distribution of the strain are discussed. All culture parcels must be opened in a laboratory and all hazard group 2/3 (ACDP, 1996) organisms require the appropriate containment level facilities. The forms in which the organism is preserved affect delivery time. If the strain is a non-indigenous plant pathogen (to the UK), the customer has to obtain a MAFF licence before the strain can be delivered. Overseas customers may require an import permit. The customer will be informed of any special restrictions, and the relevant paperwork that is required from them prior to provision of requested cultures e.g., with plant pathogens (see Section 7.6).

Restrictions also apply to the supply of dangerous organisms that could be mis-used. The UKNCC member collections take special precautions to prevent such occurrences and have special procedures in place (see http://www.ukncc.co.uk for details). Human and animal pathogens and toxin producers are not sent to the listed countries in the Ministry of Defence booklet on Biological Warfare. There are also some countries which the UKNCC cannot supply without obtaining permission from the UK Department of Trade and Industry these include Iraq, Iran, Cuba, Libya, North Korea and Syria.

7.4 Price of strains and special considerations

When ordering by telephone, customers will be informed of the current price, discounts may be given (depending on the requirements of the individual member collection) for:

- Universities/Colleges (details of the course the strain will be used on must be provided)
- Bulk purchases

Several organisations and collections exchange organisms on a free basis. Organisations with a Memorandum of Understanding, collections belonging to the European Culture Collection Organisation (ECCO) and depositors of strains may be entitled to one free culture in exchange (consult relevant collection).

The fee paid by customers is for the supply of the culture and is not a charge for the culture itself.

7.4.1 Invoicing

Invoices will normally be dispatched with the strains from most collections unless otherwise instructed or where proforma invoices have been paid in advance, however, some collections supply the strain and invoice within a specified period of time, e.g., for CCAP within one month of order delivery.

7.4.2 Charges and payment

Cultures are available from the collections for a fee (current prices may be located on the UKNCC web site). Charges are subject to change and revised annually. Please check current prices and conditions of sale with the collections. You will need to contact the collections and complete a registration document to receive hazard group 3 or 4 pathogens. The above prices are exclusive of UK Value Added Tax, which will be applied where applicable. Prices may vary without notice. European orders are charged VAT unless the VAT registration number is provided. Overseas payments can be by cheque drawn on a UK Bank or Banker's Draft. UNESCO Coupons or International Money Orders and credit card payment are accepted by some collections.

When ordering cultures, clients are advised not to quote accession numbers unless they are sure that a particular strain is most suitable for their purpose. It is frequently more satisfactory to state the purpose for which the cultures are required and to leave the choice of strain to collection staff. For educational purposes it is often sufficient to order by the generic name only, or to allow the collection to select the

best strain of a species available. Many isolates are now supplied immediately as freeze-dried ampoules, but if stocks are old they are checked for viability before despatch which may cause delay. Some cultures still have to be grown, for example, retrieval from liquid nitrogen involves thawing, incubation under suitable conditions and a purity check. ECACC distributes cell lines in the frozen state. Time of dispatch may depend on growth rate, but where feasible orders are dispatched within 2 or 3 weeks.

7.5 Quarantine and postal regulations/restrictions

Adequate precautions must be taken to ensure the safety of all those persons involved in the packaging, transport and receipt of strains. Steps must also be taken to make sure that material transported presents no hazard to plants, people, animals, or the environment. In order to reduce risk in these areas, national and international regulations have been formulated to govern the shipment of biological materials. All those who are involved in the shipment of cultures must follow the rules and regulations that apply (Chapter 2).

7.6 Plant pathogens

Most governments place restrictions on the import of strains from abroad, especially of plant pathogens, and it is frequently necessary to obtain official permission before a culture can be imported and used. It is the responsibility of all workers wishing to obtain cultures from any microbial resource collection to ensure that they comply with the appropriate regulations. Import and export restrictions for perishable non-infectious or perishable infectious biological substances by national postal services can be found in the *Official Compendium of Information of general interest concerning the implementation of the Convention and its Detailed Regulations* revised at Hamburg in 1984, International Bureau of the Universal Postal Union, Berne. This information has also been compiled by DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany in their booklet, *Instructions for the shipping of infectious and non-infectious biological substances* (Anon, 1998). Most national post offices are members of the Universal Postal Union (UPU) and will provide detailed regulations on the transport of cultures which will include when permits are required and where they can be obtained.

Strains of plant pathogens supplied to Canada and the USA must be accompanied by import mailing labels, without which entry of cultures to these countries is refused. Applications for these labels, stating the names of the organisms and the purpose for which they are required, should be made to:

Canada

Chief of the Plant Protection Division, `Agriculture Canada' Science Division, Science Service Building, Ottawa, Ontario, Canada K1AS 0C5. Tel: +1 613995 5222 Fax: +1 613 996 9223/9219

USA

USDA Agricultural Research Service, Plant Protection & Quarantine, Room 764, 6505 Belcrest Road, Hyattsville, Marylands 20782, USA. Tel: +1 301 436 8590 Fax: +1 301 436 6402

For information on transport of plant pathogens throughout Europe contact:

European and Mediterranean Plant Protection Organisation (EPPO), 1 rue le Nôtre, 75016 Paris, France. Tel: +331 45 20 77 94 Fax: +331 42 24 89 43

Clients in the UK who wish to obtain strains of non-indigenous plant pathogens must first obtain a permit from the England and Wales or the Scottish offices of the Ministry of Agriculture, Fisheries and Food (MAFF).

England and Wales

Ministry of Agriculture, Fisheries and Food, Room 340, Foss House, Kings Pool, 1-2 Peaceholme Green, York Yo1 2PX. Tel: 01904 455195

Scotland

Plant Health Section, Agricultural Science Agency, East Craigs, Edinburgh EH12 8NJ
Tel: +44 131 244 8934 Fax: +44 131 244 8940

Non-indigenous tree pathogens can only be supplied if the customer holds a current permit issued by The Forestry Commission.

Forestry Commission Headquarters, 231 Corsthorphine Road, Edinburgh EH12 7AP
Tel: +44 131 334 0303 Fax: +44 131 334 3047

On receipt of orders for such strains, clients are referred to the Ministry if they do not already hold a permit. Under the terms of its licence to maintain non-indigenous plant pathogens, UKNCC collections are required to see a copy of the Ministry permit before such strains can be supplied.

7.7 Human and animal pathogens

International Postal Regulations regarding the postage of human and animal pathogens are very strict because of the safety hazard they present (see Chapter 2). Most UKNCC collections hold a special permit for the despatch of such material. The packaging and other regulations are strictly observed. Some countries will not accept such potential human infective material through the post including the UK. Further information can also be found in *Shipping of infectious, non-infectious and genetically modified biological materials, International Regulations* DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (1998) and the UKNCC web site (http://www.ukncc.co.uk).

7.8 Dispatch

Cultures are dispatched to customers in good healthy condition after checks (if applicable). They require periods from 5 days to several weeks to grow satisfactorily. They are packed in a way to prevent damage during transit. On receipt, if the culture is provided as an active culture, bottle caps should be loosened. Cultures on agar have a limited shelf-life and should be sub-cultured after periods of 2-26 weeks (see Chapter 3 or contact the relevant collection). However, freeze-dried fungi in ampoules or vials can be kept for much longer periods, tens of years in some cases.

7.9 Quality assurance

The identities of cultures are confirmed (wherever possible). In several of the UKNCC member collections this is done before acceptance by a team of expert biosystematists and strains are checked again by these experts before and after preservation and during maintenance procedures to ensure quality is maintained. The UKNCC collections operate to conform with ISO Standards or the UKNCC quality management system see UKNCC web site for details (http://www.ukncc.co.uk).

7.10 Concerns, comments and complaints

UKNCC collections will endeavour to address all customer queries by return of post, or on the same day by fax, telephone or e-mail if appropriate and practicable.

References

Anon (1996). Categorisation of pathogens according to hazard and categories of containment. Fourth edition. Advisory Committee on Dangerous Pathogens (ACDP). London: HMSO.

Anon (1998). *Shipping of infectious, non-infectious and genetically modified biological materials, International Regulations* DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

Chapter 8

Deposits

David Smith

The UKNCC member collections welcome deposits relevant to their objectives. It is hoped that scientists will deposit interesting strains in these and other principal collections. The lodging of cultures in more than one collection is desirable for rare and important strains as insurance against accidental loss. Ideally all strains referred to in published research should be deposited in a public service collection as reference material. Deposits to the UKNCC should be strains of new taxa, type cultures, strains with interesting properties, and those cited in publications. Depositors are requested to provide as much information as possible relating to the strain.

8.1 Benefits for depositors

Collections offer a range of benefits. These include:

1. Safe preservation of your culture
2. Worldwide access to deposited strains
3. Removal of the burden of distribution
4. Validation of type strains
5. Mycoplasma testing of cells
6. Exchange of cultures

8.2 Safe deposits

A confidential safe deposit service is offered. Cultures will be stored as freeze-dried ampoules and/or in liquid nitrogen to ensure long-term viability. Customers often find this service useful for important strains during research and development prior to application for a patent. If cultures are to be identified prior to storage, the current rates for that service apply. Viability checks can be carried out during storage if requested. Cultures known to be unstable can be reprocessed at regular intervals specified by the customer.

8.3 Patent deposits

Several of the UKNCC collections are International Depositary Authorities under the Budapest Treaty of 1977 and together cover a wide range of organisms (Table 8.1).

The collections follow the standard procedures approved by the European Culture Collection Organisation (ECCO) which have been written to clarify the requirements of the Budapest Treaty.

ALLOW SUFFICIENT TIME BEFORE DISCLOSURE, PATENT DEPOSITS CAN TAKE FROM SEVERAL DAYS TO SEVERAL WEEKS.

Table 8.1 UKNCC recognised as depositary authorities

Collection	Organisms that can be deposited
CABI	Bacteria, fungi, nematodes, yeasts
CCAP	Algae, cyanobacteria and protozoa
ECACC	Animal cells and hybridomas; animal viruses; DNA probes
NCIMB	Bacteria; phages; plasmids; plant seeds; yeasts
NCPPB	Phytopathogenic bacteria
NCTC	Bacteria

Patent deposits are given a unique collection number on the day of receipt; this is classed as the date of deposit. However, the strain cannot be formally accepted, or, in most cases, the depositor notified of the number until a successful viability test has been carried out (in some instances this follows preservation). In addition, correctly completed application and accession forms must have been received, and some collections require full payment before acceptance. After successful preservation, an ampoule or culture is returned to the depositor for confirmation of identity and activities. Viability checks are not normally carried out during storage unless requested by the depositor. The Collection will suggest intervals depending on the organism and preservation method. A fee is levied for each viability check. Samples of the organisms are made available at any time to the depositor to check the retention of activities.

Stains previously deposited may be converted to Patent deposits. Depositors must provide the relevant completed forms and a viability check will be carried out on the deposited material. A sample of this will be returned to the depositor for confirmation of identity and activities. A new accession number and patent deposit date will be issued. Cultures will be released only on the written authorisation of the depositor or on receipt of a certificate of release from the relevant Patent Office depending on the type of deposit. The depositor will be informed of the name and address of the relevant Patent Office and of the requesting party.

Healthy, pure cultures suitable for preparing samples for freeze-drying and liquid nitrogen storage should be supplied. Payment must accompany the deposit for urgent requests. These procedures can be completed in optimum time (depending on the growth rate of the organism). Further information concerning the service together with copies of the application and accession forms are available from UKNCC collections on request.

PLEASE SEE APPENDIX E FOR ACCESSIONS DEPOSIT FORM

Chapter 9

UKNCC collections and services

John Day and David Smith

The collections main interests are generally in identification and preservation but they and their parental organisations often offer many more services. This chapter outlines those services and interests of the UKNCC collections. This chapter is divided into sections on: About the member collections; Identifications; Culture and preservation services; Research, Consultancy; Contract and investigation services; Information and reference facilities; Training; facilities available in UKNCC collections. Contact information is given as applicable.

9.1 About the member collections

9.1.1 CABI Bioscience (CABI) (formerly IMI)

CAB International is a not-for-profit intergovernmental organisation owned by 43 member countries. Since 1913 it has been advising and supplying expert services to industry, commerce, governments, international agencies and universities on matters of agriculture, forestry and human and animal health. CAB International is a unique organisation owned and directed by the governments of its member countries operating in publishing, information provision and bioscience. Underpinning CABI's scientific services are the working collections of fungi, bacteria and nematodes held in the CABI Bioscience UK Centre representing 22 000 strains and associated information from 147 countries. CABI Bioscience has multi-disciplinary capabilities with centres in six countries, two adjacent sites at Egham and Ascot in the UK and five overseas centres in Malaysia, Pakistan, Kenya, Switzerland and Trinidad. The CABI living collections are well placed, being embedded in an infrastructure that provides biosystematic, genotypic and phenotypic characterisation and technical support. In 1947 CABI was asked to house the UK National Collection of Fungus Cultures and up to 1989 received a Government subsidy to make the CABI collections available to the scientific community in general. Since then CABI Bioscience has been subsidising the public service collection role. In 1999 CABI member country representatives endorsed the involvement of CABI in technology exchange and capacity building in the field of *ex situ* conservation on their behalf. Although policy, research and development related to the biological resources it holds are totally in the control of CABI it collaborates with international initiatives and for example has a mechanism for compliance with the Convention on Biological Diversity (CBD). The CABI collection plays a proactive role in the operation of the UK National Culture Collection (UKNCC) and adheres to the UKNCC Quality Management System and the European Common Access to Biological Resources Information (CABRI) Guidelines. The prime objective of the collection is to provide the organism base and repository to underpin CABI Bioscience programmes but it also has other key functions e.g., to act as an International Depository Authority for strains cited in patents.

CABI Bioscience centres provide crucial linkages for project development and management, and bases for the local, regional and international research and training activities of the CABI bioscience programmes. The key areas of research are in biosystematics and biochemical and molecular characterisation, sustainable pest management, ecology and environmental and industrial microbiology. Activities in these areas provide new and interesting organisms for the collection but even more importantly enable characterisation leading to added value holdings. For further detail see the CABI web site (http://www.cabi.org).

The National Collection of Woodrotting macro-Fungi (NCWRF) is now incorporated into the CABI collection. It was originally part of the Forest Products Research Laboratory (FPRL), within the British Government's Department of science and industrial research at Princes Risborough. It became part of the Biodeterioration Section of the Building Research Establishment and was housed in the Timber protection division of the Building Research Station (BRE), Garston. The NCWRF collected wood destroying fungi important in the biodeterioration of wood and holds over 600 accessions.

9.1.2 Culture Collection of Algae and Protozoa (freshwater) (CCAP)

The CCAP accesses freshwater algal and all protozoan strains. The collection is housed at the NERC, Centre for Ecology & Hydrology, Windermere. The CCAP maintains microalgae, including cyanobacteria and free-living nonpathogenic protozoa. The CCAP is an International Depository Authority (IDA) for the above groups of organisms under the terms of the Budapest Treaty (1977). The collection holds over 1 800 strains.

9.1.3 Culture Collection of Algae and Protozoa (marine) (CCAP)

The CCAP comprises a gene bank of marine and hypersaline microalgae and cyanobacteria collected from a wide range of geographical locations and ecological niches, ranging from Antarctic seas to soda lakes in Africa. It includes some small multicellular seaweeds and most algal classes are represented. The collection holds over 500 strains and is housed in the-Dunstaffnage Marine Laboratory, Oban.

9.1.4 European Collection of Cell Cultures (ECACC)

The ECACC is part of the Centre for Applied Microbiology and Research (CAMR). CAMR is a special health authority of the Department of Health and a centre of excellence working on infectious diseases. ECACC is supported from a combination of sources, including the UK Research Councils (MRC, BBSRC, NERC), the World Health Organisation and revenue from sales and provision of technical services. The collection accepts deposits of animal, and other cell lines, hybridomas, HLA-defined human B-lymphoblastoid cell lines and DNA probes and holds over 20000 accessions.

9.1.5 The National Collections of Industrial, Food and Marine Bacteria (NCIMB)

The NCIMB is grant aided by the BBSRC. It is committed to and encourages a pro-active accessions policy for new type strains and novel bacteria of industrial and environmental significance. The grant is

administered through NCIMB Ltd. which is ISO 9002 certificated, and is dedicated to the development and application of research aimed at the solution of industrial and environmental problems world-wide. NCIMB Ltd. provides the infrastructure to offer high quality services in bacterial identifications as well as bacterial and chemical analytical and consultancy services. The collections provide expert safe and patent deposit facilities as an International Depository Authority (IDA) within the rules of the Budapest Treaty (1977) and has recently broadened its remit in the food sector by the transfer of the National Collection of Food Bacteria (NCFB) from the IFR Reading to Aberdeen. The integrated collection now holds in excess of 8500 accessions.

9.1.6 National Collection of Pathogenic Fungi (NCPF)

NCPF is an integral component of the Public Health Laboratory Service (PHLS) Mycology Reference Laboratory. It maintains and supplies a comprehensive range of fungi causing infection in humans and warm-blooded animals (over 2500 accessions). The main holdings are dermatophytes, dimorphic pathogens, yeasts and moulds from subcutaneous and systemic infections. As part of the MRL, the collection is also able to offer antifungal drug susceptibility testing, an identification service, and advice on diagnosis. Ancillary activities include the UK National External Quality Assessment Scheme for Microbiology, and involvement in various teaching and training initiatives. The MRL has close links with the University of Bristol and uses a large teaching laboratory for its annual three-day course on identification of pathogenic fungi. The main research activities are centred on taxonomy (including typing for epidemiological studies), antifungal sensitivities and diagnosis of fungal infections.

9.1.7 National Collection of Plant Pathogenic Bacteria (NCPPB)

The NCPPB is a special laboratory within the agriculture and environment directorate, plant health of the Central Science Laboratory (CSL). The primary role of the CSL is to deliver high quality scientific advice, technical support and enforcement activities underpinned by appropriate research and development to enable departmental customers to meet MAFF aims. The services are also provided to other Government departments and to public and private sector organisations on a commercial basis. The CSL is an associated institute of the Universities of York and Leeds and has links with the University of East Anglia. The collection exists primarily to maintain cultures of the world's bacterial plant pathogens for use by research, educational establishments and by industry and has over 5500 accessions.

9.1.8 National Collection of Pathogenic Viruses (NCPV)

The UK Biotechnology and Biological Sciences Research Council (BBSRC) and the Wellcome Trust agreed to jointly support the initial phase of a National Collection of Pathogenic Viruses. The Collection has operated since late 1999 under the auspices of the UKNCC, and is sited at CAMR. Operating under ISO 9001 certification, an archive of well-characterised, authenticated human pathogens will be built up to resource the supply of viruses and materials derived from them to the scientific community. Advice on policies for accession and distribution, and on scientific activities is obtained from an advisory panel composed of distinguished virologists from universities and medical

schools, government agencies and industry. An essential part of the collection's activities is concerned with the authentication of virus holdings. This is performed using traditional serological and molecular methods. Ultimately, it is intended that authenticity of stocks will be determined by direct sequencing of PCR products amplified from appropriate genomic regions. An incremental programme to make available non-infectious virus-derived materials such as DNA or RNA from purified virus or from infected cells, individual virus genes (in the form of PCR products or cloned DNA), and viral proteins is being developed. Associated services such as safe deposit and patent deposit facilities, and virus identification will be provided as the collection develops. The collection is expected to be of benefit in the development and testing of vaccines and antiviral compounds, in the development and validation of diagnostic test systems, and in the conservation of biodiversity.

9.1.9 National Collection of Type Cultures (NCTC)

The NCTC is a specialised laboratory located in the Central Public Health Laboratory (CPHL), Colindale. It accesses, preserves and supplies authentic cultures of bacteria and mycoplasmas that are pathogenic to man, or other animals, that may occur in food, water and in hospital or other health related environments and which can be preserved by freeze-drying. Non-pathogenic strains may be accepted where they are phylogenetically related (e.g., members of the same genus) to pathogenic strains. Bacteriophages may be accepted where they are active against pathogenic bacterial strains. Medically important plasmids are accepted only in host strains. Founded in 1920, it is the longest-established collection in the world offering a bacterial culture supply service. It is internationally recognised, serving as a European resource centre for Plasmids and a UNESCO Microbial Resource Centre (MIRCEN). The collection has over 10000 accessions.

9.1.10 National Collection of Yeast Cultures (NCYC)

The National Collection of Yeast Cultures (NCYC) collects yeasts associated with food materials or with particular relevance to food production, food spoilage, brewing and fundamental scientific research and currently holds over 2800 accessions. It is an International Depositary Authority (IDA) on the Budapest Treaty (1977). NCYC is based at the Institute of Food Research (IFR), Norwich and is a division of IFR Enterprises Ltd, a wholly-owned subsidiary of IFR. IFR's mission is to carry out independent basic and strategic research onfood safety, quality, nutrition and health.

9.2 Identification services

The UKNCC individual member collections offer identification by a range of techniques for many organisms including, actinomycetes, algae, animal cells, bacteria, cyanobacteria, filamentous fungi, nematodes, protozoa, mycoplasma and yeasts. Techniques used for strain identification include anatomical and mophological analysis, MIDI FAME analysis and BIOLOG, biochemical and molecular fingerprinting and sequencing. The collections that provide the services and methods used are listed under the different organisms below (Table 9.1)

Table 9.1 Identification methods used by UKNCC collections for strain identification

Organism	Technique				
	Morphological	**MIDI**	**BIOLOG**	**Biochemical**	**Molecular fingerprinting and sequencing**
Actinomycetes	NCIMB	NCIMB		NCIMB	NCIMB
Algae	CCAP			CCAP	
Animal cell lines					ECACC
Bacteria	NCIMB	CABI NCIMB NCPPB NCTC	NCIMB	CABI; NCIMB; NCPPB NCTC	NCIMB; CABI
Cyanobacteria	CCAP			CCAP	
Filamentous fungi	CABI; NCPF			CABI	CABI; NCPF
Nematodes	CABI				CABI
Protozoa	CCAP				CCAP
Mycoplasma	NCTC; ECACC				
Yeasts	NCYC: NCPF	CABI		CABI; NCYC; NCPF	NCYC; NCPF

9.3 Culture and preservation services

The UKNCC strives to optimise existing preservation protocols and development new ones. Most research is aimed at improving cryopreservation protocols with emphasis on species specific criteria. Preservation regimes (Chapter 4), Deposits (Chapter 8) and Identifications of micro-organisms (see Section 9.2) have already been discussed. MAFF, UK have sponsored a postdoctoral position based at CABI Bioscience to develop preservation protocols for recalcitrant organisms and cells. The research will add to the expertise and wealth of information generated and collected by the UKNCC member collections over the decades of their work.

The ECACC animal cell collection offers a number of services including detection and screening for contaminant bacteria, fungi and viruses. ECACC is also able to test for and eradicate mycoplasma, screening being carried out to ISO 9002, European and USDA standards. ECACC also offers a cell characterisation and authentication service. Isozyme analysis and DNA fingerprinting with Jeffreys probes is used for verification of the species of origin and identity of a cell line. A library of fingerprints to aid validation of suspect cell banks is being established. ECACC have a purpose built cell banking facility for the production of cell banks and is a designated repository for WHO generated cell banks. In addition, ECACC undertakes immortalisation procedures. Generation of B-lymphoblastoid cell lines by immortalisation with EBV is offered as a routine service to scientists in human genetic research. Immortal cell lines are also generated from primary cells by transfection with selected oncogens.

9.4 Research, consultancy, contract and investigation services

In addition to their culture collection activities, most UKNCC collections, in association with their parental organisations can offer external organisations a diverse range of consultancy and contract services. Consultancies and contract research expertise is offered in the areas of: biodeterioration, biodegradation, biological control, biodiversity, ecology, parasitology, pest management, biochemistry, molecular biology, taxonomy, and identifications, metabolite and enzyme screening, analytical chemistry, food and beverage microbiology, process development and large scale culture, stored product microbiology, plant pathology, evaluation of microbial identification kits and environmental monitoring.

9.4.1 CABI Bioscience (CABI) (formerly IMI)

The industrial laboratory at CABI bioscience offers a mould growth and challenge testing service to national and international standards. A wide range of materials are routinely tested including automotive and aircraft parts, museum and library specimens, fuels and lubricants, electrical equipment, building materials, stored foods, surgical goods and equipment and specialist composts. Externally funded research projects recent undertaken at CABI Bioscience include fungi in water distribution systems, composting and biodegradation of lignocellulosic wastes, the ecology of painted surfaces, and rapid methods of predicting and detecting microbial degradation of materials. CABI Bioscience can offer expertise in the following areas:

- Advice on sampling protocols
- Assistance in bioprospecting schemes
- Database design and development
- *Ex-situ* preservation and conservation
- Identification and classification
- Isolation from natural substrata
- Plant pathology
- Production of nomenclators, bibliographies and checklists
- Repatriation of data
- Selection and monitoring of indicator species
- Site surveys and inventories
- Strain and species characterisation
- Studies of soil biodiversity
- Sustainable use of ecosystems
- Training and capacity building
- Biochemistry and molecular biology (see below)

Biochemical and Molecular techniques

Biochemical or chemotaxonomic methods have been established at CABI Bioscience for over 10 years and contribute a significant proportion of all characterisation and identification work. Methods include

isoenzyme analysis by use of polyacrylamide-gel electrophoresis, analysis of isoprenoid quinones by TLC and HPLC and quantitative analysis of fatty acids by gas chromatography. The latter is based on a dedicated system (MIDI, Delaware, USA) and is one of the best available for rapid and accurate bacterial and yeast characterisation. Techniques have been developed to determine biological activities of compounds of biotechnological importance. Rapid screening methods for mycotoxins (e.g., ochratoxin A, patulin), phytotoxins (e.g., fusaric acid) and antibiotics (e.g., griseofulvin), together with citric acid and industrial enzymes (e.g., cellulase, lipase, pectinase, amylase) have been perfected. A database of TLC characters and UV visible spectra of over 100 secondary metabolites from fungi has been compiled and is updated as further compounds are obtained. A wide range of molecular biology techniques are currently in use and include restriction fragment length polymorphism (RFLP) analysis of fungal mitochondrial DNA, electrophoretic karyotyping by use of pulsed field gel electrophoresis (PFGE), genomic fingerprinting by use of rare-cutting restriction endonucleases and PFGE, genomic fingerprinting using nucleic acid amplification techniques such as random amplified polymorphic DNA analyses (RAPD's) / arbitary fragment length polymorphism (AFLP) and amplification and subsequent restriction of fungal ITS and IGS regions, and hybridisation and probing methods. A recent addition is the DGGE population analysis technique and a sequencer.

9.4.2 CCAP core research

CCAPs key areas of research are the development and improvement of cryopreservation protocols for algae and protozoa; study of the mechanisms of lethal and sublethal cryoinjury; algal lipid analyses. In addition, taxonomic studies utilise both traditional and modern molecular techniques. Contract research (CR) is carried out in a wide range of areas and techniques, e.g., using biochemical, biotechnological, ecological and microbiological approaches to fulfil CR projects including discrete CCAP projects and input into larger CEH projects.

9.4.3 ECACC contract research and development

ECACC can provide consultants in process development and large-scale culture. Its staff have considerable experience in designing, optimising and evaluating novel animal cell bioreactor systems. Including stirred bioreactors, high-density perfusion fixed and fluidised bed reactors.

Other research carried out at ECACC includes:

- Pharmaceutical manufacturing facilities (to GMP, including, freeze-drying, packaging)
- Vaccine manufacture
- Manufacturing of biopharmaceuticals
- Pilot fermentation capacity
- Large scale animal cell culture
- Diagnostics and detection
- Research and development production monitoring
- Analysis and control of micro-organisms in natural and man-made environments
- Equipment testing and validation for microbial integrity

- Bioremediation and waste management
- Aerobiology investigations

9.4.4 NCIMB research and services

- Environmental analyses
- Site investigations
- Contract R&D, product development
- Biocide testing
- Chemical analysis
- Contract preservation, including known number inocula
- Bacterial identification
- 16s rDNA sequence analysis
- Rapid and sensitive detection of bacteria using nucleic acid probes

Research, consultancy, contract and investigation services at other UKNCC collections

Research services at NCTC, NCYC and NCPF are often carried out through their parental organisations. Readers should contact the relevant collections direct for further information and visit individual collection web sites via the UKNCC (http://www.ukncc.co.uk).

9.5 Information

The CABI Bioscience UK Centre (Egham) Library houses a large and unique collection of literature on systematic and applied mycology including biodeterioration, fungal biotechnology, plant pathology and fungal taxonomy. It includes circa 600 current journals, over 30,000 bound volumes and books and over 130,000 reprints. Literature searches and studies can be carried out to specific individual requirements and a photocopy service is also available to provide copies of original source literature. The CABI Bioscience UK Centre (Egham) abstract journals are included in the CABI Abstracts computer database (almost 2 million records since 1973), they are also available from a range of database hosts including CAN/OLE, DIALOG, DIMDI, ESA-IRS and JKCT. For further information contact: CAB International, Wallingford, Oxon OX10 8DE, UK. Tel: 01491 832 111; Fax: 01491 833 508; E-mail: cabi@cabi.org.

CCAP has access via the freshwater biological association at Windermere to one of the finest freshwater libraries in the world (currently a partner in the EU UNIVERSE project). In addition, the Fritsch Collection of Algal Illustrations is unique and an invaluable aid to those involved in research into freshwater algae. Further information concerning the Fritsch Collection can be obtained from The Curator of the Fritsch Collection, Freshwater Biological Association, The Ferry House, Windermere, Ambleside, Cumbria LA22 OLP, UK, or by email to fritsch@ceh.ac.uk

All UKNCC member collections have built up a wealth of information in the process of their activities and can provide answers to specific questions and solutions to many problems concerning their particular activities.

9.6 Training

The UKNCC member collections offer a wide range of training opportunities including tailor-made training programmes, short courses, extended visiting scientist programmes and postgraduate studies arranged via local Universities. In addition there is a UK Federation for Culture Collections (UKFCC) course on the preservation and maintenance of micro-organisms and cell cultures supported by UKNCC staff.

9.6.1 Range of topics (see also short courses offered by UKNCC collections)

- Collection Management
- Isolation
- Preservation and maintenance
- Identification of organisms
- Training in specialist techniques
- Biological control

9.6.2 Tailor-made training

Please contact the UKNCC member collection direct if you require specialist training in topics not covered by the short courses listed. The collections offer training in collection management, isolation, identification, characterisation, growth and preservation particularly relevant to the micro-organisms or the cells they maintain. If you have a requirement for training in relation to a particular organism or cell contact the collection that deals with them directly. For:

- Fungi, nematodes, arthropods, biological control: Contact CABI
- Algae and protozoa: Contact CCAP (Freshwater)
- Customised training courses in culturing marine algae: Contact CCAP (Marine)
- Cell cultures: Contact ECACC
- Industrial, food and marine bacteria: Contact NCIMB
- Medically important fungi: Contact NCPF
- Medically important bacteria: Contact NCTC
- Yeasts: Contact NCYC
- Plant pathogenic bacteria: Contact CABI also see NCPPB below:

The NCPPB does not offer official courses but can provide one to one training in various aspects of culture collection maintenance and in research into plant pathogenic bacteria and the diseases they cause, particularly for diagnostic methods. Costs are determined by the Curator. The NCPPB's parent organisation, the Central Science Laboratory, offers several potentially relevant courses and tailor-

made research projects. CSL provides support for agriculture, food and the environment. The NCPPB Curator should be contacted in the first instance.

9.6.3 Short courses offered by individual UKNCC collections

- Basic Biochemical and Molecular Techniques (CABI)
- Basic Mycological Techniques (CABI)
- Biological Pest Management (CABI)
- Culture Collection Management and Preservation Techniques
- Fungi Associated with Water systems (CABI)
- Identification of Aspergillus and Penicillium species (CABI)
- Identification of Human Pathogenic Fungi (NCPF)
- Identification of Fungi of Agricultural and Environmental Significance (CABI)
- Identification of Industrial and Food Spoilage Fungi (CABI)
- Intermediate 5-day Cell Culture Course (ECACC)
- Introductory 3-day Cell Culture Course (ECACC)
- Mycoplasma testing (ECHOIC)
- PCR Characterisation of Filamentous fungi (CABI)

9.7 Facilities available in UKNCC collections

9.7.1 CABI-Bioscience (CABI) (formerly IMI)

Facilities for freeze-drying (Edwards Modulyo 4K, Edwards Super Modulyo 12K, Edwards Minifast Shelf Freeze-drier) Facilities for cryopreservation (Controlled rate freezer, 350l liquid nitrogen storage vessels with automatic fill system) Cryogenic light microscope. HPLC apparatus PCR machine Midi-FAME analysis Protein electrophoresis Sequencer Glasshouses Pulsed Field Electrophoresis equipment Electronic gel documentation system Light microscopes SEM with cryo-stage Industrial laboratory and facilities for materials testing and handling of fuels Plant Pathology laboratory and facilities Nematology laboratories and facilities Fully equipped teaching laboratory

Access to CABI Publishing expertise and data

9.7.2 Culture Collection of Algae and Protozoa (CCAP) (Freshwater)

Fully equipped purpose build laboratory suites, including separate, dedicated, media-prep and wash-up facility Four temperature controlled rooms/ walk in incubators Various small incubators both illuminated and non-illuminated Class III containment room Various laminar flow cabinets Cryopreservation facilities allowing controlled-rate, uncontrolled two-step cooling and vitrification Cryostorage facility Various microscopes including bright field, phase contrast and fluorescence Cryogenic light microscope Molecular biology equipment: PCR machine, gel electrophoresis apparatus, pulse-field gel electrophoresis apparatus, DNA sequencing apparatus

Access to the broader facilities of CEH including:

◆Electron microscopy (Both SEM & TEM) ◆Flow cytometry ◆Fritsch collection of algal illustrations ◆LAN, Internet connection and computing support ◆Analytical chemistry facilities

9.7.3 Culture Collection of Algae and Protozoa (CCAP) (marine)

◆ Several illuminated incubators with light/dark cycling, capable of maintaining constant temperatures (±1°C) between 0˚C and 40°C ◆ Two large walk-in constant temperature (10-30°C) rooms with lighting and robust shelving for larger scale (200-300l in total) cultivation ◆Gases can be supplied ◆ Single axenic culture capacity of up to 20 litres ◆ Laminar flow cabinets for aseptic operations ◆ Two top loading autoclaves ◆ Range of light microscopes available, one equipped with a colour TV camera

Access to the facilities of DML:

Associated with the core activities of CCAP are the general facilities of DML, including ◆EM ◆SEM ◆Centrifuges ◆HPLC, GC ◆Fluorometer ◆AA photospectrometer ◆X-ray Fluorescence Spectrometer ◆Inductively Coupled Plasma Mass Spectrometer (ICP-MS) ◆Scintillation counter and autoanalyser ◆Radiation suite ◆ Seagoing boats and extensive experimental aquarium facilities supplied with running seawater ◆Good library facilities

9.7.4 European Collection of Cell Cultures (ECACC)

◆Contract Master and Working Cell Bank storage of Animal cells, Human cells, bacteria and DNA Probes ◆Safe and Patent Deposit Facilities ◆Tissue Culture ◆Contract Mycoplasma, bacteria and fungi testing of Cell Lines ◆Cell Culture Scale-up ◆EBV Transformations ◆DNA Extraction ◆Serum Testing Facs ◆Immortalisation of animal cells ◆Genetic "fingerprinting" of animal cells ◆Primary Cell Culture facilities ◆Tissue modelling facilities ◆In-vitro animal cell modelling ◆Trans-Epithelial Electrical Resistance Measurement ◆Simulated-Microgravity (Rotary wall Vessels ◆3D Culturing using poly-hema coated Cell Culture Surfaces ◆Plasmid Amplification ◆Preparation and supply of purified nucleic acid or inactivated Pathogens for laboratories which cannot handle live micro-organisms ◆Provision for characterisation of pathogens and their products ◆Provision for raising hydridomas/purified antibody against pathogens (Automass) ◆Provision of bulk production of antibody Recombinant Protein Technology ◆Diagnosis of exotic viral isolates

Equipment/Technologies at CAMR and available to ECACC:

◆DNA Sequencer ◆Mass Spectrometer ◆Scanning Electron Microscope ◆Transmission Electron Microscope ◆Protein Modeller ◆Pathogen containment facilities-ACDP Class II, III and IV ◆WHO Reference site for Anthrax and Ebola ◆Research into a variety of pathogens including HIV, E. coli 0157, *Yersinia pestis*, *Neisseria menigitidis* etc ◆Production of Toxins, e.g., Botulinum Toxin

9.7.5 National Collection of Industrial, Food and Marine Bacteria (NCIMB)

Fully equipped purpose built laboratory suites (for ACDP hazard group 1, 2 or 3) with dedicated media preparation facilities ◆Centrifuges (Bench top and free-standing) ◆Walk in chill rooms (4°C and -20°C) ◆Liquid nitrogen vapour phase storage vessels ◆Waterbaths heated blocks ◆pH meters, balances, steam sterilizers ◆Reverse osmosis water production units ◆Autoclaves ◆ Plate pourers ◆Laminar sterile airflow and category 3 safety cabinets ◆Freeze-dryers (Edwards EF6s, supermodulo

and shelf driers) ◆MIDI FAME GC/chromatographic equipment ◆BIOLOG Automated ID system ◆Desktop computing facilities linked to a central server ◆Phastsystem (mini electrophoresis) ◆Microscopes and photomicroscopes ◆Anaerobic cabinet ◆Vacuum sealing equipment ◆HPLC ◆AA Spectrometer + graphite furnace ◆Dionex anion analyser ◆IR Spectrophotometer ◆LSA analyser ◆Gamma spectrometer ◆Electron microscopy (for inorganic soil analysis) with EDX ◆16s rDNA sequencing

9.7.6 National Collection of Pathogenic Fungi (NCPF)

◆Liquid Nitrogen storage facility ◆Molecular biology facilities ◆Serological techniques ◆Immunofluorescence microscopy ◆Stereomicroscopy ◆Photography, Macro and Micro ◆Antifungal drug susceptibility testing ◆Dedicated Laboratory for hazard group 3 fungi ◆PC based record maintenance ◆PC identification keys for some groups of fungi ◆Comprehensive library of medical mycological literature ◆Representative collection of histological preparations of mycotic diseases

9.7.7 National Collection of Plant Pathogenic Bacteria (NCPPB)

Available via the Central Science Laboratory of which NCPPB is a part:

◆ -150°C & -80°C freezers ◆Liquid Nitrogen storage facility ◆Freeze-drying equipment ◆MIDI MIS fatty acid profiling system ◆ libraries based largely on the NCPPB strains (of >1000 taxa) ◆Facilities for protein profiling ◆ PCR equipment- RFLP REP RAPDs AFLP ◆Pulsed field gel electrophoresis (PFGE) ◆ Serological apparatus ◆Immunofluorescence ◆ELISA ◆Immunofluorescent colony staining ◆Immunomagnetic capture PCR ◆BIOLOG ◆State of the art quarantine glasshouses with full environmental control for use with alien pathogens Access to: ◆Electron microscope. ◆Mass spectrometer

9.7.8 National Collection of Pathogenic Viruses (NCPV)

◆Safe Deposit Facilities ◆Tissue Culture ◆EBV Transformations ◆DNA Extraction ◆Serum Testing Facs ◆Genetic "fingerprinting" of viruses ◆Simulated-Microgravity (Rotary wall Vessels ◆3D Culturing using poly-hema coated Cell Culture Surfaces ◆Plasmid Amplification ◆Preparation and supply of purified nucleic acid or inactivated pathogens for laboratories which cannot handle live micro-organisms ◆Provision for characterisation of pathogens and their products ◆Provision of bulk production of antibody Recombinant Protein Technology ◆Diagnosis of exotic viral isolates

Equipment/Technologies at CAMR and available to ECACC:

◆DNA Sequencer ◆Mass Spectrometer ◆Scanning Electron Microscope ◆Transmission Electron Microscope ◆Protein Modeller ◆Pathogen containment facilities-ACDP Class II, III and IV ◆WHO Reference site for Anthrax and Ebola ◆Research into a variety of pathogens including HIV, E. coli 0157, *Yersinia pestis*, *Neisseria menigitidis* etc. ◆Production of toxins, e.g., botulinum toxin

9.7.9 National Collection of Type Cultures (NCTC)

◆Standard microbiolgical facilities ◆Centrifugal Freeze-driers ◆Shelf Freeze-drier ◆Liquid nitrogen storage facility ◆Category III facility including freeze-drier

Additional relevant Equipment /Techniques available within CPHL:

◆Molecular genetics unit ◆Electron microscopes ◆Full range of chemical analytical equipment ◆Within CPHL and PHLS access to specialist reference laboratories equipped to deal with specific groups of bacteria

9.7.10 National Collection of Yeast Cultures (NCYC)

◆Liquid Nitrogen storage facility ◆Freeze-drying equipment ◆Database software and web server ◆Microscopy - light and electron ◆PCR and general molecular biology equipment ◆Automated DNA sequencer ◆DNA microarrays and proteomics ◆Florescence activated cell sorter ◆Automated micromanipulator for spore dissection

Appendix A Microbial properties

The following lists of properties of UKNCC strains is divided into sections and reference to Sections in Chapter 5 are given.

Enzyme producing strains *(see Section 5.2)*

Metabolite producing strains *(see Section 5.3)*

Antibiotic producing strains (see Section 5.4)

Food strains (see Section 5.5)

Mycoparasites (fungal pathogens) (see Section 5.6)

Biological control (see Section 5.7)

Nitrogen fixers (see Section 5.8)

Strains isolated from interesting and diverse environments (see Section 5.9)

Biodeteriogens (see Section 5.10)

Food spoilage strains (see Section 5.11)

Utilisers/biodegraders/bioremediators (see Section 5.12)

Tolerant Strains (see Section 5.13)

Resistant strains to antibiotics, chemicals, radiation, environmental parameters etc. (see Section 5.13)

Sensitive strains (see Section 5.13)

Test strains (see Section 5.14)

Assay strains including assay of antibiotics and vitamins (see Section 5.15)

Special features: anatomical and morphological (see Section 5.16)

Special features: physiological (see Section 5.17)

Mating types (see Section 5.17)

Special features: chemical transformation, bioconversion and bioaccumulation (see Section 5.18)

Vectors, Phages, Transposons, Genetically modified organisms (auxotrophs, resitant, sensitive, producers), mutants (see Section 5.19)

Vaccine producers (see Section 5.4)

Because of the large number of strains listed, it has not been possible to cite individual literature references for each strain. Users can easily access bibliographic search databases on the Internet to track down references, if the relevant property and organism are entered as search criteria. Strain information can be crosschecked by searching the UKNCC strain database at: http://www.ukncc.co.uk/. The depositors of the strains described many properties of the strains, some of these properties have been confirmed by the collection. Other properties have been discovered by the collection staff. The UKNCC accepts no responsibility if a given strain does not exhibit the desired property. Many properties will only be exhibited under specific experimental conditions and micro-organisms, by their very nature, often exhibit distinct variability from the original culture. If the desired properties are not obvious immediately, subculturing or maintenance under different cultural conditions may promote production.

Enzyme producing strains

3 N-Acetyltransferase
- *Escherichia coli* NCIMB 11710

Acid protease
- *Penicillium citreoviride* CABI-IMI 169640
- *Rhizopus oligosporus* CABI-IMI 174457

Aconitic hydrolase
- *Aspergillus niger* CABI-IMI 075353

s-Adenosylmethionine synthetase
- *Saccharomyces cerevisiae* NCYC81

Adenylate cyclase
- *Corynebacterium liquefacians* NCIMB 9545

Agarase
- *Cytophaga flevensis* NCIMB 12056

Alcohol dehydrogenase
- *Candida utilis* NCYC 927

Alkaline phosphatase
- *Escherichia coli* NCIMB 10616

Amine oxidase
- *Arthrobacter sp.* NCIMB 11625

Amino acid decarboxylases
- *Escherichia coli* NCIMB 86

Amino acid racemases
- *Pseudomonas putida* NCIMB 10919

Amino acid oxidase
- *Neurospora crassa* CABI-IMI 068614ii
- *Neurospora crassa* CABI-IMI 053239ii

Aminoglycoside acetyltransferase
- *Micromonospora inyoensis* NCIMB 12731

Amorphous cellulase
- *Aspergillus fumigatus* CABI-IMI 255091

α Acetolactate decarboxylase
- *Bacillus licheniformis* NCIMB 8061
- *Bacillus licheniformis* NCIMB 8059
- *Bacillus licheniformis* NCIMB 6346

d-Amino acid oxidase
- *Trigonopsis variabilis* NCYC 770

α Amylase
- *Amorpotheca resinae* CABI-IMI 344050
- *Aspergillus awamori* CABI-IMI 303389
- *Aspergillus niger* CABI-IMI 303388
- *Bacillus subtilis* NCIMB 8565
- *Bacillus oxytoca* NCIMB 8017
- *Bacillus amyloliquefaciens* NCIMB 12077
- *Bacillus polymyxa* NCIMB 8158
- *Bacillus circulans* NCIMB 11033
- *Bacillus megaterium* NCIMB 9376
- *Bacillus megaterium* NCIMB 7581

Amylase (Thermoacidophilic)
- *Alicylobacillus acidocaldarius* NCIMB 11725

Amylase (Maltotetraose)
- *Pseudomonas stutzeri* NCIMB 11359

Amylase
- *Aspergillus niger* CABI-IMI 096215
- *Aspergillus oryzae* CABI-IMI 052146
- *Aspergillus oryzae* CABI-IMI 052143
- *Aspergillus oryzae* CABI-IMI 052142
- *Aspergillus oryzae* CABI-IMI 044242
- *Aspergillus foetidus*CABI-IMI 041871
- *Bacillus amyloliquefaciens* NCIMB 10785

Amylase
- *Bacillus polymyxa* NCIMB 8648
- *Bacillus subtilis* NCIMB 8646
- *Schwanniomyces occidentalis* NCYC 953
- *Schwanniomyces occidentalis* NCYC 1519
- *Trichoderma viride* CABI-IMI 016198
- *Trichosporon pullulans* NCYC 477

Amylocyanin
- *Streptomyces coelicolour* NCIMB 9798

L-alanine dehydrogenase
- *Halobacterium cutirubrum* NCIMB 2259

Apotrytophanase
- *Escherichia coli* NCIMB 10083

Arginase
- *Saccharomyces cerevisiae* NCYC 87

N-aringinase
- *Aspergillus niger* CABI-IMI

Arginine decarboxylase
- *Escherichia coli* NCIMB 8584

Arginine dihydrolase
- *Hafnia alvei* NCIMB 11999

Arylsulphatase
- Unnamed NCIMB 8734

Asparginse
- *Saccharomyces cerevisiae* NCYC 956

L-Asparginase
- *Escherichia coli* NCIMB 11595
- *Serratia marcescens* NCIMB 9155

Aspartate kinase
- *Thermus flavus* NCIMB 13085

Aspartate transcarbamylase
- *Escherichia coli* NCIMB 11380

Bacteriocuprein superoxide dismutase
- *Photobacterium leiognathi* NCIMB 2193

Blasticidin S deaminase
- *Aspergillus flavus* CABI-IMI 091856
- *Aspergillus flavus* CABI-IMI 091856iii

Byssochlamyopeptidase
- *Byssochlamys fulva* CABI-IMI 040021

N-carbamoyl-D-amino acid amidohydrolase
- *Agrobacterium radiobacter* NCIMB 13428

Catalase
- *Aspergillus niger* CABI-IMI
- *Lactobacillus yamanashiensis* NCIMB 10562
- *Lactobacillus yamanashiensis* NCIMB 10560
- *Lactobacillus yamanashiensis* NCIMB 10561

Catechol 2,3,-oxygenase
- *Pseudomonas putida* NCIMB 10432

Cellobiohydrolase D
- *Trichoderma reesei* CABI-IMI 192655ii

Cellulase
- *Trichoderma reesei* CABI-IMI 192656ii
- *Sordaria fIMI cola* CABI-IMI 103759
- *Trichoderma viride* CABI-IMI 016198
- *Aspergillus awamori* CABI-IMI 303389
- *Aspergillus niger* CABI-IMI 096215
- *Aspergillus niger* CABI-IMI 303388

Cellulase
- *Trichoderma reesei* CABI-IMI 192655ii
- *Trichoderma pseudokoningii* CABI-IMI 092027
- *Trichoderma reesei* CABI-IMI 192654
- *Chaetomium globosum* CABI-IMI 045550
- *Chaetomium globosum* CABI-IMI 045550iii
- *Chaetomium globosum* CABI-IMI 045550iv
- *Chaetomium globosum* CABI-IMI 045550ii *Myrothecium verrucaria* CABI-IMI 045541ii
- *Myrothecium verrucaria* CABI-IMI 045541
- *Penicillium pinophilum* CABI-IMI 371195
- *Penicillium pinophilum* CABI-IMI 371196
- *Penicillium pinophilum* CABI-IMI 371197
- *Penicillium pinophilum* CABI-IMI 371198
- *Penicillium pinophilum* CABI-IMI 371199

Cellulolytic enzymes
- *Aspergillus niger* CABI-IMI 050565ii
- *Aspergillus niger* CABI-IMI 050566

Cephalosporin C-4 carboxymethyl esterase
- *Streptomyces capillispiralis* NCIMB 12832

Cephalosporinase
- *Enterobacter cloacae* NCIMB 12091

Chitobiosidase
- *Aspergillus niger* CABI-IMI

Chitosanase
- *Streptomyces* sp. NCIMB 13037

Cholesterol esterase
- *Pseudomonas fluorescens* NCIMB 9493

Cholesterol oxidase
- *Rhodococcus* sp. NCIMB 10554
- *Rhodococcus* sp. NCIMB 10555

Cholinesterase
- Pseudomonas fluorescens NCIMB 8286

Coagulase (Staphylococcus)
- *Staphylococcus* aureus NCTC 6571
- *Staphylococcus aureus* NCTC 8178

Coenzyme Q-1
- *Pseudomonas dIMI nuta* NCIMB 9393

Coenzyme Q-10
- *Pseudomonas azotocolligans* NCIMB 9391

Collagenase
- *Pseudomonas fluorescens* NCIMB 13112

Collagenase (iophagus)
- *Vibrio alginolyticus* subsp. *Iophages* NCIMB 11038

Creatinine IMI nohydrolase
- *Flavobacterium filamentosum* NCIMB 12081

Cyclodextrin glycosyltransferase
- *Bacillus cereus* NCIMB 13123
- *Bacillus macerans* NCIMB 9368

Detergent
- *Thermomyces lanuginosus* CABI-IMI

Dextranase
- *Paecilomyces lilacinus* CABI-IMI 068202

Dextranase
- *Penicillium pinophilum* CABI-IMI 087160
- *Paecilomyces lilacinus* CABI-IMI 027830
- *Penicillium funiculosum* CABI-IMI 079195
- *Penicillium pinophilum* CABI-IMI 087160ii

Diaminopimelic decarboxylase
- *Escherichia coli* NCIMB 8666
- *Escherichia coli* NCIMB 9110

Diastase
- *Aspergillus oryzae* CABI-IMI 017299

Dimethylamine mono-oxygenase
- *Candida utilis* NCYC 321

DNAse B
- *Streptococcus pyogenes* NCTC 11200

Elastase
- *Lysobacter* sp. NCIMB 9497

Endodeoxyribonuclease
- *Saccharomyces cerevisiae* NCYC 74

Endogluconase
- *Clostridium stercorrium* NCIMB 11754

Endopolygalacturonase
- *Rhizopus stolonifer* CABI-IMI 090609

Esterase
- *Gliocladium roseum* CABI-IMI 304503
- *Gliocladium roseum* CABI-IMI 304501
- *Gliocladium roseum* CABI-IMI 304501

Ethanolamine ammonia lyase
- *Eshericihia coli* NCIMB 11362

Exo-oligo-1,6,glucosidase
- *Bacillus thermoglucosidasius* NCIMB 11955

Exoglucanase
- *Clostridium stercocrarium* NCIMB 11754

Ferrichrome reductase
- *Ustilago sphaerogena* CABI-IMI 061828

Formaldehyde dehydrogenase
- *Pseudomonas putida* NCIMB 11978

Formaldehyde dismutase
- *Pseudomonas putida* NCIMB 12939

Formate dehydrogenase
- *Alcaligenes* NCIMB 8642

β Fructosidase
- *Sphingobacterium multivorum* NCIMB 12560
- *Sphingobacterium multivorum* NCIMB 12558
- *Sphingobacterium multivorum* NCIMB 12559
- *Sphingobacterium multivorum* NCIMB 12557

α Fucosidase
- *Metarhizium anisopliae* CABI-IMI

Fucosidase
- *Clostridium perfringes* NCIMB 8693

α Galactosidase
- *Saccharomyces cerevisiae* NCYC 74
- Unamed NCIMB 11051
- *Escherichia coli* NCIMB 11032

β Galactosidase
- *Kluyveromyces marxianus* NCYC 587
- *Bacillus* sp. NCIMB 11259

β Galactosidase (Thermostable, constitutive for)
- *Bacillus stereothermophilus* NCIMB 11412

β Galactosidase (Thermostable, constitutive for)
- *Bacillus stereothermophilus* NCIMB 11411
- *Bacillus stereothermophilus* NCIMB 11410
- *Bacillus stereothermophilus* NCIMB 11409
- *Bacillus stereothermophilus* NCIMB 11407
- *Bacillus stereothermophilus* NCIMB 11413

α-o-Glucosidase
- *Amorpotheca resinae* CABI-IMI 344050

α Glucosidase
- *Bacillus amyloloquefaciens* NCIMB 12077

β Glucan endohydrolase
- *Schizosaccharomyces pombe* NCYC 132

β 1,3 Glucanase
- *Streptomyces violaceus* NCIMB 11194

β 1,6 Glucanase
- *Bacillus glucanolyticus* NCIMB 12810
- *Bacillus glucanolyticus* NCIMB 12809

Endo-1,6-beta- Glucanase
- *Cytophaga arveniscola* NCIMB 11855

β Glucanase
- *Bacillus glucanolyticus* NCIMB 12809
- *Bacillus glucanolyticus* NCIMB 12810

β 1,2-Glucanase
- *Aspergillus quadricinctus* CABI-IMI 048583ii
- *Penicillium papuanum* CABI-IMI 040587

β 1-6 Glucanase
- *Penicillium dodgei* CABI-IMI 040592ii
- *Penicillium dodgei* CABI-IMI 040592
- *Penicillium ochrochloron* CABI-IMI 061271

Glucanases
- *Penicillium melinii* CABI-IMI 119893

Glucoamylase
- *Amorpotheca resinae* CABI-IMI 344050
- *Cryptococcus tsukkubaensis* NCYC 1510
- *Aspergillus niger* CABI-IMI 060286
- *Aspergillus awamori* CABI-IMI
- *Trichosporon pullulans* NCYC 477

Glucose isomerase
- *Streptomyces echinatus* NCIMB 9598
- *Microbacterium arborescens* NCIMB 8185
- *Streptomyces griseofuscus* NCIMB 9821
- *Streptomyces phaeochromogenes* NCIMB 11741
- *Streptomyces* sp. NCIMB 11856

Glucose oxidase
- *Aspergillus niger* CABI-IMI

β Glucosidase
- *Aspergillus awamori* CABI-IMI 303389
- *Penicillium pinophilum* CABI-IMI 371195*
- *Penicillium pinophilum* CABI-IMI 371196*
- *Penicillium pinophilum* CABI-IMI 371197*
- *Penicillium pinophilum* CABI-IMI 371198*

β Glucosidase
- *Penicillium pinophilum* CABI-IMI 371199*

β Glucosidase (intracellular)
- *Trichoderma reesei* CABI-IMI 192655ii

Glucoxylan mucilage
- *Agrobacterium* sp. NCIMB 11498

Glutamine decarboxylase
- *Escherichia coli* NCIMB 8584

Glutamine synthetase
- *Candida utilis* NCYC 927

Glycerokinase
- *Bacillus stearothermophilus* NCIMB 11270
- *Bacillus stearothermophilus* NCIMB 11271

Glycerol dehydrogenase
- *Bacillus stearothermophilus* NCIMB 11401
- *Bacillus stearothermophilus* NCIMB 11400

Glycerophospholipid-cholesterol transferase
- *Aeromonas hydrophila* subsp. *hydrophila* NCIMB 13190

Histidine decarboxylase
- *Lactobacillus* sp. NCIMB 12275
- *Escherichia coli* NCIMB 8584
- *Clostridium perfringes* NCIMB 6785

L-Histidine ammonia-lyase
- *Pseudomonas putida* NCIMB 10807

Hyaluronidase
- *Sphingobacterium heparinum* NCIMB 9290
- *Staphylococcus* sp. NCIMB 10208

α Hydroxysteroid dehydrogenase
- Comamonas testosteroni NCIMB 8893

β Hydroxysteroid dehydrogenase
- Comamonas testosteroni NCIMB 8893

Hypoxanthine phosphatase
- *Cellulomonas cartae* NCIMB 11025

Inulinase
- *Sphingobacterium multivorum* NCIMB 12558
- *Sphingobacterium multivorum* NCIMB 12559
- *Sphingobacterium multivorum* NCIMB 12557
- *Sphingobacterium multivorum* NCIMB 12560

Iso amylase
- *Bacillus amyloliquefaciens* NCIMB 12077
- *Lysobacter* sp. NCIMB 9497

Isocitrate lyase
- *Pseudomonas indigofera* NCIMB 9441

2-Ketogluconoreductase
- *Arthrobacter globiformis* NCIMB 9792

7-Keto-8-amino-pelargonic acid synthetase
- *Bacillus sphearicus* NCIMB 11935

α Ketoglutarate aminotransferase
- *Flavobacterium lutescens* NCIMB 10917

Keratin sulphate endo beta galactosidase
- *Aureobasidium keratanolyticum* NCIMB 12401

Kynureninase enzymes
- *Rhizopus stolonifer* CABI-IMI 090609
- *Penicillium roquefortii* CABI-IMI 024313

β Lactamase
- *Haemophilus influenzae* NCTC 11315
- Neisseria gonorrhoeae NCTC 11148
- *Staphylococcus aureus* NCTC 8511
- *Bacillus cereus* NCIMB 8967
- *Bacillus cereus* NCIMB 7464
- *Escherichia coli* NCIMB 9472
- *Enterobacter cloacaea* NCIMB 12091
- *Bacillus licheniformis* NCIMB 11108
- *Bacillus licheniformis* NCIMB 9943
- *Bacillus cereus* NCIMB 12054

Lactase
- *Candida kefyr* NCYC 744

Lactic acid racemase
- Lactobacillus sake NCIMB 13090
- *Lactobacillus paracasei* subsp. *paracaseei* NCIMB 9713
- *Lactobacillus curvatus* NCIMB 9710

Laminarinases
- *Streptomyces* sp. NCIMB 8592
- *Lysobacter* sp. NCIMB 9497

Lecithinase C
- *Clostridium perfringes* NCIMB 6125

Lipase
- *Trichoderma viride* CABI-IMI 016198
- *Lysobacter* sp. NCIMB 9497
- *Aspergillus flavus* CABI-IMI 057389
- *Pseudomonas aeruginosa* NCIMB 12718

Lipolytic enzymes
- *Aspergillus flavus* CABI-IMI 052418ii
- *Aspergillus flavus* CABI-IMI 052148

Lysine decarboxylase
- *Escherichia coli* NCIMB 8584

L-Lysine-a-ketoglutarateaminotransferase
- *Flavobacterium lutescens* NCIMB 10917

Luciferase
- *Alexandrium tamarense* CCAP 1119/u
- *Alexandrium tamarense* CCAP 1119/s

Maltase
- *Aspergillus niger* CABI-IMI 060286

Maltohexaose-producing enzyme
- *Klebsiella pneumoniae* subsp. *Pnumoniae* NCIMB 8021
- *Pseudomonas stutzeri* NCIMB 11359

NAD dependent lactate dehydrogenase
- *Rhizopus oryzae* CABI-IMI 040564

NAD glycohydrolase
- *Pseudomonas putida* NCIMB 10521

Neoagarobiase
- *Cytophaga flevensis* NCIMB 12056

Neoagarotetra-ase
- *Cytophaga flevensis* NCIMB 12056

Neuramidase
- *Rhodopseudomonas palustris* NCIMB 8252
- *Clostridium perfringes* NCIMB 8875
- Bacillus circulans NCIMB 9480
- *Klebsiela oxytoca* NCIMB 9479
- *Arthrobacter sialophilus* NCIMB 11491

Nuclease (extracellular)
- *Vibrio* sp.NCIMB 2055

Nucleotide pyrophosphotransferases
- *Streptomyces violascens* NCIMB 9820
- *Streptomyces sepatus* NCIMB 12982

Oligosaccharyl
- *Saccharomyces cerevisiae* NCYC 956

Ornithine decarboxylase
- *Aeromonas* sp. NCIMB 13014

Pantethenase
- *Pseudomonas fluorescnes* NCIMB 12017

Pectate lyase
- *Byssochlamys fulva* CABI-IMI 040021

Pectin methylesterase
- *Aureobacterium barkeri* NCIMB 9658

Pectin transelimi nase
- *Aureobacterium barkeri* NCIMB 9658

Pectinases
- *Aspergillus foetidus* CABI-IMI 050567

Pectinesterase
- *Byssochlamys fulva* CABI-IMI 083277

Penicillin amidase
- *Escherichia coli* NCIMB 8742
- *Streptomyces* sp. NCIMB 10967
- *Streptomyces* sp. NCIMB 10990
- *Streptomyces* sp. NCIMB 10978
- *Arthrobacter viscosus* NCIMB 10268
- *Escherichia coli* NCIMB 8666
- *Escherichia coli* NCIMB 8741
- *Streptomyces* sp. NCIMB 10970
- *Streptomyces lavendulae* NCIMB 10997
- *Fusarium oxysporum* CABI-IMI 113138
- *Streptomyces* sp. NCIMB 10971
- *Mucor hiemalis* CABI-IMI 113134
- *Penicillium spinulosum* CABI-IMI 113139
- *Fusarium culmorum* CABI-IMI 113133
- *Escherichia coli* NCIMB 8743
- *Epicoccum nigrum* CABI-IMI 127257
- *Streptomyces lavendulae* NCIMB 10998
- *Mucor hiemalis* CABI-IMI 113136
- *Alternaria alternata* CABI-IMI 127255
- *Aspergillus versicolor* CABI-IMI 127256
- *Streptomyces* sp. NCIMB 10968
- *Streptomyces* sp. NCIMB 10966

Penicillinase
- *Streptomyces* sp. NCIMB 11331
- Escherichia coli NCIMB 9466
- *Escherichia coli* NCIMB 9464
- *Staphylococcus aureus* NCIMB 11195
- *Bacillus licheniformis* NCIMB 8061
- *Bacillus pumilus* NCIMB 7198
- *Bacillus licheniformis* NCIMB 6346
- *Bacillus subtilis* NCIMB 6276
- *Bacillus licheniformis* NCIMB 8059
- *Bacillus cereus* NCIMB 8967
- *Bacillus licheniformis* NCIMB 6818
- *Streptomyces sp.* NCIMB 11330
- *Enterobacter cooacae* NCIMB 12091
- *Escherichia coli* NCIMB 9472
- *Staphylococcus* sp. NCIMB 10208
- *Escherichia coli* NCIMB 9465

Pentosanases
- *Bacillus subtilis* NCIMB 8646

Pepsin
- *Aspergillus parasiticus* CABI-IMI 015957

Phosphatase
- *Yarrowia lipolytica* NCYC 825

6-Phosphogluconate dehydrogenase
- *Penicillium griseoroseum* CABI-IMI 015378

Phospholipase
- *Clostridium perfringes* NCIMB 6125
- *Clostridium perfringes* NCIMB 10663

Phospholipase
- *Clostridium perfringes* NCIMB 10662
- *Clostridium perfringes* NCIMB 10691

Phospholipase C (phosphatidylinositol specific)
- *Flavobacterium* sp. NCIMB 1314

Polygalacturonase
- *Byssochlamys fulva* CABI-IMI 083277
- *Byssochlamys fulva* CABI-IMI 040021

Polygalacturonate lyase
- *Cytophaga johnsonae* NCIMB 10150
- *Bacillus polymyxa* NCIMB 11088
- *Bacillus subtilis* NCIMB 11034

Polygulornate lyse
- *Pseudomonas* sp. NCIMB 2054

Protease
- *Aspergillus awamori* CABI-IMI 303389
- *Aspergillus oryzae* CABI-IMI 052145
- *Aspergillus oryzae* CABI-IMI 052144
- *Aspergillus niger* CABI-IMI 096215
- *Aspergillus niger* CABI-IMI 303388
- *Conidiobolus khandalensis* CABI-IMI 102045
- *Conidiobolus nanodes* CABI-IMI 092299
- *Acremonium chrysogenium* CABI-IMI 049137

Protease (extracellular)
- *Penicillium roquefortii* CABI-IMI 024313

Proteinases
- *Lysobacter* sp. NCIMB 9497
- *Janthinobacterium lividum* NCIMB 10926
- *Bacillus amyloliquefaciens* NCIMB 10785
- *Lysobacter enzymogenes subsp. Enzymogenes* NCIMB 11894
- *Pseudomonas* sp. NCIMB 10939
- *Serratia marcescens* NCIMB 10351
- *Lysobacter enzymogenes* subsp. *Enzymogenes* NCIMB 9924

Proteinases (alkalophilic)
- *Bacillus* sp. NCIMB 10323
- *Bacillus* sp. NCIMB 10321
- *Bacillus* sp. NCIMB 10300
- *Bacillus* sp. NCIMB 10299
- *Bacillus* sp. NCIMB 10286
- *Bacillus* sp. NCIMB 10284
- *Bacillus* sp. NCIMB 10281
- *Bacillus subtilis* NCIMB 10145
- *Bacillus subtilis* NCIMB 10144
- *Bacillus circulans* NCIMB 10148
- *Bacillus* sp. NCIMB 10302
- *Bacillus circulans* NCIMB 10146
- *Bacillus* sp. NCIMB 10320
- *Bacillus* sp. NCIMB 10303
- *Bacillus* sp. NCIMB 10319
- *Bacillus* sp. NCIMB 10317
- *Bacillus* sp. NCIMB 10316
- *Bacillus* sp. NCIMB 10304
- *Bacillus* sp. NCIMB 10324
- *Bacillus* sp. NCIMB 10318
- *Bacillus circulans* NCIMB 10147

Proteolytic enzymes
- *Aspergillus parasiticus* CABI-IMI 087159
- *Aspergillus parasiticus* CABI-IMI 087159ii
- *Aspergillus parasiticus* CABI-IMI 015957
- *Aspergillus flavus* CABI-IMI 052176
- *Vibrio proteolyticus* NCIMB 1326
- *Aspergillus flavus* CABI-IMI 052148

Proteolytic enzymes
- *Aspergillus flavus* CABI-IMI 052175
- *Aspergillus flavus* CABI-IMI 052173
- *Aspergillus flavus* CABI-IMI 052418ii

Protocatechuate 3:4 dioxygenase
- *Pseudomonas putida* NCIMB 12602

Pullan 4-glucanhydrolase
- *Aspergillus niger* CABI-IMI 091855

Pullanase
- *Cellulomonas mixtus* subsp. *Mixtus* NCIMB 8634
- *Klebsiella pneumoniae* subsp. *Pneumoniae* NCIMB 8021
- *Bacillus polymyxa* NCIMB 8158
- *Bacillus megasterium* NCIMB 7581
- *Klebsiella oxytoca* NCIMB 8017
- *Klebsiella pneumoniae* subsp. *pneumoniae* NCIMB 418
- Bacillus macerans NCIMB 9368

Pullanase (thermostable)
- *Bacillus cereus* NCIMB 13245
- *Bacillus cereus* NCIMB 13244
- *Bacillus cereus* NCIMB 13246

Purine beta ribonucleosidase
- *Penicillium ochrochloron* CABI-IMI 061271
- *Penicillium melinii* CABI-IMI 061836
- *Penicillium glabrum* CABI-IMI 040234
- *Penicillium indonesiae* CABI-IMI 039733
- *Aspergillus ambiguus* CABI-IMI 139274

Pyrrolidonecacarboxyl peptidase
- *Klebsiella pneumoniae* subsp. *pneumoniae* NCIMB 11162
- *Pseudomonas fluorescens* NCIMB 10806

Restriction Type 2 endonuclease
- *Streptomyces phaeochromogenes* NCIMB 11741

Restriction Alu 1endonuclease
- *Cellulomonas cartae* NCIMB 11921

Restriction Sf1L endonuclease
- *Streptomyces fimbriatus* NCIMB 13039

Restriction Bac1endonuclease
- *Alicyclobacillus acidocaldarius* NCIMB 11725

Restriction Mra1endonuclease
- *Deinococcus radiodurans* NCIMB 9279

Restriction Sgr1endonuclease
- *Streptomyces griseus* subsp. *griseus* NCIMB 13023

Restriction Nae1endonuclease
- *Saccharothrix aerocolonigenes* NCIMB 12944

Restriction Apa 1endonuclease
- *Acetobacter pasteurans* NCIMB 7215

Restriction Dgo1endonuclease
- *Streptomyces goshikiensis* NCIMB 9828

Restriction Pmy1endonuclease
- *Proteus myxofaciens* NCIMB 13273

Restriction SM1enzyme
- *Serratia marcescens* NCIMB 11919

Restriction SAL1enzyme
- *Streptomyces albus* NCIMB 11920

Ribonuclease
- *Bacillus amyloliquefaciens* NCIMB 10785

Ribonuclease U1
- *Ustilago sphaerogena* CABI-IMI 061828

RuBisCo Ribulose bisphosphate Carboxylase/ oxygenase
All algal and cyanobacterial strains
Saccharifying enzymes
Aspergillus niger CABI-IMI 060286
Salicylate hydroxylase
Burkholderia cepacia NCIMB 12757
Sarcosine dehydrogenase
Pseudomonas putida NCIMB 11978
Steroid dehydrogenase
Rhodococcus sp. NCIMB 11161
Sterol-ester hydrolase
Saccharomyces cerevisiae NCYC 667
Sterol-ester synthetase
Saccharomyces cerevisiae NCYC 667
Succinoglycan depolymerase
Cytophaga arvensicola NCIMB 11855
Sulphamidase
Sphingobacterium heparinum NCIMB 9290
Sulphoesterase
Sphingobacterium heparinum NCIMB 9290
Tannase
Aspergillus parasiticus CABI-IMI 087159
Aspergillus parasiticus CABI-IMI 087159ii
Thiaminase
Bacillus thiaminolyticus NCIMB 10047
Bacillus thiaminolyticus NCIMB 10053
Bacillus thiaminolyticus NCIMB 10046
Bacillus aneurinolyticus NCIMB 10065
Bacillus thiaminolyticus NCIMB 10051
Bacillus thiaminolyticus NCIMB 10054
Bacillus thiaminolyticus NCIMB 10050
Bacillus thiaminolyticus NCIMB 10049
Bacillus thiaminolyticus NCIMB 10048
Bacillus aneurinolyticus NCIMB 10056
Bacillus thiaminolyticus NCIMB 10052
Bacillus aneurinolyticus NCIMB 10064
Bacillus aneurinolyticus NCIMB 10063
Bacillus aneurinolyticus NCIMB 10061
Bacillus aneurinolyticus NCIMB 10060
Bacillus aneurinolyticus NCIMB 10059
Bacillus thiaminolyticus NCIMB 10055
Bacillus aneurinolyticus NCIMB 8698
Bacillus aneurinolyticus NCIMB 10058
Bacillus aneurinolyticus NCIMB 10057
L-Threonine 3-dehydrogenase
Arthrobacter sp. NCIMB 11220
Threonine synthase
Neurospora crassa CABI-IMI 075722
Trimethylamine mono-oxygenase
Candida utilis NCYC 321
Trypsin
Aspergillus parasiticus CABI-IMI 015957
Tyrosinase
Streptomyces phaeochromogenes NCIMB 8505
UDP-4-galactose epimerase
Kluyveromyces marxianus NCYC 100
Urea amydolase
Pichia dispora NCYC 697
Urease
Bacillus pasteurii NCIMB 8841
Hafnia alvei NCIMB 11999
Streptococcus intestinalis NCIMB 13249
Urease
Bacillus pasteurii NCIMB 8841
Bacillus fastidiosus NCIMB 10372
Bacillus fastidosus NCIMB 10424
Bacillus fastidosus NCIMB 10423
Bacillus fastidosus NCIMB 10425
Bacillus fastidosus NCIMB 10372
Vanillate-O-demethylase
Pseudomonas fluorescens NCIMB 12375
Xanthine dehydrogenase
Clostridium cylinosporum NCIMB 12822
Nocardiodes albus NCIMB 11454
Xylan hydrolase
Cryptococcus albidus NCYC 445
Xylanase
Bacillus polymyxa NCIMB 8158
Streptomyces sp. NCIMB 8592
Xylanase (thermostable)
Clostridium stercocarium NCIMB 11754
Yeast cell walls lytic enzymes
Cellulomonas cartar NCIMB 11921
Cytophaga johnsonae NCIMB 10150

Metabolite producing strains

Primary and secondary metabolites including: alcohols, alkaloids, amino acids, peptides and proteins, carbohydrates, hydrocarbons, ketones, lipids and fatty acids, organic acids, pigments, plant hormones and growth regulators, steroids and sterols, toxins, carcinogens, mutagens, teratogens, vitamins, coenzymes, and growth factors.

Acetaldehyde
- *Candida utilis* NCYC 707
- *Cochliobolus spicifer* CABI-IMI 091972

Acetic acid
- *Acetobacter pasteurianus* NCIMB 2224
- Acetobacter pasteurianus NCIMB 5346
- *Acetobacter pasteurianus* NCIMB 5346
- *Acetobacter pasteurianus* NCIMB 6249
- *Acetobacter pasteurianus* NCIMB 7215
- *Acetobacter* sp. NCIMB 1345
- *Lactobacillus brevis* NCIMB 947
- *Lactobacillus pentosus* NCIMB 8026

α-Acetolactate
- *Lactobacillus casei* subsp. *casei* NCIMB 11970

Acetone
- *Clostridium acetobutylicum* NCIMB 2951
- *Clostridium beijerinckii* NCIMB 8052
- *Clostridium saccharoperbutylacetonicum* NCIMB 12605
- *Clostridium saccharoperbutylacetonicum* NCIMB 12606

Acetyl choline
- *Lactobacillus plantarum* NCIMB 7220

3-Acetylvomitoxin
- *Fusarium culmorum* CABI-IMI 014764

6-O-Aetylglucose
- *Bacillus megaterium* NCIMB 8508

1'-O-Aetylpaxilline
- *Emericella striata* CABI-IMI 163899

Acidicolin
- *Verticillium lecanii* CABI-IMI 068689

Aconate
- *Streptomyces noursei* NCIMB 8593

cis-Aconitic acid decarboxylase
- *Aspergillus terreus* CABI-IMI 044243

Acronycine
- *Cunninghamella blakesleena* CABI-IMI 053585
- *Cunninghamella blakesleena* CABI-IMI 063877

Acrylic acid
- *Clostridium propionicum* NCIMB 10656

Actinonin
- *Streptomyces* sp. NCIMB 8845

Actinorhodin
- *Actinoplanes* NCIMB 127366

Actinospectacin
- *Streptomyces flavopersicus* NCIMB13020
- *Streptomyces spectabilis* NCIMB 9733

Actithilaic acid
- *Streptomyces lydicus* NCIMB 12977

Adenosyl 2-methylmethionine
- *Candida utilis* NCYC 707

Adenosyl D—methionine
- *Candida utilis* NCYC 707

Aerolysin
- *Aeromonas hydrophila* subsp. *hydrophila* NCIMB 13190

Aflatoxin
- *Aspergillus flavus* CABI-IMI 190443
- *Aspergillus flavus* CABI-IMI 015957vii
- *Aspergillus flavus* CABI-IMI 091020
- *Aspergillus flavus* CABI-IMI 242693
- *Aspergillus flavus* CABI-IMI 242684
- *Aspergillus flavus* CABI-IMI 015957vi
- *Aspergillus parasiticus* CABI-IMI 091019bi
- *Aspergillus parasiticus* CABI-IMI 015957
- *Aspergillus parasiticus* CABI-IMI 091019biii
- *Aspergillus parasiticus* CABI-IMI 091019bii
- *Aspergillus parasiticus* CABI-IMI 089717
- *Aspergillus parasiticus* CABI-IMI 091019b
- *Aspergillus parasiticus* CABI-IMI 089717ii
- *Aspergillus parasiticus* var. *globosus* CABI- IMI 120920

Aflatoxin M
- *Aspergillus flavus* CABI-IMI 089717

Aflatoxin S
- *Aspergillus flavus* CABI-IMI 089717

Aflatoxin B1 from sterigmatocystin
- *Aspergillus parasiticus* var. *globosus* CABI-IMI 120920

Alanine
- *Clostridium saccharoperbutylacetonicum* NCIMB 12606
- *Clostridium saccharoperbutylacetonicum* NCIMB 12605

Alazopeptin
- *Streptomyces griseoplanus* NCIMB 9811

Alboleersin
- *Bipolaris leersiae* CABI-IMI 089369

Alcohol (industrial production)
- *Rhizopus oryzae* CABI-IMI 309922
- *Rhizopus oryzae* CABI-IMI 309923

Alginate
- *Ascophyllum* sp.
- *Azotobacter vineladii* NCIMB 9068
- *Pseudomonas mendocina* NCIMB 11688

Allegenergic extract
- *Staphylococcus epidermidis* NCIMB 8558

Alpha Dicarboxylic acid
- *Aspergillus fumigatus* CABI-IMI 045338

Altenuic acid
- *Alternaria alternata* CABI-IMI 089344

Alternarin
- *Alternaria alternata* CABI-IMI 089343

Alternariol
- *Alternaria alternata* CABI-IMI 089342
- *Alternaria alternata* CABI-IMI 089343
- *Alternaria alternata* CABI-IMI 089344
- *Alternaria alternata* CABI-IMI 089345
- *Alternaria alternata* CABI-IMI 354944
- *Alternaria alternata* CABI-IMI 354943

Alternariol
Alternaria alternata CABI-IMI 354942
Aernarolmethylether
Alternaria alternata CABI-IMI 354943
Di-fumaryl alanine
Penicillium resticulosum CABI-IMI 040227
Alternuene
Alternaria alternata CABI-IMI 354943
Altertoxin
Alternaria alternata CABI-IMI 354943
Alternaria alternata CABI-IMI 354945
Amicetin
Steptomyces plicatus NCIMB 11305
Amicetin and amicetin B
Streptomyces vinaceusdrappus NCIMB 12980
Amino acids
Corneybacterium lilium NCIMB 10337
4-Aminoxabicyclo4,1,0hept3enee2,5,dione3 carboxamide
Streptomyces sp. NCIMB 11306
Aminocidin E
Streptomyces chrestomyceticus NCIMB 8995
Streptomyces fradiae subsp. italicus NCIMB 11002
5-Amino-imidazole carboxyamide riboside
Corneybacterium NCIMB 10266
p-Aminobenzoic acid
Clostridium beijerinckii NCIMB 8052
Amodin A
Aspergillus amstelodami CABI-IMI 071295
Amodin B
Aspergillus amstelodami CABI-IMI 071295
Amylovorin
Erwina amylovora
Anatoxin-a
Anabaena sp. CCAP 1403/21
Anditesin C
Aspergillus variecolor CABI-IMI 060316
Anditomin
Aspergillus variecolor CABI-IMI 060316
Angolide
Pithomyces cynodontis CABI-IMI 101184
Pithomyces sacchari CABI-IMI 102686
Anhydro-fusarubin
Fusarium moniliforme CABI-IMI 316818
Fusarium moniliforme CABI-IMI 316819
Fusarium moniliforme CABI-IMI 316821
Fusarium moniliforme CABI-IMI 316822
Fusarium moniliforme CABI-IMI 316823
Anhydro-fusarubin
Fusarium moniliforme CABI-IMI 316816
Fusarium moniliforme CABI-IMI 316817
Fusarium moniliforme CABI-IMI 316820
Antheridiol (mycohormone)
Achyla bisexualis CABI-IMI 141473
Aphidicolin
Verticillium lecanii CABI-IMI 068689ii
Aphidicolin
Cephalosporium aphidicola CABI-IMI 68689ii
Apramycin
Streptoaloteichus hindustanus NCIMB 12539
Arthrobactin
Arthrobacter pascens NCIMB 8910
Arugosin
Aspergillus rugulosus CABI-IMI 084338
Aspergillin
Aspergillus niger CABI-IMI 096215
Asperphenamate
Penicillium canadense CABI-IMI 095493
Asperthecin
Aspergillus quadrilineatus CABI-IMI 089348
Aspergillus quadrilineatus CABI-IMI 089349
Astaxanthin
Haematococcus lacustris CCAP 34/ID; CCAP 34/IF
Haematococcus pluvialis CCAP 34/6; CCAP 34/7; CCAP 34/8
Phaffia rhodozyma NCYC 874
Asterric acid
Penicillium glabrum CABI-IMI 096659
Asterriquinone
Aspergillus terreus CABI-IMI 044243
Atrovenetin
Penicillium herquei CABI-IMI 112950
Penicillium herquei CABI-IMI 028809
Penicillium melinii CABI-IMI 061835
Penicillium melinii CABI-IMI 061836
Penicillium melinii CABI-IMI 061837
Auranthine
Penicillium aurantiogriseum CABI-IMI 180922
Aurantiamine
Penicillium aurantiogriseum CABI-IMI 357289
Penicillium aurantiogriseum CABI-IMI 357290
Penicillium aurantiogriseum CABI-IMI 357291
Penicillium freii CABI-IMI 357296
Penicillium freii CABI-IMI 357297
Penicillium freii CABI-IMI 357398
Penicillium neoechinulatum CABI-IMI 357302
Aurasperone a
Aspergillus niger CABI-IMI 297707
Aurasperone b
Aspergillus niger CABI-IMI 297707
Aurasperone c
Aspergillus niger CABI-IMI 297707
Aurasperone d
Aspergillus niger CABI-IMI 297707
Aurasperone (iso)
Aspergillus niger CABI-IMI 297707
Aurofusarin
Fusarium culmorum CABI-IMI 089367
Fusarium culmorum CABI-IMI 092033
Fusarium culmorum CABI-IMI 089364
Fusarium culmorum CABI-IMI 089365
Fusarium culmorum CABI-IMI 089366
Auroglaucin
Aspergillus amstelodami CABI-IMI 092026
Aspergillus chevalieri CABI-IMI 091867

Auroglaucin
- *Aspergillus niveoglaucus* CABI-IMI 091871
- *Aspergillus pseudoglaucus* CABI-IMI 016122ii
- *Aspergillus pseudoglaucus* CABI-IMI 091873
- *Aspergillus pseudoglaucus* CABI-IMI 016122
- *Aspergillus pseudoglaucus* CABI-IMI 091865
- *Aspergillus repens* CABI-IMI 016144ii
- *Aspergillus repens* CABI-IMI 094150
- *Aspergillus repens* CABI-IMI 016131
- *Aspergillus repens* CABI-IMI 016114
- *Aspergillus repens* CABI-IMI 091894
- *Aspergillus repens* CABI-IMI 091869
- *Aspergillus repens* CABI-IMI 091901
- *Aspergillus repens* CABI-IMI 091900

Austdiol
- *Aspergillus ustus* CABI-IMI

Austocystin
- *Aspergillus ustus* CABI-IMI

Avermectin
- *Streptomycs avermitilis* NCIMB 12804

Averufin
- *Aspergillus parasiticus* var. *globosus* CABI-IMI 120920

Ayfactin A
- *Streptomyces viridifaciens* NCIMB 8954

Ayfactin B
- *Streptomyces viridifaciens* NCIMB 8954

Azacolutin
- *Streptomyces cinnamenous* subsp. *forma azacoluta* NCIMB 12681

Azapurin derrevatives
- *Corneybacterium ammoniagenes* NCIMB 8143

L-Azetidine-2-carboxylic acid
- *Actinoplanes ferrugineus* NCIMB12644

Azurin
- *Alcaligenes xylosoxidans* NCIMB 11015

Base from lactic acid
- *Penicillium verrucosum* CABI-IMI 297964
- *Penicillium verrucosum* CABI-IMI 297964

Base from creatine
- *Penicillium verrucosum* CABI-IMI 297964

Beauvericin
- *Beauveria bassiana* CABI-IMI

Benzophenone
- *Aspergillus terreus* CABI-IMI 16043

Bikaverin (antiprotozoal)
- *Fusarium oxysporum* CABI-IMI 250086

Biotin
- *Sporidiobolus salmonicolor* NCYC 1442
- *Sporidiobolus pararoseus* NCYC 1443
- *Sporidiobolus pararoseus* NCYC 1428

Bisdechlorogeodin
- *Penicillium glabrum* CABI-IMI 096659

Bongkekic acid
- *Pseudomonas farinofermentans* NCIMB 12451
- *Pseudomonas cocovenanans* NCIMB 9450

Borrelidin
- *Streptomyces* sp. NCIMB 9755

Botrydial
- *Botrytis cinerea* CABI-IMI 124882

Botulinum Neurotoxin
- *Clostridium botulinum* Contact NCTC

Botulinum toxin A
- *Clostridium botulinum* Contact NCTC

Botulinum toxin B
- *Clostridium botulinum* Contact NCTC

Botulinum toxin C1
- *Clostridium botulinum* Contact NCTC

Botulinum toxin C2
- *Clostridium botulinum* Contact NCTC

Botulinum toxin D
- *Clostridium botulinum* Contact NCTC

Botulinum toxin E
- *Clostridium botulinum* Contact NCTC

Botulinum toxin F
- *Clostridium botulinum* Contact NCTC

Botulinum toxin G
- *Clostridium botulinum* Contact NCTC

Brefeldin
- *Penicillium aurantiogriseum* CABI-IMI 280215

Brefeldin A
- *Penicillium simplicissium* CABI-IMI 378412
- *Penicillium verrucosum* CABI-IMI 297964

Brevianamide A
- *Penicillium verrucosum* CABI-IMI 297964
- *Penicillium viridicatum* CABI-IMI 39758ii
- *Penicillium viridicatum* CABI-IMI 293187
- *Penicillium viridicatum* CABI-IMI 039758
- *Penicillium brevicompactum* CABI-IMI 040225

Brevianamide B
- *Penicillium brevicompactum* CABI-IMI 040225
- *Penicillium viridicatum* CABI-IMI 293187
- *Penicillium viridicatum* CABI-IMI 039758ii
- *Penicillium verrucosum* CABI-IMI 297964

Brevianamide M
- *Penicillium verrucosum* CABI-IMI 297964

Bundlin
- *Streptomycin griseofucus* NCIMB 9821

2-3-Butanediol
- *Aromonas hydrophila* subsp. *hydrophila* NCIMB 9240
- *Bacillus licheniformis* NCIMB 6346
- *Bacillus licheniformis* NCIMB 8059
- *Bacillus licheniformis* NCIMB 8061
- *Bacillus polymyxa* NCIMB 8524
- *Bacillus polymyxa* NCIMB 8526
- *Enterobacter cloacae* NCIMB 8530
- *Enterobacter cloacae* NCIMB 8529
- *Klebsiella oxytoca* NCIMB 8017

Butanol
Clostridium saccharoperbutylacetonicum NCIMB 12606
Butenolide
Fusarium tricinctum CABI-IMI 309352
Butenolide (on corngrits agar)
Fusarium culmorum CABI-IMI 309344
Fusarium culmorum CABI-IMI 309345
Fusarium sporotrichioides CABI-IMI 309349
Butenolide (on YES agar)
Fusarium trincinctum CABI-IMI 309352
Fusarium trincinctum CABI-IMI 309351
Butirosin
Bacillus circulans NCIMB 12335
Bacillus circulans NCIMB 12336
n-Butyl alcohol
Clostridium acetobutylicum NCIMB 2951
Clostridium acetobutylicum NCIMB 8052
Clostridium acetobutylicum NCIMB 2951
Clostridium beijerinckii NCIMB 8052
Clostridium saccharoperbutylacetonicum NCIMB 12605
Clostridium saccharoperbutylacetonicum NCIMB 12605
Butyrate
Clostridium celerecrescens NCIMB 12829
Iso-Butyronitrile
Neisseriaceae sp. NCIMB 11651
Neisseriaceae sp. NCIMB 11650
Pseudomonas sp. NCIMB 11652
Iso-Butyraldoxime O-methyl ether
Neisseriaceae sp. NCIMB 11650
Neisseriaceae sp. NCIMB 11651
Pseudomonas sp. NCIMB 11652
Pseudomonas sp. NCIMB 11653
Psychrobacter immobilis NCIMB 11651
Psychrobacter immobilis NCIMB11650
Pseudomonas sp. NCIMB 11652
Pseudomonas sp. NCIMB 11653
Byssochlamic acid
Byssochlamys fulva CABI-IMI 040021
trans-Carveol (production from alpha pinene)
Pseudomonas sp. NCIMB 10687
Pseudomonas putida NCIMB 10684
C16-Ene-diyne
Lentinus degeneri CABI-IMI 110525
$C_{10}H_{10}O_4$
Oidiodendron rhodogenum CABI-IMI 235256
$C_{15}H_{18}O_4$
Oidiodendron rhodogenum CABI-IMI 235256
$C_{21}H_{28}O_{10}$ triacetate(trichothecene)
Fusarium scirpi CABI-IMI 045490
$C_{19}H_{24}O_9$ diacetate(trichothecene)
Fusarium scirpi CABI-IMI 045490
$C_{27}H_{28}N_2O_4$
Aspergillus glaucus CABI-IMI 053242
$C_{29}H_{35}NO_5$
Helminthosporium dematioideum CABI-IMI 074812
$C_{11}H_{10}O_5$
Aspergillus nidulans CABI-IMI 238850
$C_{11}H_{11}ClO_4$
Periconia macrospinosa CABI-IMI 024411
$C_{14}H_{12}O_6$
Cylindrocarpon sp. CABI-IMI 127996
Caffeine
Claviceps sp. CABI-IMI 380290*
Calonectrin
Fusarium culmorum CABI-IMI 014764
Calonectria nivalis CABI-IMI 014764
Canarius
Streptomyces canarius NCIMB 9468
Candicidin
Strepomycin griseus NCIMB 8891
Candidulin
Aspergillus candidus CABI-IMI 078734
β di Carboxylic acid
Aspergillus fumigatus CABI-IMI 045338
Carlosic acid
Penicillium verrucosum CABI-IMI 297964
2-Carboxy-3,5-dihydroxyphenyl acetyl carbinol
Penicillium verrucosum CABI-IMI 297964
Carlosic acid
Penicillium verrucosum CABI-IMI 297964
Carolic acid
Penicillium verrucosum CABI-IMI 297964
Carotene
Dunaliella salina CCAP 19/18
Dunaliella salina CCAP 19/25
Dunaliella sp. CCAP 19/12
Dunaliella sp. CCAP 17/15
β Carotenes
Blakeslea trispora CABI-IMI 195169
Carotenoids
Streptomyces mediolani NCIMB 10969
Neurospora crassa CABI-IMI 075722
Catenarin
Pyrenophora graminea CABI-IMI 089368
Dreschlera catenaria CABI-IMI 91984
Celesticetin
Streptomyces caelestis NCIMB 9751
Cell vibriocins
Cellvibrio sp. NCIMB 9914
Cellvibrio sp. NCIMB 9916
Cellvibrio sp. NCIMB 9915
Cellostatin
Streptomyces cellostaticus NCIMB 9830
Cellulose
Acetobacter aceti NCIMB 8940
Acetobacter aceti NCIMB 8132
Acetobacter pasteurians NCIMB 8945
Acetobacter pasteurians NCIMB 7029
Acetobacter pasteurianus NCIMB 5346
Cercosporin
Cercospora beticola CABI-IMI 077043
Cheaotoglobosin C
Penicillium verrucosum CABI-IMI 297964

Chlorine containing
Periconia macrospinosa CABI-IMI 024411
Chloroisoxazoline amino acid anti tumour
Streptomyces sviceus NCIMB 11184
3-Chloro 5,2dihydroxy3,7,8-trimethoxyflavone
Aspergillus candidus CABI-IMI 127260
Chlororaphin
Pseudomonas chlororaphis NCIMB 9392
Pseudomonas chlororaphis NCIMB 9402
Cholera toxin
Vibrio cholerae Contact NCTC
Cholic acid
Aspergillus nidulans CABI-IMI 227976
Chromismic acid
Kleibsiella pnumoniae subsp. *pnumoniae* NCIMB 11153
Chrysogenin
Penicillium chrysogenum CABI-IMI 039759
Penicillium chrysogenum CABI-IMI 024314
Penicillium griseoroseum CABI-IMI 015378
Chrysophanol
Penicillium islandicum CABI-IMI 092277
Penicillium kloeckeri CABI-IMI 040047
Citreoviridin
Aspergillus terreus var. aureus CABI-IMI 082431
Citric acid
Arthrobacter parraffineus NCIMB 10699
Aspergillus carbonarius CABI-IMI 041873
Aspergillus carbonarius CABI-IMI 041875
Aspergillus foetidus CABI-IMI 041871
Aspergillus foetidus CABI-IMI 015954
Aspergillus niger CABI-IMI 084304
Aspergillus niger CABI-IMI 297707
Candida catenulata NCYC 1369
Candida famata NCYC 389
Chaetomium globosum CABI-IMI 016203
Chaetomium globosum CABI-IMI 045550ii
Dekkera bruxellensis NCYC 395
Rhodococcus rhodoschrous NCIMB 11277
Yarrowia lipolytica NCYC 825
Citric acid (from beet molasses)
Aspergillus niger CABI-IMI 027809
Aspergillus niger CABI-IMI 075353
Aspergillus niger CABI-IMI 041874
Citric acid (in submerged culture)
Aspergillus niger CABI-IMI 051433
Iso Citric acid
Yarrowia lipolytica NCYC 825
Citrinin
Aspergillus carneus CABI-IMI 135818
Aspergillus terreus CABI-IMI 016043
Penicillium citrinum CABI-IMI 024306
Penicillium citrinum CABI-IMI 092196
Penicillium citrinum CABI-IMI 091961
Penicillium citrinum CABI-IMI 024307
Penicillium expansum CABI-IMI 293192
Penicillium hirsutum CABI-IMI 293183
Penicillium hirsutum CABI-IMI 293206
Citrinin
Penicillium verrucosum CABI-IMI 293203
Penicillium verrucosum CABI-IMI 297964
Citromycetin
Penicillium glabrum CABI-IMI 091944
Penicillium glabrum CABI-IMI 028043
Penicillium glabrum CABI-IMI 091914
Penicillium glabrum CABI-IMI 091945
Clorflavonin (patented strain)
Aspergillus candidus CABI-IMI 127260
Cobalanim
Bacillus megaterium NCIMB 8508
Cobamides
Eubacterium limnosum NCIMB 9554
Colominic acid
Escherichia coli NCIMB 10582
Comiron
Pseudomonas antimycetica NCIMB 8641
Coproporphyrinogen III
Rhodobacter capsulatus NCIMB 10006
Cosyntheticfactor 1
Streptomyces aurofaciens NCIMB 12716
Crotocin
Acremonium crotocinigenum CABI-IMI 112775
Culmorin
Fusarium culmorum CABI-IMI 089365
Fusarium culmorum CABI-IMI 014764
Calonectria nivalis CABI-IMI 014764
Culmorone
Fusarium culmorum CABI-IMI 014764
Curvulin
Bipolaris papendorfii CABI-IMI 075861
Curvulinic acid
Bipolaris papendorfii CABI-IMI 075861
Oidiodendron rhodogenum CABI-IMI 235256
Cyanocobalamine
Steptomyces olivaceus NCIMB 8238
7-Cyanosteroides
Nocardioides simplex NCIMB 8929
Cycloheximide
Streptomyces griseus NCIMB 8232
Streptomyces griseus NCIMB 8591
Cyclopaldic acid
Penicillium aurantiogriseum CABI-IMI 089375
Penicillium aurantiogriseum var *album* CABI-IMI 089312
Cyclopenin
Penicillium atramentosum CABI-IMI 089374
*Penicillium crustosum*CABI-IMI 293182
*Penicillium echinulatum*CABI-IMI 293180
Penicillium solitum CABI-IMI 293181
Penicillium aurantiovirens CABI-IMI 357292
Penicillium aurantiovirens CABI-IMI 357293
Penicillium cyclopium CABI-IMI 357294
Penicillium cyclopium CABI-IMI 357295
Penicillium freii CABI-IMI 357296
Penicillium freii CABI-IMI 357297
Penicillium freii CABI-IMI 357298

Cyclopenin
- *Penicillium neoechinulatum* CABI-IMI 357302
- *Penicillium polonicum* CABI-IMI 357303
- *Penicillium polonicum* CABI-IMI 357304
- *Penicillium polonicum* CABI-IMI 357305

Cyclopenol
- *Penicillium aurantiogriseum* CABI-IMI 089374
- *Penicillium echinulatum*CABI-IMI 293180
- *Penicillium verrucosum*CABI-IMI 297964
- *Penicillium aurantiovirens* CABI-IMI 357292
- *Penicillium aurantiovirens* CABI-IMI 357293
- *Penicillium cyclopium*CABI-IMI 357294
- *Penicillium cyclopium* CABI-IMI 357295
- *Penicillium freii* CABI-IMI 357296
- *Penicillium freii* CABI-IMI 357297
- *Penicillium freii* CABI-IMI 357298
- *Penicillium neoechinulatum* CABI-IMI 357302
- *Penicillium polonicum* CABI-IMI 357303
- *Penicillium polonicum* CABI-IMI 357304
- *Penicillium polonicum* CABI-IMI 357305

Cyclopeptin
- *Penicillium aurantiovirens* CABI-IMI 357292
- *Penicillium aurantiovirens* CABI-IMI 357293
- *Penicillium cyclopium*CABI-IMI 357294
- *Penicillium cyclopium* CABI-IMI 357295
- *Penicillium freii* CABI-IMI 357296
- *Penicillium freii* CABI-IMI 357297
- *Penicillium freii* CABI-IMI 357298
- *Penicillium neoechinulatum* CABI-IMI 357302
- *Penicillium polonicum* CABI-IMI 357303
- *Penicillium polonicum* CABI-IMI 357304
- *Penicillium polonicum* CABI-IMI 357305

α Cyclopiazonic acid
- *Penicillium verrucosum*CABI-IMI 297964

Cyclopiazonic acid
- *Aspergillus oryzae* CABI-IMI 309454
- *Penicillium commune* CABI-IMI 293201
- *Penicillium commune* CABI-IMI 293179
- *Penicillium griseofulvum*CABI-IMI 293195
- *Penicillium solitum* CABI-IMI 293210

Cyclopolic acid
- *Penicillium aurantiogriseum* var *album* CABI-IMI 089312
- *Penicillium aurantiogriseum* CABI-IMI 089375

Cynodotin
- *Cochliobolus lunatus*CABI-IMI 061535

Cytochalasin C
- *Metarhizium anisopliae* CABI-IMI 097373

Cytochalasin D
- *Metarhizium anisopliae* CABI-IMI 097373

Cytochalasin E
- *Rosellinia necatrix* CABI-IMI 108006

Cytochalasin G
- *Pseudeurotium zonatum*CABI-IMI 171019

Cytochalasins
- *Drechslera dematioidea* CABI-IMI 074812

Cytochrome P-450
- *Saccharomyces cerevisiae* NCYC 754

DD-Diaminopimelic acid (preparation of)
- *Bacillus megaterium*NCIMB 11251

Deacetylcalonectrin
- *Calonectria nivalis* CABI-IMI 014764

3Deactylcalonectrin(trichothecin)
- *Fusarium culmourm* CABI-IMI 014764

15Deacetyl8oxocalonectrin
- *Fusarium culmorum* CABI-IMI 014764

Decoynin
- *Streptomyces hygroscopius* subsp. *decoyicus* NCIMB 1052

Deoxyherqueinone
- *Penicillium herquei*CABI-IMI 028809

Deoxynivalenol (on YES agar)
- *Fusarium culmorum* CABI-IMI 309344
- *Fusarium gramiearum* CABI-IMI 285824
- *Fusarium gramiearum* CABI-IMI 285825
- *Fusarium gramiearum* CABI-IMI 285826

17-Deoxysteroids
- *Streptomyces olivaceus*NCIMB 8238

Desacetylpebrolide
- *Penicillium verrucosum* CABI-IMI 297964

Desferritriacetylfusigen
- *Aspergillus deflectus* CABI-IMI 061448

Desideus
- *Streptomyces griseus* subsp. *desideus* NCIMB 9444

Desmethoxyviridin
- *Nodulisporium hinnuleum*CABI-IMI 214826

Desoxyrosenonolactone
- *Trichothecium roseum* CABI-IMI 129425

Dessferri-ferricrocin
- *Aspergillus viridinutans* CABI-IMI 062875
- *Aspergillus viridinutans* CABI-IMI 062875ii

Detoxins
- *Streptomyces mobaraewnsis* NCIMB 11159

Dextran
- *Leuconostic pseudomesenteroides* NCIMB 8699
- *Leuconostoc citreum*NCIMB 3351
- *Leuconostoc mesenteroides* subsp. *Dextranicum* NCIMB 8723
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 6109
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 3352
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 3739
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 8029
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 8590
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 8710

Dextran
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 8724
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 3354

Dextran (class A)
- *Leuconostoc mesenteroides* subsp. *dextranicum* NCIMB 3355
- *Leuconostoc mesenteroides* subsp. *dextranicum* NCIMB 9260
- *Leuconostoc mesenteroides subsp. dextranicum* NCIMB 9313
- *Leuconostoc mesenteroides* subsp. *dextranicum* NCIMB 9312
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 8710
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 8172
- *Weissella confusa* NCIMB 9311

Dextran (class B)
- *Leuconostoc mesenteroides* subsp. *cremoris* NCIMB 8189
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 9314
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 9315
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 9316

Dextran (class C)
- *Leuconostoc mesenteroides* subsp. *dextranicum* NCIMB 2706
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 6992
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 8023
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 8013
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 9319
- *Leuconostoc mesenteroides* subsp. *mesenteroides* NCIMB 8015

Dextran (Injections)
- *Leuconostoc* mesenteroides NCTC 10817

Dextran-like polyglucose
- *Lactobacillus brevis* NCIMB 8664

Diacetoxysciripenol
- *Fusarium scirpi* CABI-IMI 112503
- *Fusarium scirpi* CABI-IMI 045490
- *Fusarium equiseti* CABI-IMI 035100

Diacetoxyscirpenol (on YES agar)
- *Fusarium sporotrichioides* CABI-IMI 309350
- *Fusarium sporotrichioides* CABI-IMI 309349

Diacetyl
- *Lactobacillus lactis* subsp. *lactis* NCIMB 8763

Dialkylresorcinol
- *Stemphylium majusculum* CABI-IMI 135459

Diaminopimelic acid
- *Escherichia coli* NCIMB 9342

Dienes
- *Nocardiodes simplex* NCIMB 8929

Dihydrobotrydial
- *Botrytis cinerea* CABI-IMI 124882

Dihydrocitrinone
- *Aspergillus carneus* CABI-IMI 135818

Dihydroshikimic acid
- *Lactobacillus collinoides* NCIMB 8847
- *Lactobacillus collinoides* NCIMB 8848

Dihydroxyacetone
- *Gluconobacter oxydans* NCIMB 621
- *Gluconobacter oxydans* NCIMB 8035
- *Gluconobacter oxydans* NCIMB 7069

7,8-Dihydroxycalonectrin
- *Fusarium culmorum* CABI-IMI 014764

3:5 Dihydroxycarboxyphenylacetylcarbinol acid
- *Penicillium brevicompactum* CABI-IMI 039824

Dihydro paraherquimide
- *Penicillium* sp. CABI-IMI 332995

5,6,Dimethyoxysterigmatocystin
- *Aspergillus* sp. CABI-IMI 069857

2,6 Dimethyl-3-methxy pyrazine
- Unnamed NCIMB 11802

2-5-Diketogluconate
- *Gluconobacter* sp. NCIMB 9099

3,15DIOH12,13Eopxytrichothec9ene
- *Fusarium culomurm* CABI-IMI 014764

3,15DIOH12,13Epoxytrichothec9en8one
- *Fusarium culomurm* CABI-IMI 014764

7,8-DIOHcalonectrin
- *Fusarium culomurm* CABI-IMI 014764

Dipicolinic acid
- *Penicillium verrucosum* CABI-IMI 297964

Diversonol
- *Penicillium diversum* CABI-IMI 040579
- *Penicillium diversum* CABI-IMI 040579iii

Duclauxin
- *Penicillium emmonsii* CABI-IMI 038805ii
- *Penicillium emmonsii* CABI-IMI 038805iii

Echinulin by isoprenylation of cycl l-alanyl-l-tryptophanyl
- *Aspergillus amstelodami* CABI-IMI 211806

Eicosapentanoic acid (EPA)
- *Chlorella minutissima* CCAP 211/52
- *Phaeodactylum tricornutum* CCAP 1052/1B

Elaiophylin
- *Streptomyces melanosporofaciens* NCIMB 12978

Elizabethin
- *Streptomyces elizabethii* NCIMB 11545

Emericellopsin A
- *Emericellopsis minima* CABI-IMI 069015

Emericellopsin B
- *Emericellopsis minima* CABI-IMI 069015

Emodic acid
- *Penicillium albicans* CABI-IMI 089373

Enterotoxin
- *Escherichia coli* NCIMB 13002
- *Escherichia coli* NCIMB 13003

Enterotoxin
- *Escherichia coli* NCIMB 13001

Equisetin
- *Fusarium equiseti* CABI-IMI 309348
- *Fusarium equiseti* CABI-IMI 309347

Erdin
Aspergillus terreus CABI-IMI 044339
Eremofortin
*Penicillium roquefortii*CABI-IMI 024313
Ergochrome secalonic acid D
Penicillium oxalicum CABI-IMI 039750
Ergokryptine
*Claviceps purpurea*CABI-IMI 126133
Ergosterol
Gaeumannomyces graminis CABI-IMI 083849
Penicillium aurantiogriseum CABI-IMI 034913ii
Penicillium aurantiogriseum CABI-IMI 034913iii
Penicillium verrucosum CABI-IMI 297964
Saccharomyces cerevisiae NCYC 667
Saccharomyces cerevisiae NCYC 73
Saccharomyces cerevisiae NCYC 74
Ergosterol and ubiquinone
Candida tropicalis NCYC 997
Ergosterol palmitate
Penicillium italicum CABI-IMI 091959
Penicillium crustosum CABI-IMI 092235
Ergotamine
Claviceps purpurea CABI-IMI 104437
Erythroglaucin
*Aspergillus chevalieri*CABI-IMI 091867
Aspergillus echinulatus CABI-IMI 091866
Aspergillus echinulatus CABI-IMI 091872
Aspergillus echinulatus CABI-IMI 091860
Aspergillus niveoglaucus CABI-IMI 091871
Aspergillus niveoglaucus CABI-IMI 032050
Aspergillus niveoglaucus CABI-IMI 032050ii
Aspergillus ruber CABI-IMI 091864
Aspergillus ruber CABI-IMI 092030
Aspergillus ruber CABI-IMI 092043
Aspergillus ruber CABI-IMI 094147
Aspergillus ruber CABI-IMI 091863
Aspergillus ruber CABI-IMI 091868
Aspergillus ruber CABI-IMI 091862
Aspergillus umbrosus CABI-IMI 091861
Erythroskyrin
Penicillium islandicum CABI-IMI 092038
Penicillium islandicum CABI-IMI 092270
*Penicillium islandicum*CABI-IMI 092277
Esters in milk
Pseudomonas fragi NCIMB 10476
Pseudomonas fragi NCIMB 10475
Ethanol
Kluyveromyces marxianus NCYC 179
Kluyveromyces marxianus NCYC 100
Saccharomyces cerevisiae NCYC 975
Saccharomyces cerevisiae NCYC 73
Saccharomyces cerevisiae NCYC 87
Schizosaccharomyces pombe NCYC 132
Zymomonas mobilis subsp *mobilis* NCIMB 8938
Ethisolide
Penicillium implicatum CABI-IMI 138002
Ethyl acetate
Candida utilis NCYC 707
Penicillium digitatum CABI-IMI 091956
Pichia anomala NCYC 682
Ethyl alcohol
Clostridium acetobutylicum NCIMB 8049
Penicillium digitatum CABI-IMI 091956
Cochliobolus spicifer CABI-IMI 091972
Ethylene oxide
Aspergillus fumigatus CABI-IMI 045338
Ethyleneoxide dicarboxylic acid
Paecilomyces variotii CABI-IMI 058427
Eugenitin
Cylindrocarpon sp. CABI-IMI 127996
Eurocidin
Streptomyces albireticuli NCIMB 9600
Equestin
Fusarium equiseti CABI-IMI 309348
Exopolysaccharide
Alteromonas atlantica NCIMB 1865
Alteromonas atlantica NCIMB 1864
Exotoxin
Pseudomonas aeruginosa NCIMB 11965
Extracellular polysaccharide
Paecilomyces lilacinus CABI-IMI 280556
F2 Toxin
Fusarium graminearum CABI-IMI 155426
Fusarium graminearum CABI-IMI 152103
Fat
Penicillium indonesiae CABI-IMI 039733
Fatty acids from decane
Pseudomonas proteofaciens NCIMB 10204
Ferridoxin
Clostridium pasteurianum NCIMB 9486
Ferrioxamine A-G
Streptomyces pilosus NCIMB 9612
Fibrinolysin
Streptococcus pyogenes NCIMB 8885
Flavasperone
Aspergillus niger CABI-IMI 297707
Flavicin
Aspergillus flavus CABI-IMI 111023
Flavin adenine dinucleotide
Brevibacterium linens NCIMB 8546
Microbacterium arborescens NCIMB 8185
Sphingomonas paucimobilis NCIMB 8195
Flaviolin
*Aspergillus foetidus*CABI-IMI 015954
*Phialophora lagerbergii*CABI-IMI 096745
Flavipin
Epicoccum nigrum CABI-IMI 068797
Aspergillus terreus CABI-IMI 089358
Fennellia flavipes CABI-IMI 089347
Flavoglaucin
Aspergillus amstelodami CABI-IMI 092026
Aspergillus chevalieri CABI-IMI 091867

Flavoglaucin
- *Aspergillus echinulatus* CABI-IMI 091860
- *Aspergillus echinulatus* CABI-IMI 091866
- *Aspergillus echinulatus*CABI-IMI 091872
- *Aspergillus glaucus*CABI-IMI 091870
- *Aspergillus niveoglaucus* CABI-IMI 032050
- *Aspergillus niveoglaucus* CABI-IMI 091871
- *Aspergillus niveoglaucus* CABI-IMI 032050ii
- *Aspergillus pseudoglaucus* CABI-IMI 016122
- *Aspergillus pseudoglaucus* CABI-IMI 091865
- *Aspergillus pseudoglaucus* CABI-IMI 016122ii
- *Aspergillus pseudoglaucus* CABI-IMI 091873
- *Aspergillus repens* CABI-IMI 091901
- *Aspergillus repens* CABI-IMI 094150
- *Aspergillus repens* CABI-IMI 016144ii
- *Aspergillus repens* CABI-IMI 016131
- *Aspergillus repens* CABI-IMI 091900
- *Aspergillus repens* CABI-IMI 091894
- *Aspergillus repens* CABI-IMI 091869
- *Aspergillus repens* CABI-IMI 016114
- *Aspergillus ruber* CABI-IMI 091868
- *Aspergillus ruber* CABI-IMI 092030
- *Aspergillus ruber* CABI-IMI 091864
- *Aspergillus ruber* CABI-IMI 094147
- *Aspergillus ruber* CABI-IMI 091863
- *Aspergillus ruber* CABI-IMI 091862
- *Aspergillus umbrosus* CABI-IMI 091861
- *Aspergillus umbrosus* CABI-IMI 091874

Flavoskyrin
- *Penicillium islandicum* CABI-IMI 092277

Fonsecin
- *Aspergillus carbonarius* CABI-IMI 041875
- *Aspergillus niger* CABI-IMI 297707

Fonsecin monomethylether
- *Aspergillus niger* CABI-IMI 297707

Formate
- *Aeromonas caviae* NCIMB 9235

Frequentin
- *Penicillium palitans* CABI-IMI 040215
- *Penicillium verrucosum* CABI-IMI 297964

Fructose (preparation of)
- *Gluconobacter oxydans* NCIMB 8034
- *Gluconobacter oxydans* NCIMB 7069
- *Gluconobacter oxydans* NCIMB 621

Fucidin
- *Acremonium fusidioides*CABI-IMI 087758
- *Acremonium fusidioides*CABI-IMI 094124
- *Acremonium fusidioides*CABI-IMI 087757
- *Fusarium coccineum*CABI-IMI 087759

Fulvic acid
- *Penicillium dodgei* CABI-IMI 040592ii
- *Penicillium dodgei* CABI-IMI 040592

Fulvic acid
- *Penicillium griseofulvum* CABI-IMI 092273
- *Penicillium verrucosum* CABI-IMI 297964

Fumagillin
- *Aspergillus fumigatus*CABI-IMI 069714

Fumaric acid
- *Aspergillus terreus* CABI-IMI 089356

Fumigacin
- *Aspergillus fumigatus* CABI-IMI 045338

Fumigatin
- *Aspergillus fumigatus* CABI-IMI 045338
- *Aspergillus fumigatus* CABI-IMI 089353

Fumitremorgin B
- *Aspergillus caespitosus*CABI-IMI 016034ii
- *Aspergillus caespitosus*CABI-IMI 016034

Fungichromin
- *Streptomyces cinnamoneus* NCIMB 8851

Fungitoxic alcohols and fatty acid
- *Aspergillus niger* CABI-IMI 050565ii

Fumonisin B1
- *Fusarium moniliforme* CABI-IMI 350432
- *Fusarium moniliforme* CABI-IMI 350433
- *Fusarium moniliforme* CABI-IMI 350434
- *Fusarium moniliforme* var *intermedium* CABI-IMI 350435
- *Fusarium moniliforme* var *intermedium* CABI-IMI 350436
- *Fusarium moniliforme* CABI-IMI 350437

Fumonisin B2
- *Fusarium moniliforme* CABI-IMI 350432
- *Fusarium moniliforme* CABI-IMI 350433
- *Fusarium moniliforme* CABI-IMI 350434
- *Fusarium moniliforme* var *intermedium* CABI-IMI 350435
- *Fusarium moniliforme* var *intermedium* CABI-IMI 350436
- *Fusarium moniliforme* CABI-IMI 350437

Fusarenone
- *Fusarium crookwellense* CABI-IMI 334774

Fusarin C
- *Fusarium crookwellense* CABI-IMI 334774
- *Fusarium moniliforme* CABI-IMI 350432
- *Fusarium moniliforme* CABI-IMI 350433
- *Fusarium moniliforme* CABI-IMI 350434
- *Fusarium moniliforme* var *intermedium* CABI-IMI 350435
- *Fusarium moniliforme* var *intermedium* CABI-IMI 350436
- *Fusarium moniliforme* CABI-IMI 350437

Fuscin
- *Oidiodendron rhodogenum* CABI-IMI 235256
- *Oidiodendron tenuissinum* CABI-IMI 089370
- *Oidiodendron tenuissinum* CABI-IMI 089371

Fusel alcohol
- *Saccharomyces cerevisiae* NCYC 956

Fusidic acid
Verticillium lamellicola CABI-IMI 093439
Galactocarlose
Penicillium fellutanum CABI-IMI 040232
Gentisic acid
Penicillium verrucosum CABI-IMI 297964
Gentisyl alcohol
Penicillium griseofulvum CABI-IMI 034909
Penicillium verrucosum CABI-IMI 297964
Geodin
Aspergillus terreus CABI-IMI 044339
Gibberellic acid
*Fusarium avanaceum*CABI-IMI 137728
Fusarium lateritium CABI-IMI 250545
Fusarium moniliforme CABI-IMI 058289
Fusarium sambucinum CABI-IMI 273414
Fusarium sambucinum CABI-IMI 267789
Giberella fujikuroi CABI-IMI 300793
Giberella fujikuroi CABI-IMI 300791
Giberella fujikuroi CABI-IMI 300792
Giberella fujikuroi var. *intermedia* CABI-IMI 300796
Giberella fujikuroi var. *intermedia* CABI-IMI 300795
Giberella fujikuroi var. *intermedia* CABI-IMI 300794
Giberella fujikuroi var. *intermedia* CABI-IMI 300789
Giberella fujikuroi var. *moniliformis* CABI-IMI 300787
Giberella fujikuroi var. *subglutinans* CABI-IMI 300788
Giberella fujikuroi var. *subglutinans* CABI-IMI 300785
Giberella fujikuroi var. *subglutinans* CABI-IMI 300784
Giberella fujikuroi var. *subglutinans* CABI-IMI 300783
Giberella fujikuroi var. *subglutinans* CABI-IMI 300782
Giberella fujikuroi var. *subglutinans* CABI-IMI 300781
Giberella sp. CABI-IMI 224130
Giberella tricincta CABI-IMI 314470
Fusarium moniliforme CABI-IMI 112801
Glioresein
Gliocladium roseum CABI-IMI 093065
Gliotoxin
Aspergillus fumigatus CABI-IMI 016030
Gliocladium flavofuscum CABI-IMI 100714
Gliovirin
Gliocladium virens CABI-IMI 283130
Glucan type mucilage
Agrobacterium sp. NCIMB 11497
Gluco-delta lactone
Aspergillus niger CABI-IMI 041876
Gluconate
Pseudomonas putida NCIMB 9229
Gluconobacter oxydans NCIMB 3734
Gluconic acid
Aspergillus carbonarius CABI-IMI 041875
Aspergillus carbonarius CABI-IMI 041873
Aspergillus niger CABI-IMI 041876
Aspergillus niger CABI-IMI 041874
Aspergillus wentii CABI-IMI 023010
Leptoxyphium fumago CABI-IMI 089362
Penicillium purpurogenum CABI-IMI 090178
Glutamic acid
Arthrobacter mysorens NCIMB 10583
Arthrobacter paraffineus NCIMB 10699
Arthrobacter sp. NCIMB 9422
Bacillus circulans NCIMB 9556
Bacillus licheniformis NCIMB 8874
Corneybacterium acetoacidophilum NCIMB 9661
Corneybacterium callunae NCIMB 10338
*Corneybacterium glutamicum*NCIMB 10334
*Corneybacterium glutamicum*NCIMB 10025
*Corneybacterium glutamicum*NCIMB 10333
Corneybacterium herculis NCIMB 9694
*Corneybacterium lilium*NCIMB 10337
Corneybacterium melassecola NCIMB 10336
Corneybacterium melassecola NCIMB 10371
Corneybacterium sp. NCIMB9544
Corneybacterium sp. NCIMB10335
Corneybacterium sp. NCIMB9662
Corneybacterium sp. NCIMB9567
Corneybacterium sp. NCIMB9566
Corneybacterium sp. NCIMB9565
Corneybacterium sp. NCIMB9543
Corneybacterium sp. NCIMB9397
Protaminobacter thiaminophaga NCIMB 12106
Pseudomonas insueta NCIMB 12105
Rhodococcus sp. NCIMB 10700
Glutamyl polypeptide
Bacillus licheniformis NCIMB 11709
Bacillus subtilis NCIMB 9590
Glycerol
Leptoxyphium fumago CABI-IMI 089362
Aspergillus wentii CABI-IMI 023010
Bascillus licheniformis NCIMB 8059
Bascillus licheniformis NCIMB 6346
Bascillus licheniformis NCIMB 8061
Cochliobolus spicifer CABI-IMI 091972
Dunaliella parva CCAP 19/9
Saccharomyces cerevisiae NCYC 200
Glycolic acid
Chlorella emersonii CCAP 211/8A
Griseofulvin *see antibiotic producers*
Guaiacol
Bacillus licheniformis NCIMB 11143
Guanosine-5-monophosphate
*Arthrobacter globiformis*NCIMB 8717
Haemolysin
Clostridium perfringes NCIMB 10662
Clostridium perfringes NCIMB 10691

Haemolysin
Clostridium perfringes NCIMB 10663
Clostridium perfringes NCIMB 6125
Staphylococcus aureus subsp. *aureus* NCIMB 9760
Heamatochrome
Chlamydomonas aplanata CCAP 11/9
Heat labile enterotoxin
Escherichia coli NCIMB 13001
Escherichia coli NCIMB 13002
Escherichia coli NCIMB 13003
Helenine
Penicillium funiculosum CABI-IMI 040582
Helminthosporin (iso)
Pyrenophora graminea CABI-IMI 089368
Helvolic acid
Aspergillus fumigatus CABI-IMI 045338
Herqueinone
Penicillium herquei CABI-IMI 089376
Penicillium herquei CABI-IMI 028809
Heveadride
Drechslera hevea CABI-IMI 080137
Histamine
Providencia rettgeri NCIMB 865
Vibrio sp. NCIMB 2147
Histidine
Arthrobacter histidinolovorans NCIMB 9541
HT-2 toxin (on YES agar)
Fusarium sporotrichioides CABI-IMI 309350
Fusarium sporotrichioides CABI-IMI 309349
Humicolin
Aspergillus asperescens CABI-IMI 046813
Hydantoinase
Agrobacterium radiobacter NCIMB 013428
Hydrogen sulphide
Budvicia aquatica NCIMB 13182
5-Hydroxyaspertin
Penicillium verrucosum CABI-IMI 297964
M Hydroxybenzoic acid
Penicillium verrucosum CABI-IMI 297964
8-Hydroxycalonectrin
Fusarium culomurm CABI-IMI 014764
15-Hydroxyculmorone
Fusarium culomurm CABI-IMI 014764
15-Hydroxyculmorin
Fusarium culomurm CABI-IMI 014764
12-Hydroxyculmorin
Fusarium culomurm CABI-IMI 014764
5-Hydroxyculmorin
Fusarium culomurm CABI-IMI 014764
Poly-beta-Hydroxybutyrate
Alcaligenes latus NCIMB 12189
Alcaligenes latus NCIMB 12188
Burkholderia cepacia NCIMB 9089
Poly-beta-Hydroxybutyrate
Burkholderia cepacia NCIMB 9088
Rhozobia sp. NCIMB 10340
8-Hydroxyellipticines
Aspergillus alliaceus CABI-IMI 051982
β-Hydroxyethylflavin
Eubacterium fisicatena NCIMB 10445
Eubacterium fisicatena NCIMB 10446
1-Hydroxy-5-methoxy-6-methyl-2(1H)-pyridinone
Pseudomonas alcaligenes NCIMB 11492
3-Hydroxy-2-napthhoic acid
Pseudomonas sp. NCIMB 12229
6-hydroxymethyleugenin
Cephalosporium aphidicola CABI-IMI 068689
9-α-Hydroxysteroids
Comamonas testosteroni NCIMB 8955
Rhodococcus rhodochrous NCIMB 9557
9-Hydroxy pre helminthosporol
Cochliobolus ativus CABI-IMI 125851
9-Hydroxyellipticines
Aspergillus alliaceus CABI-IMI 051982
Hydroxyprogesterone
Aspergillus niger CABI-IMI 076837
I-Erythritol
Penicillium brevicompactum CABI-IMI 092034
ICI 146791 Compound
Streptomyces sp. NCIMB 11649
Indigo pigment
Schizophyllum commune CABI-IMI 067963
Indigotin
Comamonas acidovorans NCIMB 2760
Comamonas acidovorans NCIMB 9153
Indigouidine
Pseudomonas indigofera NCIMB 9441
5-Inosinic acid
Brevibacterium ammoniagenes NCIMB 8143
Flavobacterium breve NCIMB 11298
Iodinin
Brevibacterium iodinium NCIMB 8179
Lysobacter antibioticus NCIMB 10773
Pseudomonas phenazinium NCIMB 11724
Pseudomonas phenazinium NCIMB 11027
Iridoskyrin
Penicillium islandicum CABI-IMI 092038
Penicillium islandicum CABI-IMI 092270
Islandicin
Penicillium islandicum CABI-IMI 092038
Penicillium islandicum CABI-IMI 092270
Islanditoxin
Penicillium islandicum CABI-IMI
Itaconic acid
Aspergillus terreus CABI-IMI 126254
Aspergillus terreus CABI-IMI 044243
Aspergillus terreus CABI-IMI 089357
Jasmonic acid
Euglena gracilis CCAP 1224/5Z
Javanicin
Fusarium solani CABI-IMI 029817
Karnatakin
Streptomyces karnatatakensis NCIMB 9981

5-ketogluconic acid
- *Gluconobacter cerinus* NCIMB 9108
- *Gluconobacter oxydans* NCIMB 8035
- *Gluconobacter oxydans* NCIMB 621
- *Gluconobacter oxydans* NCIMB 6723
- *Gluconobacter oxydans* NCIMB 7069

α-Ketoglutaric acid
- *Corynebacterium herculis* NCIMB 9694
- *Escherichia coli* NCIMB 9138
- *Kluyvera cryocrescens* NCIMB 9139
- *Pseudomonas fluorescens* NCIMB 8906
- *Rhodococcus* sp. NCIMB 10700

Ketogluconic acid
- *Pseudomonas* sp. NCIMB 8296

2-Keto-L-gluconic acid
- *Gluconobacter cerinus* NCIMB 4739
- *Serratia marcescens1* NCIMB 9591
- *Acetobacter pasteurianus* NCIMB 8034
- *Pseudomonas chlororaphis* NCIMB 9392

Killer toxin
- *Candida glabrata* NCYC 388
- *Candida pintolopesii* NCYC 526
- *Candida valida* NCYC 327
- *Debaryomyces vanrijiae* NCYC 577
- *Kluyveromyces lactis* NCYC 575
- *Kluyveromyces marxianus* NCYC 587
- *Pichia anomala* NCYC 434
- *Pichia anomala* NCYC 522
- *Pichia anomala* NCYC 682
- *Pichia anomala* NCYC 435
- *Pichia anomala* NCYC 249
- *Pichia anomala* NCYC 750
- *Pichia anomala* NCYC 432
- *Pichia anomala* NCYC 711
- *Pichia membranaefaciens* NCYC 333
- *Pichia subpelliculosa* NCYC 16
- *Saccharomyces cerevisiae* NCYC 761
- *Saccharomyces cerevisiae* NCYC 1001
- *Saccharomyces cerevisiae* NCYC 215
- *Saccharomyces cerevisiae* NCYC 217
- *Saccharomyces cerevisiae* NCYC 218
- *Saccharomyces cerevisiae* NCYC 212
- *Saccharomyces cerevisiae* NCYC 214
- *Saccharomyces cerevisiae* NCYC 190
- *Saccharomyces cerevisiae* NCYC 219
- *Saccharomyces cerevisiae* NCYC 225
- *Saccharomyces cerevisiae* NCYC 223
- *Saccharomyces cerevisiae* NCYC 220
- *Saccharomyces cerevisiae* NCYC 738
- *Saccharomyces cerevisiae* NCYC 1409
- *Saccharomyces cerevisiae* NCYC 1410
- *Saccharomyces cerevisiae* NCYC 213
- *Saccharomyces cerevisiae* NCYC 232
- *Saccharomyces cerevisiae* NCYC 230
- *Saccharomyces cerevisiae* NCYC 226
- *Saccharomyces cerevisiae* NCYC 1407
- *Saccharomyces cerevisiae* NCYC 672
- *Saccharomyces cerevisiae* NCYC 713
- *Saccharomyces cerevisiae* NCYC 235
- *Williopsis mrakii* NCYC 500
- *Williopsis saturnus* NCYC 22
- *Williopsis saturnus* NCYC 23

Kojic acid
- *Aspergillus oryzae* var. *effusus* CABI-IMI 016142
- *Aspergillus oryzae* var. *effusus* CABI-IMI 124935

Kojic acid
- *Aspergillus parasiticus* CABI-IMI 015957
- *Aspergillus tamarii* CABI-IMI 091888
- *Aspergillus tamarii* CABI-IMI 015956
- *Penicillium jensenii*CABI-IMI 086562
- *Penicillium lanosum* CABI-IMI 090463

L-dopa (by bioconversion)
- *Aspergillus ochraceus* CABI-IMI 061247iv
- *Gliocladium deliquescens* CABI-IMI 040023

Lachnelluloic acid
- *Lachnellula fuscosanguinea* CABI-IMI 250255
- *Lachnellula fuscosanguinea* CABI-IMI 250256

Lachnellulone
- *Lachnellula fuscosanguinea* CABI-IMI 250255
- *Lachnellula fuscosanguinea* CABI-IMI 250256

β-Lactamase inhibitor MM4550
- *Streptomyces olivaceus* NCIMB 12807

Lactic acid
- *Bacillus coagulans* NCIMB 10276
- *Enterococcus faecium* NCIMB 10415
- *Lactobacillus pentosus* NCIMB 8026
- *Lactobacillus pentosus* NCIMB 8026
- *Rhizopus oryzae* CABI-IMI 040564

D(-)Lactic acid
- *Bacillus laevolacticus* NCIMB 10272
- *Bacillus laevolacticus* NCIMB 10269
- *Bacillus laevolacticus* NCIMB 10270
- *Bacillus laevolacticus* NCIMB 10271
- *Lactobacillus delbrueckii* NCIMB 8130
- *Sporolactobacillus inulinus* NCIMB 9743

DL-Lactic acid
- *Bacillus coagulans* NCIMB 10276
- *Bacillus racemilacticus* NCIMB 10275
- *Bacillus racemilacticus* NCIMB 10274
- *Bacillus* sp. NCIMB 10276
- *Enterococcus durans* NCIMB662
- *Enterococcus faecium* NCIMB 11181
- *Lactobacillus amylophilus* NCIMB 11546
- *Lactobacillus amylovorus* NCIMB 13276
- *Lactobacillus helveticus* subsp. *jugurti* NCIMB 11496

L(+) Lactic acid
- *Bacillus coagulans* NCIMB 8041
- *Carnobacterium divegens* NCIMB 11952
- *Lactococcus lactis* subsp. *Lactis* NCIMB 6681

Lactobionic acid
- *Acetobacter calcoaceticus* NCIMB 10694
- *Acetobacter* sp. NCIMB 9029
- *Pseudomonas aromatica* subsp. *quercito-Pyrogallica* NCIMB 9043

Lactonic sophorolipid
- *Candida bombicola* NCYC 1449

Lagosin
- *Streptomyces roseolutus* NCIMB 9854

Lapidosin
- *Penicillium lapidosum* CABI-IMI 039743

Lavendulin
- *Streptomyces lavendulae* NCIMB 9000

L-iso Leucine
- *Brevibacterium ammoniagenes* NCIMB 8143

Levan
- *Erwinia* sp. NCIMB 10121
- *Erwinia* sp. NCIMB 10120
- *Erwinia* sp. NCIMB 10130
- *Erwinia* sp. NCIMB 10119
- *Erwinia* sp. NCIMB 10123
- *Erwinia* sp. NCIMB 9966
- *Microbacterium laevaniformans* NCIMB 9659
- *Pseudomonas fluorescens* NCIMB 9057
- *Pseudomonas fluorescens* NCIMB 9053
- *Pseudomonas fluorescens* NCIMB 9054
- *Pseudomonas fluorescens* NCIMB 9056
- *Pseudomonas fluorescens* NCIMB 9058
- *Pseudomonas fluorescens* NCIMB 9055

Limocrocin
- *Streptomyces limosus* NCIMB 12976

Lipolytic substance GA-56
- *Pseudomonas* sp. NCIMB 12719
- *Pseudomonas* sp. NCIMB 12720

Luteic acid
- *Penicillium verruculosum* CABI-IMI 068239
- *Penicillium udagawae* CABI-IMI 044338

Luteoleersin
- *Bipolaris leersiae* CABI-IMI 089369

Luteose
- *Penicillium udagawae* CABI-IMI 044338

Lycopene
- *Streptomyces chrestomyceticus* subsp. *rubescens*NCIMB 10993

Lysergic acid
- *Claviceps paspali* CABI-IMI 082998
- *Claviceps paspali* CABI-IMI 082999

Lysine
- *Escherichia coli* NCIMB 9342
- *Escherichia coli* NCIMB 9110

L-lysine
- *Brevibacterium linen* NCIMB 11438
- *Corynebacterium* sp. NCIMB11078
- *Rhodococcus* sp. NCIMB 11275

Macrosporin
- *Alternaria alternaria* CABI-IMI 354944

Magnesium protoporphyrin monomethyl ester
- *Rhodobacter capsulatus* NCIMB 10006

Malic acid
- *Cochliobolus spicifer* CABI-IMI 091972

Maltobionic acid
- *Pseudomonas aromatica* subsp. *quercito-Pyrogallica* NCIMB 9043

Mannitol
- *Aspergillus candidus* CABI-IMI 091897
- *Aspergillus candidus* CABI-IMI 089346
- *Aspergillus candidus* CABI-IMI 091885
- *Aspergillus nidulans* CABI-IMI 091903
- *Aspergillus ochraceus* CABI-IMI 061247iii
- *Aspergillus wentii* CABI-IMI 023010
- *Byssochlamys fulva* CABI-IMI 040021
- *Candida krusei* NCYC 1398
- *Cochliobolus spicifer* CABI-IMI 091972
- *Penicillium griseofulvum* CABI-IMI 075832

Mannocarlose
- *Penicillium fellutanum* CABI-IMI 040232

Meleagrin
- *Penicillium melanoconidium* CABI-IMI 357299
- *Penicillium melanoconidium* CABI-IMI 357300
- *Penicillium melanoconidium* CABI-IMI 357301

Menaquinone (hydrogenated)
- *Micrococcus* sp. NCIMB 9148

4-methoxytoluquinol
- *Lentinus degeneri* CABI-IMI 110525

6 (3-Methyl ,2-butenyl)phenazine carboxylic acid
- *Streptomyces cinnamonensis* NCIMB 12603

3-Methyl-6-methoxy-8-hydroxy-3-4-dihydrosocoumarin
- *Aspergillus caespitosus* CABI-IMI 016034
- *Aspergillus caespitosus* CABI-IMI 016034ii

3-Methylorsellinic acid
- *Aspergillus terreus* CABI-IMI 044243

Methylglutamic acid
- *Aminobacter aminovorans* NCIMB 11590

6-Methylsalicylic acid
- *Penicillium griseofulvum* CABI-IMI 028808
- *Penicillium griseofulvum* CABI-IMI 092273
- *Penicillium expansum* CABI-IMI 297959
- *Penicillium verrucosum* CABI-IM 297964

o-Methylsterigmatocystin
- *Chaetomium virescens* var. *thielavioidium* CABI-IMI 240608

Mevalonate derrivitives
- *Phytophthora cactorum* CABI-IMI 021168

Microcystin
- *Microcystis aeruginosa* CCAP 1450/4
- *M. aeruginosa* CCAP 1450/6
- *M. aeruginosa* CCAP 1450/10
- *M. aeruginosa* CCAP 1450/12

Minioluteic acid
- *Penicillium minioluteum* CABI-IMI 089377

Misionin
- *Streptomyces misionensis* NCIMB 12979

Moniliformin
- *Fusarium moniliforme* CABI-IMI 316825
- *Fusarium moniliforme* CABI-IMI 316826
- *Fusarium oxysporum* CABI-IMI 350438
- *Fusarium moniliforme* var. subglutinans CABI-IMI 350439

Moniliformin (on YES agar)
- *Fusarium avenaceum* CABI-IMI 309355
- *Fusarium avenaceum* CABI-IMI 309354

Monocerin
- *Helminthosporium monoceras* CABI-IMI 125855

Mucilage
- *Chlamydomonas asymmetrica* CCAP 11/41
- *Chlamydomonas aplanata* CCAP 11/9

Multicolic acid
Penicillium multicolor CABI-IMI 104602
Multicolosic acid
Penicillium multicolor CABI-IMI 104602
Mycelianamide
Penicillium crustosum CABI-IMI 285510
Penicillium echinulatum CABI-IMI 285508
Penicillium echinulatum CABI-IMI 285503
Penicillium expansum CABI-IMI 285521
Penicillium expansum CABI-IMI 297959
Penicillium griseofulvum CABI-IMI 075832
Penicillium griseofulvum CABI-IMI 028808
Penicillium griseofulvum CABI-IMI 092273
Penicillium janthinellum CABI-IMI 040238
Penicillium solitum CABI-IMI 285509
Penicillium verrucosum CABI-IMI 285522
Penicillium verrucosum CABI-IMI 297964
Mycogone (antagonistic substance)
Acremonium strictum CABI-IMI 210624
Mycophenolic acid
Penicillium brevicompactum CABI-IMI 092274
Penicillium brevicompactum CABI-IMI 094149
Penicillium brevicompactum CABI-IMI 017456
Penicillium brevicompactum CABI-IMI 039824
Penicillium brevicompactum CABI-IMI 092044
Penicillium brevicompactum CABI-IMI 126540
Penicillium brevicompactum CABI-IMI 092262
Penicillium brevicompactum CABI-IMI 040225
Penicillium brevicompactum CABI-IMI 092034
Penicillium roquefortii CABI-IMI 293190
Penicillium roquefortii CABI-IMI 293204
Penicillium verrucosum CABI-IMI 297964
Mycotoxin (unspecified)
Aspergillus repens CABI-IMI 173203
Aspergillus repens CABI-IMI 173204
Aspergillus repens CABI-IMI 173205
Aspergillus repens CABI-IMI 173206
Aspergillus repens CABI-IMI 173207
Myxin
*Lysobacter antibioticus*NCIMB 10773
Nalgiolaxin
*Penicillium jensenii*CABI-IMI 039804
Nalgiovense
*Penicillium jensenii*CABI-IMI 039804
Nectriapyronone
Gliocladium vermoesenii CABI-IMI 040231
Neopentaene
*Streptomyces khandalensis*NCIMB 11146
Neosolaniol (on YES agar)
Fusarium sporotrichioides CABI-IMI 309349
Fusarium sporotrichioides CABI-IMI 309350
Netropsin
Streptomyces netropsis NCIMB 9592
Niacin
Saccharomyces cerevisiae NCYC 74
Nicotinic acid
Saccharomyces cerevisiae NCYC 87
Nidulin
*Aspergillus nidulans*CABI-IMI 085473
Nigrifortine
Penicillium nigricans CABI-IMI 228699
Nisin
Lactococcus lactis subsp. *Lactis* NCIMB 8780
Lactococcus lactis subsp. *Lactis* NCIMB 8586
β Nitropropionic acid
Aspergillus oryzae CABI-IMI 052141
Aspergillus oryzae CABI-IMI 052141ii
Penicillium melinii CABI-IMI 061837
Penicillium melinii CABI-IMI 061836
Nivalenol
Fusarium crookwellense CABI-IMI 334774
Nornidulin
*Aspergillus nidulans*CABI-IMI 085473
Northerqueinone
*Penicillium herquei*CABI-IMI 089376
Notatin
Penicillium griseoroseum CABI-IMI 015378
Noxiversin
Penicillium griseoroseum CABI-IMI 058138
5-Nucleotides
Nocardioides simplex NCIMB 8929
Acinetobacter sp. NCIMB 9542
Nystatin
Streptomyces noursei NCIMB 8593
Ochratoxin
Aspergillus ochraceus CABI-IMI 132429
Ochratoxin A
Penicillium crustosum CABI-IMI 206159
Penicillium expansum CABI-IMI 297959
Penicillium verrucosum CABI-IMI 297964
Penicillium verrucosum CABI-IMI 293203
Penicillium verrucosum CABI-IMI 291193
Aspergillus fumigatus CABI-IMI 376377
Aspergillus fumigatus CABI-IMI 376378
Aspergillus fumigatus CABI-IMI 376380
Aspergillus versicolor CABI-IMI 376379
Organic acids (6)
Penicillium griseofulvum CABI-IMI 075832
Organic acids (8)
Penicillium fellutanum CABI-IMI 040232
Orsellinic acid
Penicillium madriti CABI-IMI 096506
Penicillium madriti CABI-IMI 086563

Oxalic acid
- *Aspergillus niger* CABI-IMI 243400
- *Aspergillus niger* CABI-IMI 243400
- *Aspergillus niger* CABI-IMI 091881ii
- *Aspergillus terreus* CABI-IMI 089356
- *Aspergillus terreus* CABI-IMI 089355

Oxysporone
- *Fusarium oxuysporum* CABI-IMI 211811

Palitantin
- *Penicillium expansum* CABI-IMI 297959
- *Penicillium palitans* CABI-IMI 089379
- *Penicillium palitans* CABI-IMI 089378
- *Penicillium palitans* CABI-IMI 040215
- *Penicillium palitans* CABI-IMI 092276
- *Penicillium verrucosum* CABI-IMI 297964

Paraherquimide
- *Penicillium* sp. CABI-IMI 332995

Paraherquamide
- *Penicillium* paraherquei CABI-IMI 068220

Patulin (clavacin)
- *Aspergillus clavatus* CABI-IMI 015949v
- *Aspergillus clavatus* CABI-IMI 015949iv
- *Aspergillus clavatus* CABI-IMI 015949iii
- *Aspergillus terreus* CABI-IMI 017294
- *Aspergillus terreus* CABI-IMI 017294ii
- *Aspergillus terreus* CABI-IMI 016043
- *Byssochlamys fulva* CABI-IMI 083277
- *Penicillium claviforme* CABI-IMI 293198
- *Penicillium expansum* CABI-IMI 297959
- *Penicillium expansum* CABI-IMI 293192
- *Penicillium griseofulvum* CABI-IMI 039809
- *Penicillium griseofulvum* CABI-IMI 293195
- *Penicillium griseofulvum* CABI-IMI 034908
- *Penicillium griseofulvum* CABI-IMI 028808
- *Penicillium griseofulvum* CABI-IMI 034909
- *Penicillium roquefortii* CABI-IMI 293190
- *Penicillium roquefortii* CABI-IMI 293204
- *Penicillium verrucosum* CABI-IMI 297964

Paxilline
- *Emericella striata* CABI-IMI 163899

Pencolide
- *Penicillium sclerotiorum* CABI-IMI 104602

Penicillic acid
- *Penicillium atramentosum* CABI-IMI 293185
- *Penicillium aculeatum* CABI-IMI 089372
- *Penicillium aurantiogriseum* CABI-IMI 034913iii
- *Penicillium aurantiogriseum* CABI-IMI 040236
- *Penicillium aurantiogriseum* CABI-IMI 034913ii
- *Penicillium verrucosum* CABI-IMI 297964
- *Penicillium cyclopium* CABI-IMI 357294
- *Penicillium cyclopium* CABI-IMI 357295
- *Penicillium raistrickii* CABI-IMI 378416

Penicillic acid
- *Penicillium melanoconidium* CABI-IMI 357299
- *Penicillium melanoconidium* CABI-IMI 357300
- *Penicillium melanoconidium* CABI-IMI 357301
- *Penicillium neoechinulatum* CABI-IMI 357302
- *Penicillium pulvivorum* CABI-IMI 378411
- *Penicillium polonicum* CABI-IMI 357303
- *Penicillium polonicum* CABI-IMI 357304
- *Penicillium polonicum* CABI-IMI 357305

Penitrem A
- *Penicillium crustosum* CABI-IMI 293182
- *Penicillium expansum* CABI-IMI 297959
- *Penicillium janczewskii* CABI-IMI 228669
- *Penicillium melanoconidium* CABI-IMI 357299
- *Penicillium melanoconidium* CABI-IMI 357300
- *Penicillium melanoconidium* CABI-IMI 357301
- *Penicillium verrucosum* CABI-IMI 297964

Pennigritrem $C_{37}H_{44}ClNO_6$
- *Penicillium nigricans* CABI-IMI 228699

Pepstain
- *Streptomyces argenteolus* subsp. *toyonokensis* NCIMB 12426

Peptide hepatotoxin
- *Oscillularia agardhii* CCAP 1459/22

Phenazine
- *Burkholderia cepacia* NCIMB 9089
- *Burkholderia cepacia* NCIMB 9090
- *Burkholderia cepacia* NCIMB 9091
- *Burkholderia cepacia* NCIMB 9092
- *Burkholderia cepacia* NCIMB 9690
- *Burkholderia cepacia* NCIMB 9088
- *Burkholderia cepacia* NCIMB 9093

Phenazine 1,6, dicarboxylic acid
- *Pseudomonas phenazinium*NCIMB 11431

Phenazine alpha-carboxylic acid
- *Pseudomonas aureofaciens* NCIMB 9625

Phenethyl alcohol
- *Candida albicans* NCYC 1363

L-Phenylalanine anhydride
- *Penicillium janczewskii* CABI-IMI 104603

Phloroglucin like compoun
- *Pseudomonas* sp. NCIMB 11241

Phosphomannan
- *Pichia holstii* NCYC 560

Phycobiliproteins
- All CCAP cyanobacterial strains

Physcion
- *Aspergillus echinulatus* CABI-IMI 091860
- *Aspergillus niveoglaucus* CABI-IMI 032050ii
- *Aspergillus echinulatus* CABI-IMI 091872
- *Aspergillus echinulatus* CABI-IMI 091866

Physcion
- *Aspergillus niveoglaucus* CABI-IMI 032050
- *Aspergillus chevalieri* CABI-IMI 091867
- *Aspergillus ruber* CABI-IMI 091862
- *Aspergillus ruber* CABI-IMI 091863
- *Aspergillus ruber* CABI-IMI 091864
- *Aspergillus ruber* CABI-IMI 094147
- *Aspergillus umbrosus* CABI-IMI 091861
- *Aspergillus umbrosus* CABI-IMI 091874
- *Aspergillus niveoglaucus* CABI-IMI 091871
- *Penicillium herquei* CABI-IMI 028809

Physcion anthranol
- *Penicillium herquei* CABI-IMI 028809

Pigment (black)
- *Pseudomonas aeruginosa* NCIMB 11284
- *Pseudomonas aeruginosa* NCIMB 11285

Pigment (blue)
- *Pseudomonas fluorescens* NCIMB 8917

Pigment (dark-brown water soluble)
- *Acetobacter liquefaciens* NCIMB 9418
- *Acetobacter liquefaciens* NCIMB 9417
- *Acetobacter liquefaciens* NCIMB 9136

Pigment (dark-brown)
- *Bacillus atrophaeus* NCIMB 12899

Pigment (pink slouble)
- *Thermoactinomyces thalpophilus* NCIMB 11365

Pigment (red diffusible fluorescent)
- *Arthrobacter* sp. NCIMB 5

Pigment (red-brown diffusible)
- *Pseudomonas fluorescens* NCIMB 13041

Pigment (red-brown soluble)
- *Streptomyces albovinaceus* NCIMB 13010

Pigment (violet water insoluble)
- *Micrococcus* sp. NCIMB 9148

Pimaydolide
- *Pithomyces maydicus* CABI-IMI 046232
- *Pithomyces maydicus* CABI-IMI 098084

Pink stain
- *Streptomyces* sp. NCIMB 10987

Pink stain production on PVC
- *Streptoverticillium waksmannii* NCIMB 10991

Pink-Mauve pigment (exposed to long wave UV)
- *Epicoccum nigrum* CABI-IMI 336106

Pithoimycolides
- *Pithomyces chartarum* CABI-IMI 074473

Plicacetin
- *Streptomyces plicatus* NCIMB 11305

Polyglutamic acid
- *Bacillus licheniformis* NCIMB 11709
- *Bacillus subtilis* NCIMB 9590

Polysaccharide
- *Lysobacter gummosus* NCIMB 11896
- *Penicillium udagawae* CABI-IMI 095152
- *Pseudomonas elodea* NCIMB12171
- *Pseudomonas elodea* NCIMB11942
- *Zooglea ramigera* NCIMB 11941

Polysaccharide (alginate-like)
- *Azotobacter vinelandii* NCIMB 11263

Polysaccharide (curdlan type)
- *Agrobacter radiobacter* NCIMB 8149

Polysaccharide (gelling XM6)
- *Klebsiella pneumoniae subsp. pneumoniae* NCIMB 11870

Polysaccharide (hetero, extrcellular, anionic)
- *Pseudomonas* sp. NCIMB 11592

Polysaccharide (thermogelable)
- *Agrobacterium* sp. NCIMB 12233

Polysaccharide (extracellular)
- *Arthrobacter viscosus* NCIMB 9728
- *Klebsiella pneumoniae* subsp. *pneumoniae* NCIMB 9111
- *Klebsiella pneumoniae* subsp. *pneumoniae* NCIMB 8806
- *Arthrobacter viscosus* NCIMB 9729
- *Klebsiella pneumoniae* subsp. *pneumoniae* NCIMB 8805

Polysaccharide (also salt responsive, atypical) alkali deacetylated
- *Xanthomonas campestris* NCIMB 11803

Porphyrins
- *Amycolatopsis rugosa* NCIMB 11010

PR toxin
- *Penicillium roquefortii* CABI-IMI 024313
- *Penicillium expansum* CABI-IMI 297959
- *Penicillium verrucosum* CABI-IMI 297964

Proferrorosamine A
- Unnamed NCIMB 9782

Propane
- *Cryptococcus albidus* NCYC 445

Protein
- *Arthrospira maxima* CCAP 1475/9
- *Candida utilis* NCYC 359
- *Candida utilis* NCYC 707
- *Candida utilis* NCYC 769
- *Candida utilis* NCYC 193
- *Kluyveromyces marxianus* NCYC 587
- *Kluyveromyces marxianus* NCYC 970
- *Pichia pastoris* NCYC 175
- *Saccharomyces cerevisiae* NCYC 695
- *Trichoderma reesei* CABI-IMI 192655ii
- *Yarrowia lipolytica* NCYC 825

Protein A
- *Staphylococcus aureus* NCTC 8530

Protein A (A-layer)
- *Aeromonas salmonicida* subsp. *salmonicida* NCIMB 13077
- *Staphylococcus aureus* subsp.*aureus* NCIMB 11787

Protein antigen
- *Streptococcus mutans* NCIMB 11516

Pseudonigeron(1-3)alpha-D-glucan
- *Aspergillus niger* CABI-IMI 050566ii

Pseudotropin (from tropin, syner. with Bacillus)
- *Streptococcus* sp. NCIMB 9633

Pseudotropin (from tropin, syner. with Streptoc)
- *Bacillus* sp. NCIMB 9632

Puberculic acid
- *Penicillium aurantiogriseum* CABI-IMI 034913iii
- *Penicillium aurantiogriseum* CABI-IMI 034846
- *Penicillium aurantiogriseum* CABI-IMI 034913ii

Pulvinone derrivitives
- *Aspergillus terreus* CABI-IMI 044243

Pumilin
Bacillus pumulis NCIMB 8738
Pyocyanin
Pseudomonas aeruginosa NCIMB 10434
Pseudomonas aeruginosa NCIMB 6750
Pseudomonas aeruginosa NCIMB 13118
Pyomelanin
Pseudomonas aeruginosa subsp. *Erythrogenes* NCIMB 11835
Pyorubin
Pseudomonas aeruginosa NCIMB 6750
Pseudomonas aeruginosa subsp. *Erythrogenes* NCIMB 11835
Pyoverdin
Pseudomonas aeruginosa NCIMB 6750
Pyrenophorin
*Stemphylium radicinum*CABI-IMI 105654
Quadrilineatin
Aspergillus quadrilineatus CABI-IMI 089313
Aspergillus quadrilineatus CABI-IMI 089348
Aspergillus quadrilineatus CABI-IMI 089351
Aspergillus quadrilineatus CABI-IMI 089349
Aspergillus quadrilineatus CABI-IMI 089350
Questin
Penicillium glabrum CABI-IMI 096659
Questinol
Penicillium glabrum CABI-IMI 096659
Qurantiogliocladin
Gliocladium roseum CABI-IMI 093065
Radarins A
Aspergillus sulphureus CABI-IMI 211397
Radarins D
Aspergillus sulphureus CABI-IMI
Radicinin
Alternaria radicinin CABI-IMI 105653
Alternaria radicinin CABI-IMI 105654
Resorcinol
Penicillium brevicompactum CABI-IMI 092034
Resorcinol derrivitives
Penicillium brevicompactum CABI-IMI 092034
Riboflavin
Eremothecium ashbyi CABI-IMI 031269
Eremothecium ashbyi CABI-IMI 014783
RNA (double stranded)
Pseudomonas syringae NCIMB 11266
RNA (highly polymerised ribosomal)
Escherichia coli NCIMB 10115
Roquefortine A
Penicillium hirsutum CABI-IMI 293183
Roquefortine B
Penicillium expansum CABI-IMI 297959
Penicillium verucosum CABI-IMI 297964
Roquefortine C
Penicillium roquefortii CABI-IMI 293190
Penicillium griseofulvum CABI-IMI 293195
Roquefortine C
Penicillium chrysogenum CABI-IMI 293188
Penicillium claviforme CABI-IMI 293198
Penicillium hirsutum CABI-IMI 293183
Penicillium expansum CABI-IMI 293192
Penicillium roquefortii CABI-IMI 293189
Penicillium hirsutum CABI-IMI 293184
Penicillium roquefortii CABI-IMI 293204
Penicillium hirsutum CABI-IMI 293206
Penicillium crustosum CABI-IMI 293182
Penicillium italicum CABI-IMI 293196
Penicillium expansum CABI-IMI 297959
Penicillium verrrucosum CABI-IMI 297964
Rosenonolactone
Trichothecium roseum CABI-IMI 129425
Rosololactone
Trichothecium roseum CABI-IMI 129425
Rubralone
Streptomyces echinoruber NCIMB 12831
Rubratoxin
Penicillium rubrum CABI-IMI 040036
Rubratoxin A
Penicillium rubrum CABI-IMI 136128
Penicillium rubrum CABI-IMI 136127
Penicillium rubrum CABI-IMI 136126
Rubratoxin B
Penicillium rubrum CABI-IMI 112715
Penicillium rubrum CABI-IMI 136128
Rubrofusarin
Fusarium culmorum CABI-IMI 089365
Fusarium culmorum CABI-IMI 089364
Fusarium culmorum CABI-IMI 089366
Aspergillus carbonarius CABI-IMI 041875
Aspergillus niger CABI-IMI 297707
Fusarium culmorum CABI-IMI 089367
Fusarium culmorum CABI-IMI 092033
Rubroglaucin
Aspergillus umbrosus CABI-IMI 091861
Aspergillus ruber CABI-IMI 092043
Aspergillus ruber CABI-IMI 091868
Aspergillus niveoglaucus CABI-IMI 032050
Aspergillus ruber CABI-IMI 091862
Aspergillus echinulatus CABI-IMI 091860
Aspergillus ruber CABI-IMI 094147
Aspergillus echinulatus CABI-IMI 091866
Aspergillus ruber CABI-IMI 092030
Gliocladium roseum CABI-IMI 093065
Rubroskyrin
Penicillium islandicum CABI-IMI 092270
Penicillium islandicum CABI-IMI 092038
Rubrosulphin
Aspergillus sulphureus CABI-IMI 128939
Rubrotiorin
Penicillium hirayamae CABI-IMI 078255
Rugulosin
Penicillium kloeckeri CABI-IMI 040047
Penicillium rugulosum CABI-IMI 040041
Penicillium rugulosum CABI-IMI 089381
Penicillium rugulosum CABI-IMI 092223
Penicillium rugulosum CABI-IMI 089382
Penicillium rugulosum CABI-IMI 089380

Rugulovasine
Penicillium expansum CABI-IMI 297959
Penicillium verrucosum CABI-IMI 297964
Salnopyrone A (in presence of chick pea tissue)
Ascocyhta rabiei CABI-IMI 331919
Salanopyrone C (in presence of chick pea tissue)
Ascocyhta rabiei CABI-IMI 331919
Sambucinol
Fusarium culmorum CABI-IMI 014764
Sambucoin
Fusarium culmorum CABI-IMI 014764
Saxitoxins
Alexandrium tamarense CCAP 1119/4
Alexandrium tamarense CCAP 1119/5
Scatole
Clostridium scatologenes NCIMB 8855
Sclerin
*Aspergillus carneus*CABI-IMI 135818
Sclerotiorin
Penicillium sclerotiorum CABI-IMI 039742
Penicillium sclerotiorum CABI-IMI 092040
Penicillium hirayamae CABI-IMI 078255
Penicillium sclerotiorum CABI-IMI 040574
Penicillium sclerotiorum CABI-IMI 092039
Penicillium sclerotiorum CABI-IMI 040569
Scytalone
Phialophora lagerbergii CABI-IMI 096745
Secalonic acid (ergochrome)
Penicillium oxalicum CABI-IMI 039750
α-Butylhomo- Serine
Clostridium saccharoperbutylacetonicum NCIMB 12605
o-Butylhomo-Serine
Clostridium saccharoperbutylacetonicum NCIMB 12605
O-Carbaryl-D-Serine
Streptomyces polychromogenes NCIMB 8791
L-Serine
*Arthrobacter citreus*NCIMB 8915
Shamixanthon
Aspergillus variecolor CABI-IMI 112543
Shigatoxin
Shigella sp. *Contact* NCTC
Sirodesmin A
Sirodesmium diversum CABI-IMI 102519
Sirodesmin B
Sirodesmium diversum CABI-IMI 102519
Sirodesmin C
Sirodesmium diversum CABI-IMI 102519
Sirodesmin G
Sirodesmium diversum CABI-IMI 102519
Skyrin
Penicillium rugulosum CABI-IMI 040041
Penicillium rugulosum CABI-IMI 092223
Penicillium rugulosum CABI-IMI 089380
Penicillium islandicum CABI-IMI 092277
Penicillium rugulosum CABI-IMI 089382
Penicillium kloeckeri CABI-IMI 040047
Penicillium rugulosum CABI-IMI 089381
Slime
Lactobacillus sp. NCIMB 12119
Erwinia herbicola NCIMB 11521
Erwinia herbicola NCIMB 11522
Klebsiella pneumoniae subsp. *pneumoniae* NCIMB 11467
Pediococcus danosus NCIMB 10563
Erwinia herbicola NCIMB 11524
Slime (from fructose)
Xanthobacter autotrophicus NCIMB 10809
Sorbose
Gluconobacter oxydans NCIMB 621
Gluconobacter oxydans NCIMB 7069
Gluconobacter oxydans NCIMB 8035
L-Sorbosone
Streptomyces cellulosae NCIMB 12600
Micrococcus luteus NCIMB 8553
Spiculisporic acid
Penicillium minioluteum CABI-IMI 089377
Spinulosin
Penicillium spinulosum CABI-IMI 091950
Aspergillus fumigatus CABI-IMI 089354
Penicillium spinulosum CABI-IMI 091954
Aspergillus fumigatus CABI-IMI 045338
Penicillium spinulosum CABI-IMI 091955
Sporidesmins
Pithomyces chartarum CABI-IMI 074473
Sporidesmolides I
Pithomyces chartarum CABI-IMI 074473
Pithomyces chartarum CABI-IMI 074473
Sporidesmolides III
Pithomyces chartarum CABI-IMI 074473
Sporidesmolides IV
Pithomyces chartarum CABI-IMI 074473
Pithomyces maydicus CABI-IMI 098084
Sporidesmolides V
Pithomyces chartarum CABI-IMI 074473
Staphylococcal beta toxin
Staphylococcus aureus subsp. *aureus* NCIMB 11857
Stellatin
Aspergillus variecolor CABI-IMI 075219
Stemlon
Pleospora herbarum CABI-IMI 130782
Sterigmatocystin
Chaetomium virescens var. *thielavioideum* CABI-IMI 240608
Aspergillus rugulosus CABI-IMI 136775
Aspergillus versicolor CABI-IMI 041924
Aspergillus versicolor CABI-IMI 016139
Sterigmatocystin
Aspergillus parasiticus var. globus CABI-IMI 120920
o-Methyl-sterigmatocystin
Chaetomium virescens var. thielavioideum CABI-IMI 240608
Steroids
Paecilomyces lilacinus CABI-IMI 027830
Sterol binding polysaccharides
Saccharomyces cerevisiae NCYC 431
Penicillium roquefortii CABI-IMI 024313

Sterol binding polysaccharides
Saccharomyces cerevisiae NCYC 79
Sterols
Saccharomyces cerevisiae NCYC 739
Stipitatic acid
Penicillium emmonsii CABI-IMI 038805ii
Penicillium emmonsii CABI-IMI 038805iii
Streptolysin
Streptococcus pyogenes NCIMB 8884
Subtilin
Bacillus subtilis NCIMB 8054
Succinic acid
Aspergillus terreus CABI-IMI 089356
Aspergillus terreus CABI-IMI 017294
Aspergillus terreus CABI-IMI 017294ii
Aspergillus terreus CABI-IMI 091911
Cochliobolus spicifer CABI-IMI 091972
Leptoxyphium fumago CABI-IMI 089362
trans-2,3-epoxysuccinic acid
Lentinus degeneri CABI-IMI 110525
Succinoglycan
Agrobacterium radiobacter NCIMB 8149
Sulochrin
Penicillium glabrum CABI-IMI 096659
Aspergillus terreus CABI-IMI 016043
Surfactant
Pseudomonas fluorescens NCIMB 11712
Synnematin B
Acremonium chrysogenium CABI-IMI 091579
Acremonium chrysogenium CABI-IMI 049137
Emericellopsis minima CABI-IMI 058330
Emericellopsis minima CABI-IMI 092625
T-2 toxin (on YES agar)
Fusarium sporotrichioides CABI-IMI 309349
Fusarium sporotrichioides CABI-IMI 309350
Tajixanthone
Aspergillus variecolor CABI-IMI 112543
Tardin
Penicillium rugulosum CABI-IMI 028333
Tartaric acid
Gluconobacter oxydans NCIMB 7069
Gluconobacter oxydans NCIMB 8035
Gluconobacter oxydans NCIMB 621
Tenuazoic acid
Alternaria alternata CABI-IMI 089344
Alternaria alternata CABI-IMI 089345
Penicillium aurantiogriseum CABI-IMI 293185
Penicillium chrysogenum CABI-IMI 293188
Penicillium crustosum CABI-IMI 285510
Penicillium expansum CABI-IMI 297959
Penicillium griseofulvum CABI-IMI 285525
Penicillium roqueforti CABI-IMI 293190
Penicillium solitum CABI-IMI 285509
Penicillium verrucosum CABI-IMI 297964
Penicillium verrucosum CABI-IMI 285523
Tenuazoic acid
Penicillium verrucosum CABI-IMI 285522
Peniciullium expansum CABI-IMI 285521
Terramide A
Aspergillus terreus CABI-IMI 044339
Terramide C
Aspergillus terreus CABI-IMI 044339
Terrein
Aspergillus terreus CABI-IMI 044339
Terrestric acid
Penicillium crustosum CABI-IMI 089386
Penicillium crustosum CABI-IMI 089385
Penicillium crustosum CABI-IMI 293182
Penicillium crustosum CABI-IMI 028040
Penicillium expansum CABI-IMI 297959
Penicillium hirsutum CABI-IMI 293206
Penicillium hirsutum CABI-IMI 293184
Penicillium hirsutum CABI-IMI 293183
Penicillium verrucosum CABI-IMI 297964
Tetanospasmin
Clostridium tetani Contact NCTC
Tetanolysin
Clostridium tetani Contact NCTC
Tetraene
Streptomyces xantholiticus NCIMB 9857
Tetraenins A and B
Streptomyces fragmentans NCIMB 10721
Tetramycoin B
Chainia grisea subsp. *fusca* NCIMB10962
Tetrathionate
Thiobacillus thioparus NCIMB 8454
Tetrodotoxin
Shewanella alga NCIMB 13178
Alteromonas tetraodonis NCIMB 13177
Tetronic acid (antitumour / antibacterial activity)
Aspergillus panamensis CABI-IMI 019393iii
Aspergillus panamensis CABI-IMI 019393ii
Thermorubin
Thermoactinomyces thalpophilus NCIMB 11368
Thermostable macromolecules
Thermus aquaticus NCIMB 11243
Thermothiocin
Thermoactinomyces coremialis NCIMB 11405
Thiolutin
Streptomyces kasugaensis NCIMB 12717
Toxin
Fusarium graminearum CABI-IMI 183761
Fusarium graminearum CABI-IMI 183762
Microcystis aeruginosa CCAP 1450/10
Oscillatoria agardhii CCAP 1459/22
Cephalotrichum microsporus CABI-IMI 173202
Fusarium culmorum CABI-IMI 202042
Fusarium sulpureum CABI-IMI 206026
Toxin (lethal)
Clostridium perfringes NCIMB 10691
Clostridium perfringes NCIMB 10663

Toxin (lethal)
Clostridium perfringes NCIMB 10662
Toxin (b)
Staphylococcus epidermidis NCIMB 701245
Toxin (Staphylococcal beta)
Staphylococcus aureus NCIMB 11857
Trichodermin
Trichoderma polysporum CABI-IMI 140624
Trichoderma sporulosum CABI-IMI 040624
Trichodermol (roridin C)
Trichoderma polysporum CABI-IMI 140624
Trichoderma sporulosum CABI-IMI 040624
Trichothecin
Trichothecium roseum CABI-IMI 050661
Trichothecium roseum CABI-IMI 050660
Trichothecium roseum CABI-IMI 057973
Trichothecium roseum CABI-IMI 129425
Tricothecenes
Fusarium culmorum CABI-IMI 014764
Trimethylene glycol
Citrobacter freundii NCIMB 3735
Tryptophan
Escherichia coli NCIMB 11074
Escherichia coli NCIMB 10200
Bacillus subtilis NCIMB 10485
L-Tryptophan
Escherichia coli NCIMB 11074
Tryptophol
Candida albicans NCYC 1363
Tylosin
Streptomyces fradiae NCIMB10812
Ustic acid
Aspergillus ustus CABI-IMI 089359
Aspergillus ustus CABI-IMI 089361
Aspergillus ustus CABI-IMI 089360
Uuroglaucin
*Aspergillus glaucus*CABI-IMI 091870
L-Valine
Klebsiella pneumoniae subsp. *pneumoniae* NCIMB 8152
Varianose
Penicillium funiculosum CABI-IMI 040586
Veratryl alcohol (in lignin degredation)
Phanerochaete chrysosporium CABI-IMI 232175
Veratryl alcohol
Phanerochaete chrysosporium CABI-IMI 310787
Vermiculine
Talaromyces wortmannii CABI-IMI 275985
Talaromyces wortmannii CABI-IMI 364857
Vermopyrone
Gliocladium vermoesenii CABI-IMI 040231
Verruculogen
Aspergillus caespitosus CABI-IMI 016034
Aspergillus caespitosus CABI-IMI 016034ii
Verrucofortine
Penicillium aurantiovirens CABI-IMI 357292
Penicillium aurantiovirens CABI-IMI 357293
Penicillium cyclopium CABI-IMI 357294
Penicillium cyclopium CABI-IMI 357295
Penicillium polonicum CABI-IMI 357303
Penicillium polonicum CABI-IMI 357304
Penicillium polonicum CABI-IMI 357305
Penicillium tricolor CABI-IMI 357306
Verrucosidin
Penicillium melanoconidium CABI-IMI 357299
Penicillium melanoconidium CABI-IMI 357300
Penicillium polonicum CABI-IMI 357303
Penicillium polonicum CABI-IMI 357304
Penicillium polonicum CABI-IMI 357305
Penicillium aurantiogriseum CABI-IMI 357289
Penicillium aurantiogriseum CABI-IMI 357290
Penicillium aurantiogriseum CABI-IMI 357291
Penicillium melanoconidium CABI-IMI 357301
Versiconal acetate
Aspergillus parasiticus var. *globosus* CABI-IMI 120920
Versimide insecticide activity
Aspergillus versicolor CABI-IMI 129488
Aspergillus versicolor CABI-IMI 129489
Violacein
Chromobacterium violaceum NCIMB 8890
Violacin
Streptomyces yerevanensis NCIMB 9589
Viomellein
Aspergillus sulphureus CABI-IMI 128939
Penicillium cyclopium CABI-IMI 357294
Penicillium cyclopium CABI-IMI 357295
*Penicillium expansum*CABI-IMI 297959
Penicillium freii CABI-IMI 357296
Penicillium freii CABI-IMI 357297
Penicillium freii CABI-IMI 357298
Penicillium tricolor CABI-IMI 357306
Penicillium tricolo CABI-IMI 357307
Penicillium verrucosum CABI-IMI 297964
Penicillium viridicatum CABI-IMI 357308
Penicillium viridicatum CABI-IMI 293187
Penicillium viridicatum CABI-IMI 293205
Viopurpurin
Aspergillus sulphureus CABI-IMI 128939
Viridamine
Penicillium viridicatum CABI-IMI 357308
Viridic acid
Penicillium viridicatum CABI-IMI 357308
Viridicatic acid
Penicillium crustosum CABI-IMI 206159

Viridicatin
- *Penicillium aurantiogriseum* CABI-IMI 293186
- *Penicillium solitum* CABI-IMI 293181
- *Penicillium aurantiogriseum* CABI-IMI 293185
- Penicillium aurantiovirens CABI-IMI 357292
- *Penicillium aurantiovirens* CABI-IMI 357293
- *Penicillium cyclopium* CABI-IMI 357294
- *Penicillium cyclopium* CABI-IMI 357295
- *Penicillium freii* CABI-IMI 357296
- *Penicillium freii* CABI-IMI 357297
- *Penicillium freii* CABI-IMI 357298
- *Penicillium polonicum* CABI-IMI 357303
- *Penicillium polonicum* CABI-IMI 357304
- *Penicillium polonicum* CABI-IMI 357305

Viridicatol
- *Penicillium aurantiovirens* CABI-IMI 357292
- *Penicillium aurantiovirens* CABI-IMI 357293
- *Penicillium cyclopium* CABI-IMI 357294
- *Penicillium cyclopium* CABI-IMI 357295
- *Penicillium frei* CABI-IMI 357296
- *Penicillium freii* CABI-IMI 357297
- *Penicillium freii* CABI-IMI 357298
- *Penicillium neoechinulatum* CABI-IMI 357302
- *Penicillium polonicum* CABI-IMI 357303
- *Penicillium polonicum* CABI-IMI 357304
- *Penicillium polonicum* CABI-IMI 357305

Viridin
- *Trichoderma viride* CABI-IMI 024039

Viridiol
- *Nodulisporium hinnuleum* CABI-IMI 214826
- *Gliocladium deliquescens* CABI-IMI 101523

Viriditoxin
- *Aspergillus viridinutans* CABI-IMI 062875
- *Aspergillus viridinutans* CABI-IMI 062875ii

Viridogreuisen
- *Streptomyces griseoviridis* NCIMB 9853

Vitamin B and carbamides
- *Butyribacterium limosum* NCTC 10469

Vitamin B_{12}
- *Sphingomonas paucimobilis* NCIMB 8195
- *Streptomyces argenteolus* NCIMB 9625
- *Streptomyces griseus* NCIMB 9004
- *Amycolatopsis rugosa* NCIMB 8926
- *Amycolatopsis rugosa* NCIMB 11009
- *Amycolatopsis rugosa* NCIMB 11010
- *Bacillus megaterium* NCIMB 8508
- *Clostridium tetanomorphum* NCIMB 11547
- *Eubacterium limosum* NCIMB 9554
- *Streptomyces* sp. NCIMB 10105
- *Propionibacterium fruendenreichii* subsp. *shermanii* NCIMB 9416
- *Propionibacterium fruendenreichii* subsp. *freunde`I*NCIMB 5959

Vitamin B_{12}
- *Propionibacterium fruendenreichii* subsp. *Shermanii* NCIMB 10585
- *Streptomyces olivaceus* NCIMB 8238
- *Propionibacterium freudenreichii* NCTC 10470

Vomitoxin
- *Fusarium moniliforme* CABI-IMI 316829

Wax esters
- *Acinetobacter* sp. NCIMB 9205
- *Acinetobacter* sp. NCIMB 10487

Wortmannin
- *Penicillium wortmannii* CABI-IMI 044277

Wortmannolone
- *Penicillium wortmannii* CABI-IMI 044277

Xanthalycins A and B
- *Streptomyces xantholiticus* NCIMB 9857

Xanthomegin
- *Penicillium aurantiogriseum* CABI-IMI 293186
- *Penicillium viridicatum* CABI-IMI 293205
- *Penicillium viridicatum* CABI-IMI 293187
- *Aspergillus sulphureus* CABI-IMI 128939
- *Penicillium cyclopium* CABI-IMI 357294
- *Penicillium cyclopium* CABI-IMI 357295
- *Penicillium freii* CABI-IMI 357296
- *Penicillium freii* CABI-IMI 357298
- *Penicillium tricolor* CABI-IMI 357306
- *Penicillium tricolor* CABI-IMI 357307
- *Penicillium viridicatum* CABI-IMI 357308

Xanthothricin
- *Pseudomonas farinofermentans4* NCIMB 12451
- *Pseudomonas cocovenenans* NCIMB 9450

Zearalenone
- *Fusarium crookwellense* CABI-IMI 334774
- *Fusarium culmorum* CABI-IMI 309345
- *Fusarium equiseti* CABI-IMI 309348
- *Fusarium equiseti* CABI-IMI 309347
- *Fusarium moniliforme* CABI-IMI 316832
- *Fusarium moniliforme* CABI-IMI 316831

Zearalenone (on YES agar)
- *Fusarium culmorum* CABI-IMI 309344

β-Trans-Zearalenol
- *Fusarium crookwellense* CABI-IMI 334774

trans-Zearalenol
- *Fusarium crookwellense* CABI-IMI 334774

Zymosterol
- *Saccharomyces cerevisiae* NCYC 667

Antibiotic producing strains

Antimicrobials to fungi, bacteria, protozoa and viruses, also see metabolites

Aburamycin
- *Streptomyces minutiscleroticus* NCIMB 10996

Achromycin
- *Streptomyces alboniger* NCIMB 13007
- *Steptomyces elizabethii* NCIMB 11545

Actinomycin C complex
- *Steptomyces parvus* NCIMB 9608
- *Steptomyces citreofluorescens* NCIMB 9806

Actinomycin D
- *Steptomyces parvulus* NCIMB 11240
- *Steptomyces murinusi* NCIMB 12701

Actinomycin X complex
- *Steptomyces galbus* NCIMB 13005
- *Steptomyces fluorescens* NCIMB 9851
- *Steptomyces antibioticus* NCIMB 8504

Actinospectacin
- *Streptomyces spectabilis* NCIMB 9750

Actioctin
- *Streptomycin* sp. NCIMB 8697

Adriamycin
- *Streptomyces peuceticus* subsp. *caesius* NCIMB 10974

Agrocins
- *Agrobacterium radiobacter* NCPPB 2407

Alamethicin
- *Trichoderma viride* CABI-IMI

Albicidin
- *Xanthomonas albilineans* NCIMB

Aminocyclitol
- *Micromonospora* sp. NCIMB 11642

Aminocyclitol containing antibiotic
- *Streptomyces fradiae* NCIMB12817

Aminoglycoside G-418
- *Micromonospora rhodorangea* NCIMB 12741

6-Aminopenicillinic acid
- *Acinetobacter* sp. NCIMB 9424
- *Arthrobacter viscosus* NCIMB 10268
- *Escherichia coli* NCIMB 10209
- *Emericellopsis minima* CABI-IMI 069015

Amphomycin
- *Streptomyces canus* NCIMB 9627

Amphotericin A and B
- *Streptomyces nodosus* NCIMB 12816

Angustmycin
- *Streptomyces hygroscopius subsp. angustmyceticus* NCIMB 9719

Antiamoebin
- *Emericellopsis poonensis*

Antibacterial activity
- *Antustrodesmis braunii* CCAP 202/7B
- *Coccolithus pelagicus* CCAP 913/2
- *Chlorella vulgaris* CCAP 211/11h
- *C. stigmatophora* CCAP 211/20
- *Chlorella* sp. CCAP 211/53
- *Chlamydomonas applanes* CCAP 11/2
- *Chlamydomonas proteus* CCAP 11/21
- *C. reinhardtii* CCAP 11/32B
- *Chlorococcum macrostigmatum* CCAP 213/9

Antibacterial activity
- *Chlorogonium capillabum* CCAP 12/2B
- *Cricosphaera elongata* CCAP 961/3
- *Cryptomonas calceiformis* CCAP 979/6
- *Nannochloris maculula* CCAP 251/3
- *N. oculula* CCAP 251/6
- *Ochrospaera neapolitana* CCAP 932/1
- *Oocystis marssonii* CCAP 257/1
- *Pavlova gyrans* CCAP 940/4
- *Pediosbrum tetras* CCAP 261/6
- *Phaeodactylum tricornutum* CCAP 1052/1A
- *Phaeodactylum tricornutum* CCAP 1052/6
- *Porphyridium purpureum* CCAP 1380/3
- *Scenedesmis communa* CCAP 276/4B
- *Strebolenema* sp. CCAP 1337/1
- *Syncrypta glomerifera* CCAP 958/1
- *Tetraselmis chui* CCAP 8/6
- *T. hazeni* CCAP 66/7
- *T. impellucida* CCAP 66/32
- *T. marina* CCAP 163/1B
- *T. striata* CCAP 66/3
- *T. suecica* CCAP 66/22D
- *T. tetrathele* CCAP 66/1C
- *Tetraselmis* sp. CCAP 66/8

Antibiotic general
- *Streptomyces clavuligerus* NCIMB 11260
- *Streptomyces clavuligerus* NCIMB 11261
- *Bacillus polymyxa* NCIMB 11496
- *Blepharisma japonicum* CCAP 1607/2

Antifungal agent PA166
- *Streptomyces glaucus* NCIMB 12677

Antifungal agent1968
- *Streptomyces aminiohilus* NCIMB 9827

Antifungal Antibiotic 67-121 complex
- *Actinoplanes caerulus* NCIMB 12727

Antifungal activity
- *Amphidinium carferae* CCAP 1102/2
- *Amphidinium operculatum* CCAP 1102/3
- *Anabaena variabilis* CCAP 1403/8
- *Antristrodesmus marinus* CCAP 202/24
- *Audouinella parvula* CCAP 1360/9
- *Brachiomonas submarina* CCAP 7/4
- *Chlamydomonas plethora* CCAP 11/84B
- *Dermocarpa violaceae* CCAP 1416/1
- *Nonochloris* sp. CCAP 251/5
- *Phaeodactylum tricornutum* CCAP 1052/6
- *Porphyridium purpureum* CCAP 1380/1A
- *Pseudomonas lindbergii* NCIMB 11760
- *Scotiella* sp. CCAP 277/1
- *Streptomyces corchorusii* NCIMB 9979
- *Streptomyces* sp. NCIMB 12678
- *Streptomyces griseocarneus* NCIMB 9623
- *Selenastrum capricornutum* CCAP 278/4
- *Streblonema* sp. CCAP 1337/1
- *Syncrypta glomerifera* CCAP 958/1
- *Tetraselmis suecica* CCAP 66/4
- *Tetraselmis tetrathele* CCAP 66/1C

Antifungone
Streptoverticillium sp. NCIMB 8790
Antimycoplasmal compound
Actinokineospora riparia NCIMB 13255
Antiserum group N
Lactococcuc lactis subsp. *lactis* NCIMB 6681
Antitumour antibiotic
Penicillium emmonsii CABI-IMI 038805ii
Penicillium emmonsii CABI-IMI 038805iii
Anti viral agent
Penicillium brevicompactum CABI-IMI 143520
Penicillium funiculosum CABI-IMI 040582
Axenomycin
Streptomyces lisandri NCIMB 10985
Azaserine
Streptomyces fragilis NCIMB9795
Bacillomycin B
Bacillus subtilis NCIMB 8872
Bacilysin
Bacillus subtilis NCIMB 9593
Bacitracin
Bacillus licheniformis NCIMB 8874
Bacillus licheniformis NCIMB 11672
Bacteriocine-type 3
Enterococcus faecium NCIMB 2702
Bamicetin
Streptomyces plicatus NCIMB 11305
Bandamycin
Streptomyces goshikiensis NCIMB 9828
Beta-lactam
Streptomyces sp. NCIMB 11533
Blastomycin
Streptomyces blastmyceticus NCIMB 9800
Bleomycin
Streptomyces mobaraensis NCIMB 11159
Bleomycin A
Streptoverticillium sp. NCIMB 12695
Bleomycin B
Streptoverticillium sp. NCIMB 12695
Bluensomycin
Streptomyces bluensis NCIMB 9754
BM7821
Saccharothrix australiensis NCIMB 13188
Bryamcin
Streptomyces hawaiiensis NCIMB 9410
Caldariomycin
Leptoxyphium fumago CABI-IMI 089363
Leptoxyphium fumago CABI-IMI 089362
Capreomycin
Saccharothrix mutabilis subsp *cpreolus* NCIMB 9611
Carbomycin
Streptomyces albireticuli NCIMB 9600
Streptomyces tendae NCIMB 9614
Streptomyces thermotolerans NCIMB 13006
Streptomyces halstedii NCIMB 9344
Streptomyces filamentosus NCIMB 13018
Carbomycin A
Streptomyces macrosporeus NCIMB 9630
Cephalosporin
Streptomyces clavuligerus NCIMB 12785
Escherichia coli NCIMB 8666
Cephalosporin C
Acremonium chrysogenium CABI-IMI 049137
Emericellopsis minima CABI-IMI 092625
Cephalosporium sp. CABI-IMI 049137
Cephalosporin N
Emericellopsis minima CABI-IMI 069015
Cephalosporium sp. CABI-IMI 049137
Acremonium chrysogenium CABI-IMI 091579
Cephalosporin P
Acremonium chrysogenium CABI-IMI 091579
Cephalosporium sp. CABI-IMI 049137
Cephamycin C
Streptomyces cattleya NCIMB 11928
Chalcidin
Micromonospora chalcea subsp. *chalcea* NCIMB 12895
Champamycin A
Streptomyces champavatii NCIMB 12859
Champamycin B
Streptomyces champavatii NCIMB 12859
Champavatin
Streptomyces champavatii NCIMB 12859
Chloramphenicol
Kitasatoa nagasakensis NCIMB 11314
Kitasatoa purpurea NCIMB 11311
Kitasatoa diplospora NCIMB 11312
Streptomyces phaeochromogenes NCIMB 11741
Kitasatoa kauaiensis NCIMB 11313
Streptomyces venezuelae NCIMB 8231
Streptomyces venezualae NCIMB 8231
7-Chloro-6-dimethyltetracycline
Streptomyces aureofaciens NCIMB 12716
Chlortetracycline
Streptomyces lisitanus NCIMB 9451
Streptomyces lisitanus NCIMB 9585
Streptomyces aureofaciens NCIMB 12762
Streptomyces aureofaciens NCIMB 12761
Streptomyces aureofaciens NCIMB 12760
Streptomyces aureofaciens NCIMB 12759
Streptomyces aureofaciens NCIMB 12716
Streptomyces aureofaciens NCIMB 8677
Streptomyces viridifaciens NCIMB 8954
Streptomyces aureofaciens NCIMB 8324
Cinnamycin
Streptomyces cinnamoneus subsp. Cinnamoneus NCIMB 8851
Clavulanic acid
Streptomyces clavuligerus NCIMB 12785
Coerulomycin
Streptomyces coeulescens NCIMB 9615
Complex MSD-235
Streptomyces avidinii NCIMB 11996
Coumermycin
Streptomyces spinocoumarensis NCIMB 11892
Coumermycin A
Streptomyces rishiriensis NCIMB 11890
Cremomycin
Streptomyces cremeus NCIMB 10030

Daunomycin
- *Streptomyces insignis* NCIMB 12377
- *Streptomyces peuceticus* NCIMB 10972

Daunosaminil-daunomycin
- *Streptomyces peuceticus subsp. carneus* NCIMB 10986

6 Demethyltetracycline
- *Streptomyces aureofaciens* NCIMB 12760
- *Streptomyces aureofaciens* NCIMB 12716
- *Streptomyces aureofaciens* NCIMB 12759
- *Streptomyces aureofaciens* NCIMB 12761
- *Streptomyces aureofaciens* NCIMB 12762

Demethylchlortetracycline
- *Streptomyces aureofaciens* NCIMB 12759
- *Streptomyces aureofaciens* NCIMB 12762
- *Streptomyces aureofaciens* NCIMB 12761
- *Streptomyces aureofaciens* NCIMB 12760

Demethyltetracycine
- *Streptomyces* sp. NCIMB 9501
- *Streptomyces* sp. NCIMB 9502

Diactimycin
- *Dactylosporangium vinaceum* NCIMB 12891
- *Dactylosporangium matsuzakiense* NCIMB 12890

Dihydrodaunomycin
- *Streptomyces peuceticus* subsp. *carneus* NCIMB 10986

Dihydrodaunomycinone
- *Streptomyces peuceticus* subsp. *carneus* NCIMB 10986

Diploccin
- *Streptococcus* sp. NCIMB 8887

Distamycin A
- *Streptomyces distallicus* NCIMB 8936

Distamycin B
- *Streptomyces distallicus* NCIMB 8936

Distamycin C
- *Streptomyces distallicus* NCIMB 8936

Duramycin
- *Streptomyces cinnamoneus subsp. forma azocoluta* NCIMB 12681

Echinomycin
- *Streptomyces echinatus* NCIMB 9598

Elaiomycin
- *Streptomyces gelaticus* NCIMB 9848

Endomycin
- *Streptomyces endus* NCIMB 9819

Enteromycin
- *Streptomyces albireticuli* NCIMB 9600

Erizomycin
- *Streptomyces griseus* subsp. *erizensis* NCIMB 10029

Erythromycin
- *Saccharopolyspora erythraea* NCIMB 8665
- *Saccharopolyspora erythraea* NCIMB 8594

Everninomicin
- *Micromonospora carbonacea subsp.carbonacea* NCIMB 12663
- *Micromonospora carbonacea subsp. Aurantiaca* NCIMB 12664

Flavensomycin
- *Streptomyces cavourensis* NCIMB 8918

Fortimycin complex
- *Micromonospora olivasterospora* NCIMB 12659

Fradicin
- *Streptomyces fradiae* NCIMB 8233

Fungicidin
- *Streptomyces fungicidicus* NCIMB 12680

Funicin
- *Aspergillus funiculosus* CABI-IMI 054397ii

G-418
- *Micromonospora grisea* NCIMB 12883

Gancidin
- *Streptomyces gancidicus* NCIMB 12858

Gardimycin
- *Actinoplanes garbadinensis* NCIMB 12637
- *Actinoplanes liguriae* NCIMB 12636
- *Micromonospora echinospora subsp. Echinospora* NCIMB 12744
- *Micromonospora echinospora subsp. Ferruginea* NCIMB 12728
- *Micromonospora purpurea* NCIMB 12882
- *Micromonospora echinospora subsp.pallida* NCIMB 12660

Gentamycin complex
- *Micromonospora* sp. NCIMB 11642

Geosmin
- *Streptomyces* sp. NCIMB 11173
- *Streptomyces* sp. NCIMB 11174

Glebomycin
- *Streptomyces bluensis* NCIMB 9754

Glycopeptide
- *Streptomyces candidus* contact NCIMB

Gramicidin
- *Bacillus brevis* NCIMB 8598
- *Bacillus brevis* NCIMB 8146
- *Bacillus migulans* NCIMB 7096

Granaticin A and B
- *Streptomyces violaceoruber* NCIMB 9622

Griseofulvin
- *Khuskia oryzae* CABI-IMI 122490
- *Penicillium italicum* CABI-IMI 293196
- *Penicillium crustosum* CABI-IMI 206159
- *Penicillium janczewskii* CABI-IMI 96660
- *Penicillium griseofulvum* CABI-IMI 075832
- *Penicillium raistrickii* CABI-IMI 137808
- *Penicillium raistrickii* CABI-IMI 187604
- *Penicillium janczewskii* CABI-IMI 228669
- *Penicillium janczewskii* CABI-IMI 191499
- *Penicillium raistrickii* CABI-IMI 378416
- *Penicillium radiolobatus* CABI-IMI 378410
- *Penicillium radiolobatus* CABI-IMI 378412
- *Penicillium radiolobatus* CABI-IMI 378413
- *Penicillium radiolobatus* CABI-IMI 378414
- *Penicillium radiolobatus* CABI-IMI 378415

Griseofulvin
Penicillium radiolobatus CABI-IMI 378416
Penicillium griseofulvum CABI-IMI 293195
Griseoviridin
Streptomyces griseus NCIMB 9853
Halomycin
Micromonospora halophytica subsp.halophytica NCIMB 2223
Micromonospora halophytica subsp. nigra NCIMB 2225
Heptaene Anti-fungal
Streptomyces coelicolor NCIMB 9798
Hydroxystreptomyvcin
Streptomyces griseocarneus NCIMB 9623
Streptomyces sp. NCIMB 9378
Hygromycin A
Streptomyces crystallinus NCIMB 12860
Interferon
Human leukaemic T cell lymphoblast ECCAC 88042803
Kanamycin
Streptomyces kanamyceticus NCIMB 9343
Kirromycin
Streptomyces collinus NCIMB 11904
L-Deoxynojirimycin
Bacillus subtilis subsp. *niger* NCIMB 8058
Libanomycin
Streptomyces libani NCIMB 11011
Streptomyces libani NCIMB 11012
Streptomyces libani NCIMB 10982
Streptomyces libani subsp. *rufus* NCIMB 10976
Lincomycin
Streptomyces lincolnensis NCIMB 9413
Streptomyces vellosus NCIMB 11180
Lincomycin sulphates
Aspergillus niger CABI-IMI 041876
Lincomycin sulphoxides
Aspergillus carbonarius CABI-IMI 041875
Lipiarmycin
Avtinoplanes deccanensis NCIMB 12643
Lipoglycopeptide
Streptomyces carneum NCIMB 13202
Lipoxamycin
Streptomyces virginae NCIMB 10450
Lomofungin
Streptomyces lomondensis NCIMB 10094
Lucensomycin
Streptomyces lucensis NCIMB 12679
Lydimycin
Streptomyces lydicus NCIMB12977
M-741
Streptomyces septatus NCIMB 12982
Macrocin
Streptomyces fradiae NCIMB10812
Madumycin
Saccharothrix flava NCIMB 11447
Mannosidohydroxystreptomycin
Streptomyces sp. NCIMB 9378
Marcescin
Serratia plymuthica NCIMB 8266
Matamycin
Streptomyces bellus NCIMB 9818
Dtreptomyces matensis NCIMB 9826
Mediocidin
Strewptomyces mediocidicus NCIMB 9836
Megalomycin
Micromonospora megalomicea subsp. nigra NCIMB 12662
Melanomycin
Streptomyces melanogenes NCIMB 9559
Streptomyces melanogenes NCIMB 9835
Melanosporin
Streptomyces melanosporofaciens NCIMB 12978
Micromonosporin
Micromonspora sp. NCIMB 12855
Micromonspora sp. NCIMB 12734
Moldcidin
Streptomyces griseofuscus NCIMB 9821
Monamycin
Streptomyces jamaicensis NCIMB 10166
Mycolutein
Streptomyces distallicus NCIMB 8936
Nebramycin
Streptoalloteichus hindustanus NCIMB 12539
Streptomyces tenebrarius NCIMB 11028
Negamycin
Streptomyces pupeofuscus NCIMB 9822
Neomycin
Streptomyces albogriseolus NCIMB 9604
Streptomyces fradiae NCIMB 8233
Neutramycin
Streptomyces luteoverticillatus NCIMB 9720
Nitrosporin
Streptomyces nitrosporeus NCIMB 9717
Nocardicin A
Actinosynnema mirum NCIMB 13271
Nocardin
Amycolata autotrophica NCIMB 8939
Noglamycin
Streptomyces nogalater NCIMB 9489
Nonactin
Streptomyces tsusimeansis NCIMB 12715
Nor-plicacetin
Streptomyces plicatus NCIMB 11305
Notomycin
Streptomyces rishiriensis NCIMB 11890
Novobiocin
Streptomyces niveus NCIMB 9219
Streptomyces spheroides NCIMB 11891
Oleandomycin
Streptomyces antibioticus NCIMB 11506
Olivacein
Streptomyces olivaceus NCIMB 8238
Oxytetracyline
Streptomyces alboflavus NCIMB 9453
Streptomyces henetus NCIMB 11003
Streptomyces platensis NCIMB 9607
Streptomyces rimosus NCIMB 8229
Streptomyces varsoviensis NCIMB 9522
Pactamyces
Streptomyces pactum NCIMB 9445
Panamycin
Streptomyces alboniger NCIMB 13007

Penicillanic acid derivatives
Escherichia coli NCIMB 11670
Penicillic acid *see metabolites*
Penicillin (Flemming isolate)
Penicillium chrysogenum CABI-IMI 024317
Penicillin
Penicillium chrysogenum CABI-IMI 026211
Penicillium chrysogenum CABI-IMI 293188
Penicillin (commercial)
Penicillium chrysogenum CABI-IMI 037767
Penicillin (in submerged culture)
Penicillium griseoroseum CABI-IMI 015378
Penicillium chrysogenum CABI-IMI 026210
Penicillium chrysogenum CABI-IMI 040233
Penicillium griseoroseum CABI-IMI 017968
Penicillin (in surface culture)
Penicillium chrysogenum CABI-IMI 017969
Penicillin N
Acremonium chrysogenium CABI-IMI 091579
Streptomyces clavuligerus NCIMB 12785
Pentaene antibiotic
Streptomyces goshikiensis NCIMB 9828
Streptomyces xantholiticus NCIMB 9857
Pentamycin
Streptomyces griseofuscus NCIMB 9821
Perfringocin
Clostridium perfringes NCIMB 11105
Picromycin
Streptomyces felleus NCIMB 12974
Piericidin
Streptomyces pactum NCIMB 9445
Piericidin A and B
Streptomyces mobraensis NCIMB 11159
Pimaricin
Streptomyces natalensis NCIMB 10038
Pluramycin A and B
Streptomyces pluricolorescens NCIMB 9813
Polyanionic aututoxic
Alteromonas aurantia NCIMB 2049
Polyene anti-fungal
Actinoplanes NCIMB 12727
Polymyxin
Bacillus polymyxa NCIMB 8228
Bacillus polymyxa NCIMB 8527
Bacillus polymyxa NCIMB 8094
Polyoxin A and B
Streptomyces cacaoi subsp. *asoensis* NCIMB 12769
Streptomyces cacaoi subsp. *asoensis* NCIMB 12770
Pradimicin A (=BU-3608 or BMY-28567)
Actinomadura hibisca NCIMB 13253
Pradimicin B (=BMY-28634)
Actinomadura hibisca NCIMB 13253
Pradimicin M
Actinomadura hibisca NCIMB 13253
Pradimicin N
Actinomadura hibisca NCIMB 13253
Pradimicin O
Actinomadura hibisca NCIMB 13253
Pradimicin P
Actinomadura hibisca NCIMB 13253
Primocarcin
Streptomyces diastatochromogenes NCIMB 10210
Proactinomycin
Streptomyces sp. NCIMB 8766
Prodigiosin
Streptomyces spectabilis NCIMB 9733
Puromycin
Streptomyces alboniger NCIMB 13007
Purpuromycin
Actinoplanes ianthinogenes NCIMB 12639
Raisnomycin
Streptococcus kentuckensis NCIMB 9624
Resistomycin
Streptococcus resistomycifus NCIMB 9843
Rhodomycin A and B
Streptomyces cinereoruber NCIMB 9797
Rifamycin
Amycolatopsis mediterranei NCIMB 9613
Actinomadura cremea subsp. *rifamycini* NCIMB 12768
Rifamycin compex
Micromospora lacustris NCIMB 12767
Rifamycin S
Micromospora chalcea subsp. *chalcea* NCIMB 12800
Rifamycin SV
Micromospora chalcea subsp. *chalcea* NCIMB 12800
Rimocidin
Streptomyces romosus NCIMB 8229
Ristocetin
Amycolatopsis orientalis subsp. *Iurida* NCIMB 9601
Rosaramycin
Micromospora rosaria NCIMB 12896
Rubomycin
Streptomyces coeruleorubidus NCIMB 9620
Rubradirin
Streptomyces achromogenes subsp. Rubradiris NCIMB 9516
Rufinosporin
Micromonospora narashino NCIMB 12729
Rufrochromomycin
Streptomyces rufochromogenes NCIMB 12729
Septacidin
Streptomyces fimbriatus NCIMB 13039
Sisomycin
*Micromonospora danubiensis*NCIMB 11566
Micromonospora inyoensis NCIMB 12731
Micromonospora rosea NCIMB 11567

Sisomycin
Micromonospora sp. NCIMB 11643
Micromonospora grisea NCIMB 12883
Streptomyces viridosporus NCIMB 9824
Sparsomycin
Streptomyces sparsogenes NCIMB 9449
Spectinomycin
Streptomyces flavopersicus NCIMB 13020
Statalon Antiviral
Penicillium brevicompactum CABI-IMI 143520
Streptocin
Streptomyces griseus NCIMB8591
Streptolydigin
Streptomyces lydicus NCIMB12977
Streptolysin
Streptococcus pyogenes NCIMB 8884
Streptomycin
Streptomyces griseus subsp. *griseus* NCIMB 8136
Streptomyces griseus subsp. *griseus* NCIMB 8237
Streptomyces griseus subsp. *griseus* NCIMB 8232
Streptomyces griseus subsp. *griseus* NCIMB 8506
Streptomyces griseus subsp. *griseus* NCIMB 13023
Streptomyces griseus subsp. *griseus* NCIMB 9001
Streptomyces griseus subsp. *griseus* NCIMB 11001
Streptothricin
Streptomyces lavendofoliae NCIMB 9823
Streptomyces lavendulae NCIMB 6959
Streptomyces lavendulae NCIMB 6960
Streptomyces lavendulae NCIMB 9840
Streptomyces lavendulae NCIMB 9000
Streptovaricins
Streptomyces spectabilis NCIMB 9733
Streptozoticin
Streptomyces achromogenes subsp. *streptozoticus* NCIMB 9753
TA
Myxococcus xanthus NCIMB 12806
Teichomycins
Actinoplanes teichomycins NCIMB 12640
Tennecetin
Streptomyces chattanoogensis NCIMB 9809
Tertiomycin A
Streptomyces albireticuli NCIMB 9600
Tetracycline
Streptomyces avellaneus NCIMB 11000
Streptomyces lusitanus NCIMB 9585
Streptomyces aureofaciens NCIMB 8234
Streptomyces viridifaciens NCIMB 8954
Streptomyces lusitanus var. *tetracyclini* NCIMB 9500
Streptomyces lusitanus NCIMB 9451
Streptomyces avellaneus NCIMB 11000
Streptomyces aureofaciens NCIMB 12716
Streptomyces aureofaciens NCIMB 9124
Streptomyces aureofaciens NCIMB 9123
Streptomyces aureofaciens NCIMB 9122
Streptomyces aureofaciens NCIMB 9121
Tetracycline
Streptomyces aureofaciens NCIMB 9120
Streptomyces lusitanus var. *tetracyclini* NCIMB 9700
Thermocin
Bacillus stearothermophilus NCIMB 11401
Bacillus stearothermophilus NCIMB 11400
Thiamycin
Streptomyces michiganensis subsp. *Amylolyticus* NCIMB 10973
Thienamycin
Streptomyces cattleya NCIMB 11928
Streptomyces argenteolus NCIMB 9625
Tobramycin
Streptoalloteichus hindustanus NCIMB 12539
Toyomycin
Streptomyces toyocaensis NCIMB 9808
Trochomycin
Streptomyces olivoverticillatus NCIMB 9714
Tubercidin
Streptococcus sparsogenes NCIMB 9449
Tubermycins A znd B
Streptococcus misakiensis NCIMB 9852
Tyrocidin
Bacillus brevis NCIMB 8146
Bacillus brevis NCIMB 8598
U-42
Streptomyces sviceus NCIMB11184
U-43120
Streptomyces paulus NCIMB 12786
Unknown Against plant pathogenic fungi
Streptomyces cinnamoneus subsp. *forma azacoluta* NCIMB 12681
Unknown Against rice blast
Streptomyces tsusimaensis NCIMB 12715
Vallinomycin
Streptomyces tsusimaensis NCIMB 12715
Vancomycin
Amycolatopsis orientalis subsp. *orientalis* NCIMB 12945
Venturicidin
Streptomyces sp. NCIMB 9199
Streptomyces sp. NCIMB 9200
Streptomyces sp. NCIMB 9198
Verdamycin A and I
Micromonospora grisea NCIMB 12883
Vinactin
Streptomyces sp. NCIMB 8852
Viomycin
Streptomyces griseus subsp. *purpureus* NCIMB 9345
Streptomyces floridae NCIMB 12830
Streptomyces sp. NCIMB 9755
Xanthomycin
Penicillium crustosum CABI-IMI 206159
Xanthomycins A and B
Streptomyces pseudogriseolus NCIMB 9411
Zaomycin
Streptomyces zaomyceticus NCIMB 9850

Food and beverage strains

Including strains used directly for food and strains used in food fermentation, process and production.

Acidophilus milk
- *Lactobacillus acidophilus* NCIMB

Ale strains
- *Saccharomyces cerevisiae* NCYC 88
- *Saccharomyces cerevisiae* NCYC 1108
- *Saccharomyces cerevisiae* NCYC 1125
- *Saccharomyces cerevisiae* NCYC 1128
- *Saccharomyces cerevisiae* NCYC 1103
- *Saccharomyces cerevisiae* NCYC 1110
- *Saccharomyces cerevisiae* NCYC 1013
- *Saccharomyces cerevisiae* NCYC 1054
- *Saccharomyces cerevisiae* NCYC 1053
- *Saccharomyces cerevisiae* NCYC 1127
- *Saccharomyces cerevisiae* NCYC 227
- *Saccharomyces cerevisiae* NCYC 230
- *Saccharomyces cerevisiae* NCYC 231
- *Saccharomyces cerevisiae* NCYC 236
- *Saccharomyces cerevisiae* NCYC 240
- *Saccharomyces cerevisiae* NCYC 241
- *Saccharomyces cerevisiae* NCYC 238
- *Saccharomyces cerevisiae* NCYC 1052
- *Saccharomyces cerevisiae* NCYC 232
- *Saccharomyces cerevisiae* NCYC 1129

Animal feed
- *Aspergillus foetidus*CABI-IMI 015954
- *Aspergillus foetidus*CABI-IMI 041871

Aquaculture feed
(strains may contain high amounts of EPA 20:5(n-3) and DHA 22:6(n-3))
- *Brachionus plicatilus* CCAP 5010/1
- *Chaetoceros calcitrans* CCAP 1010/5
- *Chlorella salina* CCAP 211/25
- *Chlorella* sp. CCAP 211/46
- *Isochrysis galbana* CCAP 927/1
- *Isochrysis* sp. (Tahitian) CCAP 927/14
- *Nannochloris atomus* CCAP 254/4a
- *Nannochloropsis gaditana* CCAP 849/5
- *Nannochloris atomus* CCAP 251/4b
- *Nannochloropsis oculata* CCAP 849/1
- *Nannochloropsis* sp. CCAP 211/78
- *Pavlova lutheri* CCAP 931/1
- *Rhinomonas reticulata* var. *reticulata* CCAP 978/28
- *Rhinomonas reticulata* var. *reticulata* CCAP 995/2
- *Skeletonema costratum* CCAP 1077/5
- *Tetraselmis chui* CCAP 8/6
- *Tetraselmis suecica* CCAP 66/4
- *Thalassiopsira pseudomana* CCAP 1085/3

Awamori alcoholic beverage
- *Aspergillus awamori* CABI-IMI 015953 ii
- *Aspergillus awamori* CABI-IMI 015953

Baking strains
- *Saccharomyces cerevisiae* NCYC 490
- *Saccharomyces cerevisiae* NCYC 491
- *Saccharomyces cerevisiae* NCYC 77
- *Saccharomyces cerevisiae* NCYC 79
- *Saccharomyces cerevis* CABI-IMI 039916
- *Saccharomyces cerevisiae* NCYC 525
- *Saccharomyces cerevisiae* NCYC 694

Baking strains
- *Saccharomyces cerevisiae* NCYC 995
- *Saccharomyces cerevisiae* NCYC 1529
- *Saccharomyces cerevisiae* NCYC 1530
- *Saccharomyces cerevisiae* NCYC 1765
- *Saccharomyces cerevisiae* NCYC 996
- *Saccharomyces cerevisiae* NCYC 489

Beef flavouring strain
- *Thamnidium elegans* CABI-IMI 283871

Beef tenderising strain
- *Thamnidium elegans* CABI-IMI 283871

Beer (Whitbread strain)
- *Saccharomyces cerevis* CABI-IMI 061263

Butter Flavouring
- *Lactococcus lactis* subsp. *lactis* NCIMB 10493

Cheese Starter Cheddar culture
- *Enterococcus durans* NCIMB8587

Cheese Starter culture
- *Lactobacillus plantarum* NCIMB 13242

Cheese Starter Swiss culture
- *Lactobacillus delbrueckii* subsp *lactis* NCIMB 8140

Cheese way (utilisation)
- *Poprionibacterium freundenreichii* subsp. *Shermaniui* NCIMB 10585

Distilling strains
- *Saccharomyces cerevisiae* NCYC 90
- *Saccharomyces cerevisiae* NCYC 431
- *Saccharomyces cerevisiae* NCYC 87
- *Saccharomyces cerevisiae* NCYC 360

Health food/nutrient suppliments
- Various strains of *Spirullina*, *Arthrospira*, *Chlorella* – contact CCAP direct for information

Koji (soy sauce)
- *Lactobacillus casei* subsp. *rhamnosus* NCIMB 9282

Lager strains
- *Saccharomyces uvarium* NCYC 1365
- *Saccharomyces carlsbergensis* NCYC 679
- *Saccharomyces carlsbergensis* NCYC 1048
- *Saccharomyces carlsbergensis* NCYC 1057
- *Saccharomyces carlsbergensis* NCYC 1116
- *Saccharomyces carlsbergensis* NCYC 1296
- *Saccharomyces carlsbergensis* NCYC 540
- *Saccharomyces carlsbergensis* NCYC 1305
- *Saccharomyces uvarium* NCYC 1322
- *Saccharomyces uvarium* NCYC 1324
- *Saccharomyces carlsbergensis* NCYC 56
- *Saccharomyces carlsbergensis* NCYC 452
- *Saccharomyces uvarium* NCYC 1342
- *Saccharomyces carlsbergensis* NCYC 11
- *Saccharomyces carlsbergensis* NCYC 1262
- *Saccharomyces carlsbergensis* NCYC 1297
- *Saccharomyces uvarium* NCYC 2398

Lager strains
- *Saccharomyces uvarium* NCYC 1323
- *Saccharomyces carlsbergensis* NCYC 450

Meat (fermented)
- *Lactobacillus leichmannii* NCIMB 8118

Mushrooms (fruiting bodies)
- *Pleurotus ostreatus* NCWRF 40C
- *Agaricus* sp.
- *Pleurotus ostreatus* CABI-IMI 341687
- *Lyophyllum* sp. CABI-IMI 377721

Oyster mushrooom
- *Pleurotus djamor* CABI-IMI 341688

Peas (used in production of canned)
- *Rhizobium leguminosarum* NCIMB 11478

Penicillium powder for bluing cheese
- *Penicillium crustosum*CABI-IMI 143338

Single cell protein
- *Chaetomium virescens*CABI-IMI 185905
- *Rhodococcus* sp. NCIMB 11276

Soy sauce
- *Rhizopus oryzae* CABI-IMI 021602
- *Rhizopus japonicus* CABI-IMI 021600
- *Aspergillus parasiticus* CABI-IMI 087159
- *Aspergillus parasiticus* CABI-IMI 087159ii

Wine strain
- *Saccharomyces cerevisiae* NCYC 104
- *Saccharomyces cerevisiae* NCYC 1406
- *Saccharomyces cerevisiae* NCYC 1410
- *Saccharomyces cerevisiae* NCYC 1409

Wine strain (Australian wine)
- *Saccharomyces cerevisiae* NCYC 816

Wine strain (Avize-Cramant)
- *Saccharomyces cerevisiae* NCYC 357

Wine strain (Bordeux strain)
- *Saccharomyces cerevisiae* NCYC 1411

Wine strain (Champagne)
- *Saccharomyces cerevisiae* NCYC 482

Wine strain (French cider)
- *Saccharomyces cerevisiae* NCYC 1431

Wine strain (Hungarian)
- *Saccharomyces cerevisiae* NCYC 177

Wine strain (Jerez sherry)
- *Saccharomyces cerevisiae* NCYC 620
- *Saccharomyces cerevisiae* NCYC 619
- *Saccharomyces cerevisiae* NCYC 621

Wine strain (Madeira strain)
- *Saccharomyces cerevisiae* NCYC 1412

Wine strain (Mead wine)
- *Saccharomyces cerevisiae* NCYC 356

Wine strain (mesophilic)
- *Saccharomyces cerevisiae* NCYC 1408

Wine strain (Plum mead)
- *Saccharomyces cerevisiae* NCYC 358

Wine strain (Reisling)
- *Saccharomyces cerevisiae* NCYC 463
- *Saccharomyces cerevisiae* NCYC 430

Wine strain (Sake strain)
- *Saccharomyces cerevisiae* NCYC 1407

Wine strain (Sake yeast)
- *Saccharomyces cerevisiae* NCYC 478
- *Saccharomyces cerevisiae* NCYC 479

Wine strain (Sauternes strain)
- *Saccharomyces cerevisiae* NCYC 1413

Wine strain (Sherry yeast)
- *Saccharomyces cerevisiae* NCYC 671

Wine strain (Sherry yeast)
- *Saccharomyces cerevisiae* NCYC 672

Wine strain (Sri Lankan palm)
- *Saccharomyces cerevisiae* NCYC 2401
- *Saccharomyces cerevisiae* NCYC 2402

Wine strain (Steinberg wine)
- *Saccharomyces cerevisiae* NCYC 684

Wine strain (tarragona strain)
- *Saccharomyces cerevisiae* NCYC 1414

Wine strain (Tokay strain)
- *Saccharomyces cerevisiae* NCYC 1415

Yoghurt culture
- *Lactobacillus helveticus* NCIMB 8652
- *Lactobacillus delbrueckii* subsp. *Bulgaricus* NCIMB 11778
- *Streptococcus salivarius* subsp. *thermophilus* NCIMB 10387

Mycoparasites (fungal pathogens)

Agaric mushroom
- *Cladobotryum varium* CABI-IMI 374366*

Agaricus bisporus
- *Fusarium* sp. CABI-IMI 381120*

Agaricus bisporus
- *Verticillium fungicola* CABI-IMI 268001

Agaricus sp.
- *Hormiactis alba* CABI-IMI 098734
- *Hormiactis alba* CABI-IMI 142892
- *Hormiactis alba* CABI-IMI 142893
- *Hormiactis alba* CABI-IMI 119312

Aspergillus oryzae
- *Penicillium purpurogenum* CABI-IMI 091926

Heterobasidium annosum
- *Nectriopsis broomeana* CABI-IMI 71367

Mushroom
- *Melanospora damnosa* CABI-IMI 262513
- *Verticillium fungicola* CABI-IMI 290546

Mushroom sporophores
- *Verticillium fungicola* CABI-IMI 188936

Penicillium frequentans
- *Nematogonium ferrugineum* CABI-IMI 246537
- *Nematogonium ferrugineum* CABI-IMI 246538

Penicillium purpurescens
- *Piptocephalis xenophila* CABI-IMI 205708

Penicillium waksmanni
- *Pitocephalis xenophila* CABI-IMI 056692ii

Pilobolus crystallinus
- *Syncephalis sphaerica* CABI-IMI 212171

Rhizoctonia
- *Trichoderma viride* CABI-IMI 170657

Russula sp.
- *Myrothecium induntum* CABI-IMI 158885
- *Myrothecium induntum* CABI-IMI 008983iii
- *Myrothecium induntum* CABI-IMI 008983i

Biological control agents

There are many pathogens that have for potential for use as biological control agents, only a few representive examples have been included below, more can be found on the UKNCC website (www.ukncc.co.uk) by entering either strain or target organism information into the search criteria fields (i.e. host, order, genus etc.).

Acridids (potential)
- *Metarhizium flavoviride* CABI-IMI 351848

Citrus weevil (potential)
- Beauveria brongniartii CABI-IMI 354855

Coffee disease (potential)
- *Verticillium lecanii* CABI-IMI 337420

Crown gall
- *Agrobacterium radiobacter* NCIMB 13308

Grasshopper / locust
- Beauveria bassiana CABI-IMI 331274

Gryllidae (potential)
- *Metarhizium anisopliae* CABI-IMI 351846

Locust
- *Metarhizium anisopliae* CABI-IMI 074660

Mangoe anthracnose disease
- *Bacillus cereus* NCIMB 13166
- *Pseudomonas fluorescens* NCIMB 13167

Nematodes
- *Streptomyces costaricanus* NCIMB 13455

Onion white rot
- *Trichoderma longibrachiatum* CABI-IMI 297702

Orthoptera (potential)
- *Metarhizium anisopliae* CABI-IMI 351847

Pythium ultimum
- *Gliocladium virens* CABI-IMI 283130

Root borer (potential)
- *Beauveria bassiana* CABI-IMI 348041

Root grub (potential)
- *Beauveria densa* CABI-IMI 168426

Scapanes australis
- *Metarhizium anisopliae* CABI-IMI 298095

Sclerotinia sclerotiorum
- *Trichoderma harzianum* CABI-IMI 361087

Sclerotinia sclerotiorum
- *Trichoderma koningii* CABI-IMI 361084

Sclerotinia sclerotiorum
- *Trichoderma koningii* CABI-IMI 361086

Sclerotinia sp.
- *Trichoderma harzianum* CABI-IMI 361092

Sitona discoideus (potential)
- *Beauveria bassiana* CABI-IMI 351847

Strawberry disease (fungal)
- *Bacillus pumilus* NCIMB 13374
- *Pseudomonas fluorescens* NCIMB 13373

Teleogryllus (potential)
- *Metarhizium anisopliae* CABI-IMI 351831

Unspecified (potential)
- *Trichoderma harzianum* CABI-IMI 361076
- *Trichoderma harzianum* CABI-IMI 361077
- *Trichoderma harzianum* CABI-IMI 361080
- *Cochliobolus heterostrophus* CABI-IMI 311811
- *Sphaerulina mimosae-pigrae* CABI-IMI 352347
- *Verticillium lecanii* CABI-IMI 337422
- *Verticillium lecanii* CABI-IMI 339529

Nitrogen fixers

The UKNCC collections hold many strains that have potential for use in horticulture. Please consult the relevent collection for further information (for example: CABI, CCAP, NCPPB, NCIMB)

Fixer

Acetobacter diazotrophicus NCIMB 12985
Agromonas oligotrophica NCIMB 12152
Alcaligenes latus NCIMB 12189
Azospirillum brasilense NCIMB 11860
Azospirillum lipoferum NCIMB11861
Azotomonas agillis NCIMB 11693
Bacillus azotofixans NCIMB 12093
Bacillus gibsonii NCIMB 11495
Bacillus macerans NCIMB 701122
Bacillus macerans NCIMB 9368
Bacillus polymyxa NCIMB 10386
Bacillus polymyxa NCIMB 701166
Beijerinckia acida NCIMB 9883
Beijerinckia congensis NCIMB 9512
Beijerinckia derxii NCIMB 9513
Beijerinckia fluminensis NCIMB 9881
Beijerinckia mobilis NCIMB 9880
Beijerinckia indica NCIMB 8597
Beijerinckia indica subsp. lacticogenes NCIMB 8846
Citrobacter diversus NCIMB 11445
Clostridium pasteurianum NCIMB 9486
*All heterocystous c*yanobacteria belonging to the genera: *Anabaena, Anabaenopsis, Aphanizomenon, Calothrix,Cylindrospermum, Gleotrichia, Fischerella, Nostoc, Pseudanabaena, Tolypothrix, Scytonema*
Derxia gummosa NCIMB 9046
Desulfovibrio desulfuricans subsp. *desulfuricans* NCIMB 8388
Enterobacter aerogenes NCIMB 11460
Enterobacter cloacae NCIMB 11463
Escherichia coli NCIMB 11526
Erwinia herbicola NCIMB 11525
Klebsiella pneumoniae subsp. *pnuemoniae* NCIMB 11468
Klebsiella pneumoniae subsp. *pneumoniae* NCIMB 11469
Klebsiella terrigena NCIMB 9894
Pantoea agglomerans NCIMB 11524
Rhizobium fredii NCIMB 12104
Rhizobium galegae NCIMB 13151
Rhizobium leguminosarum NCIMB 12867
Rhizobium lupini NCIMB 12869
Rhizobium phaseoli NCIMB 12871
Rhizobium trifolii NCIMB 12866
Rhizobium leguminosarum NCIMB 11478 (canning peas)
Vibrio diazotrophicus NCIMB 2173
Klebsiella oxytoca NCIMB 12204
Herbespirillium seropedicae NCIMB 12540
Vibrio natriegens NCIMB 2274
Vibrio sp. NCIMB 2272
Xanthobacter autotrophicus NCIMB 12468
Xanthobacter flavus NCIMB 10071

Plant nodulator

Bradyrhizobium japonicum NCIMB 12870
Rhizobium leguminosarum NCIMB 12867
Rhizobium lupini NCIMB 12869
Rhizobium meliloti NCIMB 12868
Rhizobium phaseoli NCIMB 12871
Rhizobium trifoli NCIMB 12866
Sinorhizobium xinjiangensis NCIMB 13266

Strains isolated from extreme or interesting environments, symbionts and tolerant strains

Acidic effluent from a tin mine
- *Euglena mutabilis* CCAP 1224/41

Algal bloom
- *Asterionella* Contact CCAP

Alkali (from pulp manufacture)
- *Trichoderma viride* CABI-IMI 295977

Alkaline water isolate
- *Cyanophora paradoxa* CCAP981/1

Anaerobe (obligate)
- *Bacillus fastidiosus* NCIMB 10016

Antartcic isolates
- see tolerant strains

Antartica isolate (chasmoendolithic)
- *Desmococcus endolithicum* CCAP 417/2

Aquatic bloom
- *Chrysochromulina breviturrita* Contact CCAP
- *Stentor* sp. Contact CCAP
- *Synura* Contact CCAP

Aqueous liquor of stored coal tar
- *Pseudomonas fluorescens* NCIMB 12374

Coal
- *Acidiphilium cryptum* NCIMB 11690
- *Bacillus cereus* NCIMB 10024

Coal mine effluent
- Thiobacillus ferrooxidans NCIMB 8455

Coal mine soil
- *Streptoverticillium* sp. NCIMB 12695

Coal spoil material
- *Aspergillus spectabilis* CABI-IMI 216611

Coal tip
- *Acrophialophora fusispora* CABI-IMI 144749
- *Aspergillus fumigatus* CABI-IMI 131020
- *Corynascus sepedonium* CABI-IMI 131037
- *Malbranchea sulfurea* CABI-IMI 131007
- *Talaromyces leycettanus* CABI-IMI 178525
- *Thermoascus crustaceus* CABI-IMI 131006
- *Thielavia terrestris* CABI-IMI 131014
- *Trichophaea abundans* CABI-IMI 128503

Conveyor belt (textile) in coal mine
- *Cephalotrichum purpureofuscum* CABI-IMI 98505

Copriphilous organisms
- *Gymnascella confluens* CABI-IMI 100873
- *Gymnascella confluens* CABI-IMI 100873ii
- *Isaria felina* CABI-IMI 054853
- *Kernia nitidia* CABI-IMI 151090
- *Lophotrichus ampullus* CABI-IMI 188081
- *Memnoniella echinata* CABI-IMI 075133
- *Mycosphaerella* CABI-IMI 163345
- *Oedocephalum glomerulosum* CABI-IMI 074901
- *Syncephalis sphaerica* CABI-IMI 212171

Copriphilous (animal dung)
- *Mortierella indohii* CABI-IMI 242504
- *Mortierella indohii* CABI-IMI 242505

Copriphilous (antelope dung)
- *Chaetomium indicum* CABI-IMI 287277
- *Chaetomium strumarium* CABI-IMI 291757
- *Neocosmospora vasinfecta* CABI-IMI 170137

Copriphilous (bat dung)
- *Mortierella reticulata* CABI-IMI 336529

Copriphilous (buffalo dung)
- *Geotrichum candidum* CABI-IMI 190584
- *Lophotrichus ampullus* CABI-IMI 171509
- *Coemansia erecta* CABI-IMI 279145
- *Mortierella capitata* CABI-IMI 280010
- *Mucor flavus* CABI-IMI 280011

Copriphilous (burro dung)
- *Gelasinospora heterospora* CABI-IMI 267933
- *Neocosmospora vasinfecta* var. *africana* CABI-IMI 267939
- *Sporormiella minima* CABI-IMI 267941

Copriphilous (camel dung)
- *Orbicula parietina* CABI-IMI 268927

Copriphilous (carnivore dung)
- *Kernia nitidia* CABI-IMI 151088
- *Kernia nitidia* CABI-IMI 151082
- *Thelebolus caninus* CABI-IMI 144397
- *Thelebolus caninus* CABI-IMI 144378
- *Thelebolus crustaceus* CABI-IMI 144389
- *Thelebolus crustaceus* CABI-IMI 144390
- *Thelebolus crustaceus* CABI-IMI 144395

Copriphilous (cat dung)
- *Crinella muscae* CABI-IMI 040634

Copriphilous (chicken dung)
- *Cephaliophora tropica* CABI-IMI 074900

Copriphilous (cockroach dung)
- *Heterocephalum aurantiacum* CABI-IMI 236332

Copriphilous (cow dung)
- *Chaetomium succineum* CABI-IMI 287289
- *Coniocheata tetraspora* CABI-IMI 260594
- *Gelasinospora indica* CABI-IMI 267935
- *Gelasinospora pseudoreticulata* CABI-IMI 230600
- *Kernia hyalina* CABI-IMI 151079
- *Kernia nitidia* CABI-IMI 151081
- *Neocosmospora vasinfecta* var. *africana* CABI-IMI 267940
- *Nigrosabulum globosum* CABI-IMI 230591
- *Penicillium novae-zeelandiae* CABI-IMI 204086
- *Preussia cylindrica* CABI-IMI 166524
- *Sporormiella minima* CABI-IMI 175093
- *Sporormiella schadospora* CABI-IMI 230589
- *Thielavia terricola* CABI-IMI 153731

Copriphilous (deer dung)
- *Cephalotrichum nanum* CABI-IMI 068394
- *Cephalotrichum purpureofuscum* CABI-IMI 071294

Copriphilous (deer dung)
- *Chaetomium lentum* CABI-IMI 291748
- *Chatomium cuniculorum* CABI-IMI 172753
- *Pilaria anomala* CABI-IMI 109387
- *Pilobolus umbonatus* CABI-IMI 240956
- *Piptocephalis arrhiza* CABI-IMI 196613
- *Thelebolus caninus* CABI-IMI 144386
- *Thelebolus microsporus* CABI-IMI 144391

Copriphilous (dog dung)
- *Crinella umbellata* CABI-IMI 338439
- *Fimaria trochospora* CABI-IMI 230598
- *Microascus cinereus* CABI-IMI 086909
- *Mucor mucedo* CABI-IMI 122485

Copriphilous (elephant dung)
- *Chaetomium lentum* CABI-IMI 291749
- *Chaetomium subspirale* CABI-IMI 287290
- *Humicola grisea* var. *thermoidea* CABI-IMI 126329
- *Mycoarachis inversa* CABI-IMI 148374
- *Sporormiella isomera* CABI-IMI 170135
- *Sporormiella isomera* CABI-IMI 170136

Copriphilous (field mouse dung)
- *Botryotrichum piluliferum* CABI-IMI 092395
- *Mucor mucedo* CABI-IMI 133976

Copriphilous (frog dung)
- *Thamnidium elegans* CABI-IMI 337533

Copriphilous (giraffe dung)
- *Podosordaria australis* CABI-IMI 080526

Copriphilous (goat dung)
- *Heterocephalum aurantiacum* CABI-IMI 241265
- *Microascus cirosus* CABI-IMI 075819
- *Pilobolus crystallinus* CABI-IMI 240957
- *Thamnostylum piriforme* CABI-IMI 337630
- *Veroneae botryosa* CABI-IMI 115127

Copriphilous (guinea pig dung)
- *Gymnascella hyalinospora* CABI-IMI 099725
- *Gymnascella nodulosa* CABI-IMI 168760

Copriphilous (hare dung)
- *Chaetomium convolutum* CABI-IMI 073521
- *Coniocheata polysperma* CABI-IMI 223876
- *Podosordaria jugoyasan* CABI-IMI 223877

Copriphilous (herbovore dung)
- *Chaetocladium brefeldii* CABI-IMI 336114
- *Chaetomium subspirale* CABI-IMI 287291
- *Mucor hiemalis* f. *silvaticus* CABI-IMI 309639
- *Mycoarachis inversa* CABI-IMI 148375
- *Syncephalis coernui* CABI-IMI 240688
- *Thelebolus crustaceus* CABI-IMI 144387
- *Thelebolus crustaceus* CABI-IMI 144388

Copriphilous (horse dung)
- *Chaetocladium brefeldii* CABI-IMI 191243
- *Chaetocladium brefeldii* CABI-IMI 266093

Copriphilous (horse dung)
- *Chaetomium globosum* CABI-IMI 287298
- *Chaetomium thermophilium* var. *Coprophilium* CABI-IMI 126331
- *Chaetomium unipiculatum* CABI-IMI 293980

Copriphilous (horse dung)
- *Coprinus hexagonosporus* CABI-IMI 161417
- *Crinella muscae* CABI-IMI 206881
- *Mycotypha microspora* CABI-IMI 108621
- *Nigrosabulum globosum* CABI-IMI 148373
- *Piptocephalis cylindrospora* CABI-IMI 077593ii
- *Preussia isomera* CABI-IMI 223878
- *Stibella thermophila* CABI-IMI 295313

Copriphilous (human)
- *Mucor variabilis* CABI-IMI 117670

Copriphilous (kangaroo dung)
- *Kernia bifurcotricha* CABI-IMI 141564
- *Mucor circinelloides* CABI-IMI 103844

Copriphilous (lizard dung)
- *Benjaminiella youngi* CABI-IMI 336111
- *Benjaminiella youngi* CABI-IMI 337532
- *Benjaminiella poitrasii* CABI-IMI 377366
- *Crinella umbellata* CABI-IMI 325631
- *Ellisomyces anomalus* CABI-IMI 211277
- *Mortierella reticulata* CABI-IMI 236323
- *Mucor mousanensis* CABI-IMI 290247
- *Mucor mucedo* CABI-IMI 337631
- *Radiomyces embreei* CABI-IMI 338452
- *Radiomyces spectabilis* CABI-IMI 338433
- *Radiomyces spectabilis* CABI-IMI 338434
- *Radiomyces spectabilis* CABI-IMI 142387
- *Thamnidium elegans* CABI-IMI 325630
- *Thamnidium elegans* CABI-IMI 336112
- *Thamnidium elegans* CABI-IMI 337663
- *Thamnostylum nigricans* CABI-IMI 208235
- *Thamnostylum nigricans* CABI-IMI 208234
- *Thamnostylum piriforme* CABI-IMI 290425
- *Thamnostylum piriforme* CABI-IMI 290426
- *Thamnostylum piriforme* CABI-IMI 336113
- *Thamnostylum piriforme* CABI-IMI 337632
- *Thamnostylum piriforme* CABI-IMI 337634

Copriphilous (malayan squirrel dung)
- *Piptocephalis lepidula* CABI-IMI 339043

Copriphilous (marmot dung)
- *Sporormiella sIMI lis* CABI-IMI 230590

Copriphilous (millipede droppings)
- *Verticillium catenulatum* CABI-IMI 113078

Copriphilous (mircocavia dung)
- *Pleuroascus nicholsonni* CABI-IMI 230599

Copriphilous (moose dung)
- *Chaetomidium cephalothecoides* CABI-IMI 180791
- *Chaetomium trilaterale* var. *chiversii* CABI-IMI 387306
- *Cheatomium cupreum* CABI-IMI 250968
- *Coemansia erecta* CABI-IMI 281612
- *Crinella Naumovii* CABI-IMI 133977
- *Dichotomocladium elegans* CABI-IMI 211271
- *Dichotomocladium robustum* CABI-IMI 211274
- *Gymnascella nodulosa* CABI-IMI 168761
- *Helicostylum pulchrum* CABI-IMI 203381
- *Kickxella albastrina* CABI-IMI 213918
- *Mortierella scerotiella* CABI-IMI 133978
- *Mycotypha microspora* CABI-IMI 282443
- *Phycomyces nitens* CABI-IMI 281611
- *Pilaria caucasica* CABI-IMI 134107
- *Piptocephalis tieghemia* CABI-IMI 339051
- *Piptocephalis debaryana* CABI-IMI 338753
- *Piptocephalis lepidula* CABI-IMI 338765
- *Piptocephalis lepidula* CABI-IMI 339044
- *Piptocephalis microcephala* CABI-IMI 213565
- *Radiomyces embreei* CABI-IMI 338449
- *Radiomyces embreei* CABI-IMI 081586
- *Radiomyces spectabilis* CABI-IMI 338435
- *Thamnidium elegans* CABI-IMI 264665
- *Thamnidium elegans* CABI-IMI 264662
- *Thamnostylum lucknowense* CABI-IMI 211280
- *Thamnostylum repens* CABI-IMI 208236
- *Thamnostylum repens* CABI-IMI 211270

Copriphilous (mouse dung)
- *Actinomucor elegans* CABI-IMI 171226
- *Piptocephalis brijmohanii* CABI-IMI 339038

Copriphilous (opossum dung)
- *Pilaria anomala* CABI-IMI 105546

Copriphilous (pack rat dung)
- *Chaetomium torulosum* CABI-IMI 387304
- *Ellisomyces anomalus* CABI-IMI 338443
- *Eremomyces bilateralis* CABI-IMI 151076
- *Radiomyces embreei* CABI-IMI 338451
- *Radiomyces embreei* CABI-IMI 338450

Copriphilous (partridge dung)
- *Chaetomium trilaterale* CABI-IMI 287305
- *Penicillium claviforme* CABI-IMI 071357

Copriphilous (pony dung)
- *Poronia puntata* CABI-IMI 245188

Copriphilous (porcupine dung)
- *Kernia hippocrepida* CABI-IMI 151078

Copriphilous (rabbit dung)
- *Actinomucor elegans* CABI-IMI 86138
- *Chaetocladium brefeldii* CABI-IMI 234455
- *Chaetocladium brefeldii* CABI-IMI 080480

Copriphilous (rabbit dung)
- *Chaetocladium brefeldii* CABI-IMI 266092
- *Chaetocladium brefeldii* CABI-IMI 266091
- *Chaetocladium brefeldii* CABI-IMI 266090
- *Chaetocladium brefeldii* CABI-IMI 266088
- *Chaetomium bostrychodes* CABI-IMI 039725
- *Chaetomium subspirale* CABI-IMI 287292
- *Chatomium cuniculorum* CABI-IMI 155487
- *Cheatomium gangligerum* CABI-IMI 291744
- *Crinella muscae* CABI-IMI 073461
- *Gliocladium roseum* CABI-IMI 101020
- *Gymnascella hyalinospora* CABI-IMI 099726
- *Gymnascella nodulosa* CABI-IMI 168759
- *Isaria felina* CABI-IMI 281451
- *Lophotrichus incarnatus* CABI-IMI 135564
- *Mucor peasmaticus* CABI-IMI 381705
- *Onychophora corpophila* CABI-IMI 275663
- *Pilaria anomala* CABI-IMI 043023
- *Pilaria anomala* CABI-IMI 109386
- *Pilaria anomala* CABI-IMI 101020
- *Piptocephalis tieghemia* CABI-IMI 339474
- *Piptocephalis arrhiza* CABI-IMI 196614
- *Piptocephalis brijmohanii* CABI-IMI 164897
- *Piptocephalis debaryana* CABI-IMI 205704
- *Piptocephalis debaryana* CABI-IMI 205703
- *Piptocephalis freseniana* CABI-IMI 199156
- *Piptocephalis freseniana* CABI-IMI 199157
- *Piptocephalis indica* CABI-IMI 339042
- *Piptocephalis indica* CABI-IMI 199159
- *Piptocephalis lepidula* CABI-IMI 196611
- *Piptocephalis lepidula* CABI-IMI 196612
- *Piptocephalis repens* CABI-IMI 149887
- *Piptocephalis repens* CABI-IMI 149887ii
- *Spororminula tenerifae* CABI-IMI 311150
- *Sutravarana samala* CABI-IMI 205842
- *Syncephalis nodosa* CABI-IMI 203383
- *Syncephalis nodosa* CABI-IMI 197709
- *Thamnidium elegans* CABI-IMI 079897
- *Thamnidium elegans* CABI-IMI 264663
- *Thelebolus caninus* CABI-IMI 144379
- *Thelebolus crustaceus* CABI-IMI 068073

Copriphilous (rabbit hutch dung)
- *Cephalotrichum nanum* CABI-IMI 080254
- *Thelebolus crustaceus* CABI-IMI 079997

Copriphilous (rat dung)
- *Benjaminiella poitrasii* CABI-IMI 081585
- *Benjaminiella youngi* CABI-IMI 337208

Copriphilous (rat dung)
- *Cokeromyces recurvatus* CABI-IMI 077585
- *Crinella umbellata* CABI-IMI 054855
- *Piptocephalis tieghemia* CABI-IMI 339621
- *Utharomyces epallocaulis* CABI-IMI 211283
- *Utharomyces epallocaulis* CABI-IMI 213005
- *Utharomyces epallocaulis* CABI-IMI 211282
- *Utharomyces epallocaulis* CABI-IMI 211588

Copriphilous (rock hyrax dung)
- *Podosordaria austro-americana* CABI-IMI 174498

Copriphilous (rodent dung)
- *Dichotomocladium elegans* CABI-IMI 211272
- *Dispira parvispora* CABI-IMI 101210iii
- *Mucor faisalabadensis* CABI-IMI 304471
- *Piptocephalis cylindrospora* CABI-IMI 339466
- *Radiomyces embreei* CABI-IMI 338453
- *Utharomyces epallocaulis* CABI-IMI 213007
- *Utharomyces epallocaulis* CABI-IMI 236319
- *Utharomyces epallocaulis* CABI-IMI 236320
- *Utharomyces epallocaulis* CABI-IMI 240373

Copriphilous (sheep dung)
- *Coprinus utrifer* CABI-IMI 161422
- *Iodophanus carneus* CABI-IMI 070912ii
- *Penicillium novae-zeelandiae* CABI-IMI 204087
- *Piptocephalis lepidula* CABI-IMI 211369

Copriphilous (squirrel dung)
- *Pirella circinans* CABI-IMI 338448

Copriphilous (toad dung)
- *Coemansia erecta* CABI-IMI 312319

Copriphilous (unspecified)
- *Basidiobolus microsporus* CABI-IMI 093345
- *Chaetomidium fimeti* CABI-IMI 116692
- *Chaetomium brasiliense* CABI-IMI 338828
- *Chaetomium brasiliense* CABI-IMI 042201
- *Piptocephalis cylindrospora* CABI-IMI 339465

Copriphilous (wessel dung)
- *Thamnidium elegans* CABI-IMI 236139

Copriphilous (wolf dung)
- *Thelebolus caninus* CABI-IMI 144375
- *Thelebolus microsporus* CABI-IMI 144392

Copriphilous (woodchuch dung)
- *Hapsidospora irregularis* CABI-IMI 173727

Copriphilous (zebra dung)
- *Chaetomium strumarium* CABI-IMI 291758
- *Hapsidospora irregularis* CABI-IMI 291760

Copriphilous (Mus formosanus dung)
- *Utharomyces epallocaulis* CABI-IMI 213006

Deep sea (70m)
- *Anabaena variabilis* CCAP 1403/31

Desert
- *Radiospheara negevensis* fo. *Minor* CCAP 247/3
- *Radiospheara negevensis* CCAP 247/2

Drainage water from a coal mine
- Methylomonas rubrum NCIMB 11913

Eelworms
- *Harposporium bysmatosporum* CABI-IMI 087014
- *Harposporium helicoides* CABI-IMI 087013

Epiphytic
- *Audouniella alariae* CCAP 1365/2

Epiphytic (on Bonnesmaisomia asparagopsis)
- *Audouniella tetraspoa* CCAP 1360/12

Epiphytic (on Chordaria flagelliformis)
- *Chromastrum collopodum* CCAP 1365/7

Epiphytic (on Cladophora prolifera)
- *Phormidium foveolarum* CCAP 1446/8

Epiphytic (on Ectocarpus sp.)
- *Chromastrum moniliforme* CCAP 1365/5

Epiphytic (on Haloptris scoparia)
- *Chromastrum humile* CCAP 1365/3

Epiphytic (on Heterosiphonia plumosa)
- *Audouniella endophytica* CCAP 1360/5

Epiphytic (on lichen)
- *Coccomyxa arvenensis* CCAP 216/1
- *Coccomyxa viridis* CCAP 216/14

Epiphytic (on moss)
- *Dictyosphaerium minutum* CCAP 222/3

Epiphytic (on Odonthalia dentata)
- *Audouniella pectinata* CCAP 1350/11

Epiphytic (on Oedogonium sp.)
- *Mischococcus sphaerocephalus* CCAP 847/1

Epiphytic (on Polysiphonia sp.)
- *Audouniella darviesii* CCAP 1350/5

Epiphytic (on Polysiphonia sp.)
- *Audouniella dasyae* CCAP 1350/2

Epiphytic (on Polysiphonia sp.)
- *Chromastrum kylinoides* CCAP 1365/4

Epiphytic (on Polysiphonia violacea)
- *Chromastrum reductum* CCAP 13561

Epizoic (on bryozoan)
- *Audouniella pectinata* CCAP 1350/10

Epizoic (on hydrozoan)
- *Audouniella pectinata* CCAP 1350/9

Fermenter system at elevated temperature
- *Aspergillus fumigatus* CABI-IMI 293740

Foam sample
- *Porocladium aquaticum* CABI-IMI 199900

Freshwater
- *Amoeba proteus* CCAP 1503/9

Gas-works effluent
- *Comamonas testosteroni* NCIMB 8893

Hot spring
- *Naegleria clarki* CCAP 1518/15
- *Sulfolobus acidocaldarius* NCIMB 11770
- *Naegleria clarki* CCAP 1518/15
- *Naegleria clarki* CCAP 1518/15

Hot spring
- *Naegleria clarki* CCAP 1506/1
- *Sulfolobus acidocaldarius* NCIMB 11770

Nematodes
- *Dactyella illequens* CABI-IMI 372759
- *Fusarium oxysporum* CABI-IMI 369179*
- *Fusarium* sp. CABI-IMI 369180*

Nematode gut symbiont
- *Xenorhabdus nematophilus* NCIMB 9965

Nematodphagous (Hepialis sp.)
- *Cordyceps gracilis* CABI-IMI 016573

Nematophagous
- *Harposporium anguillulae* CABI-IMI 232243
- *Harposporium arthrosporum* CABI-IMI 232244
- *Harposporium crasum* CABI-IMI 174854

Pickled cured fish (spoilage)
- *Polyschema sagarii* CABI-IMI 291231

River foam
- *Articulospora tetracladia* CABI-IMI 209374
- *Ceuthospora phacidioides* CABI-IMI 209750
- *Lemonniera terrestris* CABI-IMI 209372
- *Mariannaea camptospora* CABI-IMI 133904
- *Tricladium brunneum* CABI-IMI 177447
- *Tricladium malaysianum* CABI-IMI 177449
- *Tricladium spendens* CABI-IMI 209373
- *Triposermum campelopardus* CABI-IMI 123907
- *Varicosporium helicosporum* CABI-IMI 177446a
- *Varicosporium macrosporeum* CABI-IMI 177446b
- *Flavobacterium* sp. NCIMB 10741

Rock
- *Ramalina decipeus* CABI-IMI 400185*

Rock isolate (Antarctica isolate)
- *Hemichloris antartica* CCAP 239/2
- *Hemichloris antartica* CCAP 239/3
- *Hemichloris antartica* CCAP 239/1

Rumen bacteria
- *Amycolatopsis rugosa* NCIMB 8926
- *Butyrivibrio fibrisolvens* NCIMB 702429
- *Clostridium cellobioparum* NCIMB 10669
- *Eubacterium fissicatena* NCIMB 10445
- *Fibrobacter succinogenes* NCIMB 702575
- *Megasphaera elsdenii* NCIMB 8927
- *Ruminococcus flavifaciens* NCIMB 702216
- *Selenomonas ruminantium* subsp. *ruminantium* NCIMB 702385
- *Weissella paramesenteroides* NCIMB700870

Sacking
- *Preussia vulgaris* CABI-IMI 089575

Sewage
- *Aeromonas caviae* NCIMB 9235
- *Alcaligenes* sp NCIMB 10413
- *Alteromonas* sp. NCIMB 1914
- *Aquaspirillum* sp NCIMB 8997
- *Aquaspirillum* sp NCIMB 8998

Sewage
- *Aquaspirillum* sp NCIMB 8999
- *Arthrobacter mysorens* NCIMB 10583
- *Aspergillus stellatus* CABI-IMI 146289
- *Aureobacterium barkeri* NCIMB 9658
- *Bifidobacterium angulatum* NCIMB 702237
- *Bifidobacterium angulatum* NCIMB 702238
- *Bifidobacterium angulatum* NCIMB 702239
- *Bifidobacterium angulatum* NCIMB 702249
- *Brevibacterium liquefaciens* NCIMB 9545
- *Corynebacterium glutamicum* NCIMB 10025
- *Corynebacterium glutamicum*NCIMB 10025
- *Corynebacterium sp.* NCIMB 9379
- *Corynebacterium* sp. NCIMB10335
- *Escherichia coli* NCTC 10483
- *Lysobacter* sp. NCIMB 1784
- *Microbacterium laevaniformans* NCIMB 702288
- *Microbacterium laevaniformans* NCIMB 702288
- *Neocosmospora tenuicristata* CABI-IMI 277708
- *Penicillium crustosum* CABI-IMI 146306
- *Pseudomona* sp. NCPBB 2464
- *Pseudomonas aeruginosa* NCIMB 10116
- *Pseudomonas aeruginosa* NCIMB 10545
- *Pseudomonas* sp. NCIMB 9045
- *Pseudomonas* sp. NCIMB 10558
- *Pseudomonas* sp. NCPBB 2465
- *Vibrio navarrensis* NCIMB 13120

Sewage (brewary)
- *Cellumonas cellulans* NCIMB 11921

Sewage (municipal)
- *Lactobacillus sharpeae* NCIMB 11720

Sewage beds
- *Fusarium aquaeductuum* CABI-IMI 205331

Sewage digester (aerobic)
- *Desulfovibrio vulgaris* subsp. *vulgaris* NCIMB 11727

Sewage field
- *Botrydium granulatum* var. *kolkwitzianum* CCAP 805/4

Sewage lagoon
- *Thiobacillus denitrificans* NCIMB 9548

Sewage oxidation pond
- *Anabaena* sp CCAP 1403/13A

Sewage pond
- *Rhodospirillum fulvum* NCIMB 11762

Sewage sludge (anearobic)
- *Clostridium subterminale* NCIMB 12758

Sewage sludge
- *Lactosphaera pasteurii* NCIMB 13421
- *Methylocystis echinoides* NCIMB 13100

Sewage sludge (thermally treated)
- *Sphaerobacter thermophilus* NCIMB 13135

Sewage tank
- *Thiobacillus denitrificans* NCIMB 9547

Sewage treatment plant
- *Aeromonas sobria* NCIMB 2089
- *Aeromonas sobria* NCIMB 2090
- *Desulfovibrio gigas* NCIMB 12906
- *Prodiscophrya collini* CCAP 1618/2

Sulphur springs
- *Stichococcus bacillaris* CCAP 379/13
- *Stichococcus bacillaris* CCAP 379/17
- *Stichococcus bacillaris* CCAP 379/22
- *Stichococcus bacillaris* CCAP 379/20
- *Stichococcus bacillaris* CCAP 379/19
- *Stichococcus bacillaris* CCAP 379/18
- *Stichococcus bacillaris* CCAP 379/24
- *Stichococcus bacillaris* CCAP 379/14
- *Stichococcus bacillaris* CCAP 379/25
- *Stichococcus bacillaris* CCAP 379/12
- *Stichococcus bacillaris* CCAP 379/11
- *Stichococcus bacillaris* CCAP 379/10
- *Stichococcus bacillaris* CCAP 379/9
- *Stichococcus bacillaris* CCAP 379/8
- *Stichococcus bacillaris* CCAP 379/7
- *Stichococcus bacillaris* CCAP 379/16
- *Stichococcus bacillaris* CCAP 379/15
- *Stichococcus bacillaris* CCAP 379/21
- *Stichococcus bacillaris* CCAP 379/23

Symbiont (leaf nodule symbiont)
- *Klebsiella pneumoniae subsp. pnumoniae* NCIMB 9478

Symbiont (lichen mycobiont
- *Dictyostelium mucoroides* CABI-IMI 074707

Symbiont (lichen photobiont)
- *Coccomyxa mucigena* CCAP 216/4
- *Coccomyxa peltigerae* CCAP 216/5
- *Coccomyxa pringshecabii* CCAP 216/7
- *Coccomyxa solarinae bisporae* CCAP 216/10
- *Coccomyxa solarinae crocae* CCAP 216/11a

Symbiont (lichen photobiont)
- *Coccomyxa solarinae saccatae* CCAP 216/12
- *Dilabifilum incrustans* CCAP 415/1
- *Myrmecia pyriformis* CCAP 250/1
- *Trebouxia crenulata* CCAP 219/1b
- *Trebouxia crenulata* CCAP 219/2
- *Trebouxia* sp. CCAP 213/1b

Symbiont (lichen)
- *Trebouxia* sp. CCAP 213/1B

Symbiont (*Obelia geniculata*)
- *Audouniella boryana* CCAP 1360/15

Symbiont (with *Hydra viridis*)
- *Chloerella* sp. CCAP 211/61

Symbiotic (plant rhizosphere)
- *Bradyrhizobium japonicum NCIMB* 12870

Termite
- *Bacteroides termitides* NCIMB

Thermal water (Bath, England)
- *Willaertia magna* CCAP 1594/1

Biodeteriogens

See also: Biodegraders/Utilisers, Food spoilage organisms and Environmental isolates

Acetic preserve
- *Monilinia acetobutans* CABI-IMI 180570

Aircraft fuel tank
- *Penicillium minioluteum* CABI-IMI 147406
- *Syncephalostrum racemosum* CABI-IMI 362748*

Alkali pulp manufacture
- *Trichoderma harzianum* CABI-IMI 297033
- *Trichoderma harzianum* CABI-IMI 295979

Animal specimen in formalin
- *Penicillium janthinellum* CABI-IMI 117655

Army socks
- *Mucor oblongisporus* CABI-IMI 100706

Art
- *Gliomastix luzulae* CABI-IMI 133983

Artificial leather
- *Penicillium viridicatum* CABI-IMI 091952

Artificial leather cloth
- *Penicillium commune* CABI-IMI 091957

Asbestos cord
- *Cephalotrichum nanum* CABI-IMI 076251

Asbestos substitute building
- *Stachybotrys atra* CABI-IMI 292096

Aviation fuel
- *Amorpotheca resinae* CABI-IMI 342896
- *Amorpotheca resinae* CABI-IMI 331223
- *Amorpotheca resinae* CABI-IMI 325811
- *Amorpotheca resinae* CABI-IMI 331941
- *Amorpotheca resinae* CABI-IMI 135416
- *Amorpotheca resinae* CABI-IMI 325704
- *Amorpotheca resinae* CABI-IMI 325706
- *Amorpotheca resinae* CABI-IMI 344050
- *Amorpotheca resinae* CABI-IMI 325812
- *Amorpotheca resinae* CABI-IMI 331939
- *Amorpotheca resinae* CABI-IMI 331942
- *Amorpotheca resinae* CABI-IMI 331973
- *Amorpotheca resinae* CABI-IMI 325705
- *Amorpotheca resinae* CABI-IMI 296266
- *Aspergillus fumigatus* CABI-IMI 181419
- *Aspergillus fumigatus* CABI-IMI 184361
- *Aspergillus fumigatus* CABI-IMI 184363
- *Paecilomyces variotii* CABI-IMI 184364
- *Penicillium brevicompactum* CABI-IMI 329660
- *Penicillium canescens* CABI-IMI 184360

Baby powder
- *Mucor racemosus* CABI-IMI 316960

Balsa blocks in river
- *Zopfiella leucotricha* CABI-IMI 153733

Balsa wood submerged in patuxent river
- *Trichocladium achrasporium* CABI-IMI 132773

Batter pudding
- *Aspergillus wentii* CABI-IMI 040641

BEA vanguard wing tank
- *Amorpotheca resinae* CABI-IMI 138247

Binocular lens
- *Aspergillus tonophilus* CABI-IMI 108299

Bitumen treated cardboard
- *Amorpotheca resinae* CABI-IMI 090126

Blue stain (wood spoilage)
- *Ceratocystis pilifera* CABI-IMI 274362

Bromthymol blue indicator solution
- *Penicillium spinulosum* CABI-IMI 091919

Canteen cover
- *Myrothecium verrucaria* CABI-IMI 140593

Cannery (walls and ceiling)
- *Exophiala pisciphila* CABI-IMI 288044

Canvas
- *Memnoniella echinata* CABI-IMI 061273ii
- *Memnoniella echinata* CABI-IMI 061273

Canvas bag (Officer's)
- *Myrothecium verrucaria* CABI-IMI 140592

Canvas in mine
- *Aspergillus ustus* CABI-IMI 089359

Cellophane (buried in soil)
- *Oidiodendron rhodogenum* CABI-IMI 068735

Cellophane (weathering treated)
- *Penicillium sclerotiorum* CABI-IMI 092040

Cellophane in sand
- *Botryotrichum piluliferum* CABI-IMI 081303
- *Botryotrichum piluliferum* CABI-IMI 068736

Cellophane paper
- *Aspergillus versicolor* CABI-IMI 045554

Cellulose board
- *Chaetomium globosum* CABI-IMI 107512

Cellulose board
- *Scopulariopsis brevicaulis* CABI-IMI 107513

Cellulose fibres
- *Penicillium kloeckeri* CABI-IMI 202320

Cellulosic material
- *Penicillium albicans* CABI-IMI 233074

Cellulosic material (buried in forest soil)
- *Fennellia flavipes* CABI-IMI 321308

Cellulosic material (India)
- *Chaetomium cymbiforme* CABI-IMI 252060
- *Cheatomium fungicola* CABI-IMI 318396
- *Chaetomium cymbiforme* CABI-IMI 318414

Cellulosic soil buried in forest material
- *Fennellia flavipes* CABI-IMI 171883

Charcoal
- *Hypocenomyce australis* CABI-IMI 401280

Cigar tobacco
- *Dictyoarthrinium rabaulense* CABI-IMI 181940

Cloth
- *Cladosporium sphaerospermum* CABI-IMI 094153
- *Aspergillus terreus* CABI-IMI 089355
- *Aspergillus versicolor* CABI-IMI 094152

Coal mine spoilage
- *Aspergillus spectabilis* CABI-IMI 316611

Concrete wall
- *Arthrobotrys javinica* CABI-IMI 255232

Contact lens
- *Verticillium lecanni* CABI-IMI 176057

Copper electroplating solution
- *Penicillium crustosum* CABI-IMI 166526

Copper sulphate solution
- *Penicillium ochrochloron* CABI-IMI 039806
- *Penicillium spinulosum* CABI-IMI 087416

Cotton
- *Chaetomium globosum* CABI-IMI 045550
- *Chaetomium globosum* CABI-IMI 045550ii
- *Chaetomium globosum* CABI-IMI 045550iv
- *Chaetomium globosum* CABI-IMI 045550iii

Cotton (American)
- *Aspergillus terreus* CABI-IMI 089357
- *Aspergillus terreus* CABI-IMI 044339

Cotton (Egyptian)
- *Aspergillus terreus* CABI-IMI 089356

Cotton fabric
- *Stachybotrys atra* CABI-IMI 082021
- *Memnoniella echinata* CABI-IMI 016201

Cotton textile
- *Stachybotrys atra* CABI-IMI 115289

Cotton yarn
- *Aspergillus versicolor* CABI-IMI 094159
- *Aspergillus tamarii* CABI-IMI 092019

Creosoted wood
- *Amorpotheca resinae* CABI-IMI 089357
- *Amorpotheca resinae* CABI-IMI 089838
- *Amorpotheca resinae* CABI-IMI 149211

Crude oil
- *Exophiala dermatitidis* CABI-IMI 294929

Cutting fluid
- *Geotrichum candidum* CABI-IMI 329542
- *Penicillium expansum* CABI-IMI 329544

Dextrin paste
- *Penicillium spinulosum* CABI-IMI 091953

Diesel
- *Amorpotheca resinae* CABI-IMI 179266
- *Penicillium corylophilum* CABI-IMI 290817
- *Penicillium spinulosum* CABI-IMI 320722
- *Trichoderma harzianum* CABI-IMI 290819
- *Acremonium strictum* CABI-IMI 299023

Diso naval fuel
- *Tritirachium oryzae* CABI-IMI 320903

Distilled water container
- *Penicillium spinulosum* CABI-IMI 091954
- *Penicillium spinulosum* CABI-IMI 091950

Distilled water container
- *Penicillium spinulosum* CABI-IMI 091955

Earthenware plant pot
- *Oedocephalum glomerulosum* CABI-IMI 140796

Electrical equipment
- *Cladosporium herbarum* CABI-IMI 170353

Electrical fuse
- *Aspergillus versicolor* CABI-IMI 091890
- *Penicillium commune* CABI-IMI 091918

Electrical wire sleeving
- *Absidoa californica* CABI-IMI 227977

Explosives
- *Acremonium strictum* CABI-IMI 077044

Film (deteriorated)
- *Corynascus sepedonium* CABI-IMI 316334

Flannel bag
- *Aspergillus ustus* CABI-IMI 089360

Formalin tanks (surface)
- *Penicillium simplicissium* CABI-IMI 252091

Fuel filters
- *Penicillium corylophilum* CABI-IMI 273248
- *Penicillium hirsutum* CABI-IMI 273250

Gelatine factory effluent
- *Rhizopus stolonifer* CABI-IMI 324540

Glycerine
- *Penicillium solitum* CABI-IMI 039819

Glyphosphate solution
- *Verticillium lecanni*CABI-IMI 293226

Hardwood
- *Perenniporia fraxinea* CABI-IMI 081803

Heat seal foil material
- *Penicillium chrysogenum* CABI-IMI 175765

Hydaulic oil
- *Aspergillus fumigatus* CABI-IMI 160349

Ice cream carton
- *Trichoderma harzianum* CABI-IMI 049268

Industrial waste
- *Monascus purpueus* CABI-IMI 317626

Iron bark in cooling tower
- *Penicillium erubescens* CABI-IMI 190275

Italian talcum powder
- *Cladosporium cladosporioides* CABI-IMI 185487

Jet fuel
- *Acremonium strictum* CABI-IMI 178506

Kerosene filter pad
- *Cheatomium fungicola* CABI-IMI 087410

Kerosene fuel filter
- *Aspergillus fumigatus* CABI-IMI 173460
- *Pestalotiopsis aquatica* CABI-IMI 087402
- *Amorpotheca resinae* CABI-IMI 088973
- *Amorpotheca resinae* CABI-IMI 088972
- *Amorpotheca resinae* CABI-IMI 088971
- *Amorpotheca resinae* CABI-IMI 088968
- *Yarrowia lipolytica* CABI-IMI 093743

Label (museum)
- *Crinella umbellata* CABI-IMI 136605

Leather
Paecilomyces variotii CABI-IMI 114930
Penicillium diversum CABI-IMI 040579iii
Penicillium diversum CABI-IMI 040579
Leather boot
Penicillium emmonsii CABI-IMI 089713
Linseed oil based red primer
Penicillium canescens CABI-IMI 166199
Lubricating oil
Fusarium oxysporum CABI-IMI 290814
Chaetomium globosum CABI-IMI 290815
Manilla fibre
Ulocladium chartarum CABI-IMI 116369
Marine diesel oil
Amorpotheca resinae CABI-IMI 322372
Metal working fluid
Amorpotheca resinae CABI-IMI 322170
Fusarium solani CABI-IMI 289157
Geotrichum candidum CABI-IMI 289156
Scopulariopsis brevicaulis CABI-IMI 321976
Chaetomium globosum CABI-IMI 314227
Mild steel corrosion
Shewanella putrefaciens NCIMB 12579
Shewanella putrefaciens NCIMB 12577
Military equipment (deterioratinng)
Penicillium implicatum CABI-IMI 040578ii
Mine timber
Penicillium duclauxii CABI-IMI 200309
Mines
Penicillium crustosum CABI-IMI 297891
Mould proofing
Aspergillus versicolor CABI-IMI 04554iv
Aspergillus versicolor CABI-IMI 045554
Matress cover
Chaetomium atrobrunneum CABI-IMI 291742
Mussels in preserving fluid
Aspergillus versicolor CABI-IMI 188634
*Scopulariopsis brevicaulis*CABI-IMI 188635
Nylon
Tritirachium oryzae CABI-IMI 045535
Oil emulsions (biodegredation)
Pseudomonas oleovorans NCIMB 6576
Oil palm trunk
Thielaviopsis paradoxa CABI-IMI 378941
Thielaviopsis paradoxa CABI-IMI 378942
Ointment
Amorpotheca resinae CABI-IMI 049621
Optical instrument
Penicillium capsulatum CABI-IMI 040576
Paint
Penicillium kloeckeri CABI-IMI 210615
Cladosporium sphaerospermum CABI-IMI 210616
Cladosporium herbarum CABI-IMI 131128
Cladosporium sphaerospermum CABI-IMI 214669
Paint
Cladosporium sphaerospermum CABI-IMI 084420
Paint (emulsion - degredation of thickener)
Bacillus sphaericus NCIMB 10839
Bacillus sphaericus NCIMB 10841
Providencia rettgeri NCIMB 10842
Paint (emulsion with chromate)
Penicillium minioluteum CABI-IMI 178519
Penicillium spinulosum CABI-IMI 178523
Paint (phenolic tung-Zn oxide/chromate)
Aspergillus flavus CABI-IMI 178520
Paint (red lead)
Penicillium expansum CABI-IMI 163739
Penicillium brevicompactum CABI-IMI 163740
Painted beach box
Penicillium corylophilum CABI-IMI 222984
Painted urface metal amplifier case
Aspergillus versicolor CABI-IMI 096330
Painted wood
Aureobasidium pullans CABI-IMI 045533ii
Aureobasidium pullans CABI-IMI 045533
Cladosporium sphaerospermum CABI-IMI 061886
Curvularia lunata CABI-IMI 063375
Paintwork
Cladosporium sphaerospermum CABI-IMI 235764
Palm oil
Paecilomyces variotii CABI-IMI 227571
Paper - 17th century rag-paper document
Mycotypha microspora CABI-IMI 031239
Paper
Chaetomium globosum CABI-IMI 016203
Chaetomium nigricolor CABI-IMI 144976
Penicillium rubrum CABI-IMI 040036
Penicillium sclerotiorum CABI-IMI 146640
Penicillium viridicatum CABI-IMI 297902
Stachybotrys atra CABI-IMI 072155
Trichoderma harzianum CABI-IMI 061381
Paper (damp)
Ulocladium botrytis CABI-IMI 123533
Paper pulp
Penicillium funiculosum CABI-IMI 061385
Penicillium funiculosum CABI-IMI 061383
Penicillium variabile CABI-IMI 061384
Paracetamol tablet
Penicillium aurantiogriseum CABI-IMI 305618
Parachute nylon
Penicillium janthinellum CABI-IMI 075589
Paradise nuts
Crinella muscae CABI-IMI 200308
Paraffin wax
Aspergillus versicolor CABI-IMI 091859

p-digunidino-benzoyl-l-glutamic acid hydrochloride
Penicillium spinulosum CABI-IMI 029467
Penicillium powder for blueing cheese (Denmark)
Penicillium crustosum CABI-IMI 173028
Pilot plant usng kerosene for copper extraction
Penicillium spirillum CABI-IMI 152804
Pinus strobus boards
Cephaloascus fragrans CABI-IMI 068865
Plaster and wallpaper
Botryotrichum piluliferum CABI-IMI 051266
Rhinotrichum lanosum CABI-IMI 051266
Plastic
Aspergillus fumigatus CABI-IMI 095927
Aspergillus terreus CABI-IMI 095928
Aureobasidium pullans CABI-IMI 145194
Cunninghamella elegans CABI-IMI 313951
Penicillium pinophilum CABI-IMI 087160ii
Penicillium pinophilum CABI-IMI 087160
Plastic covered nylon sail cover
Drechslera dematioidea CABI-IMI 100720
Polycaprolactone polyurethane
Trichoderma harzianum CABI-IMI 287340
Polyester based Polyurethane sheet
Alternaria alternata CABI-IMI 299022
Polyester polyurethane
Gliocladium roseum CABI-IMI 313952
Trichoderma harzianum CABI-IMI 314381
Cunninghamella elegans CABI-IMI 313951
Polyester polyurethane foam
Aspergillus ustus CABI-IMI 314382
Aspergillus versicolor CABI-IMI 314386
Fusarium culmorum CABI-IMI 314383
Fusarium solani CABI-IMI 314385
Penicillium chrysogenum CABI-IMI 314384
Penicillium chrysogenum CABI-IMI 314387
Polyether polyurethane
Penicillium simplicissium CABI-IMI 313953
Penicillium simplicissium CABI-IMI 313950
Cunninghamella elegans CABI-IMI 313951
Polypropylene divers hose
Paecilomyces lilacinus CABI-IMI 319302
Polyurethane
Alternaria alternata CABI-IMI 166751
Aspergillus fischeri CABI-IMI 319453
Aspergillus fischeri var. *spinosus* CABI-IMI 301296
Trichoderma harzianum CABI-IMI 286366
Ulocladium chartarum CABI-IMI 127962
Polyurethane coated fabric
Chaetomium globosum CABI-IMI 184731
Chaetomium cochliodes CABI-IMI 183700
Polyurethane coated fabric
Mucor racemosus CABI-IMI 184727
Mucor mucedo CABI-IMI 184726
Penicillium chrysogenum CABI-IMI 184732
Polyurethane foam
Aspergillus ustus CABI-IMI 319457
Paecilomyces lilacinus CABI-IMI 174677
Aspergillus fischeri var. spinosus CABI-IMI 319455
Aspergillus fumigatus CABI-IMI 319454
Penicillium expansum CABI-IMI 319460
Penicillium chrysogenum CABI-IMI 319452
Penicillium simplicissium CABI-IMI 319456
Polyurethane footware
Trichoderma harzianum CABI-IMI 324751
Trichoderma harzianum CABI-IMI 324750
Polyurethanel products
Aspergillus versicolor CABI-IMI 299019
Polyurethanes used in soil burial experiments
Gliocladium roseum CABI-IMI 304503
Gliocladium roseum CABI-IMI 304501
Gliocladium roseum CABI-IMI 304501
Poplin fabric
Fennellomyces linderi CABI-IMI 208237
Potassium thiocyanate containing mercuric nitrate
Paecilomyces farinosus CABI-IMI 307991
Preservative treated telegraph pole
Geomyces pannorum CABI-IMI 212118
Preservatives in paint (resistant)
Bacillus pumulis NCIMB 12791
Bacillus pumulis NCIMB 12821
Preservatives in paint (resistant)
Bacillus subtilis NCIMB 12792
Escherichia coli NCIMB 12793
PVA emulsion
Cladosporium cladosporioides CABI-IMI 178517
*Cladosporium cladosporioides*CABI-IMI 178510
*Cladosporium cladosporioides*CABI-IMI 178509
Penicillium crustosum CABI-IMI 178511
PVA emulsion paint
*Penicillium chrysogenum*CABI-IMI 178514
PVC
Cunninghamella elegans CABI-IMI 313951
Penicillium pinophilum CABI-IMI 114933
PVC covered wallpaper
Aspergillus ustus CABI-IMI 113731
Penicillium pinophilum CABI-IMI 113730
Penicillium rubrum CABI-IMI 113729
Aspergillus terreus CABI-IMI 113732
Penicillium janthinellum CABI-IMI 106294
Quinine containing medicine
Paecilomyces dactylethromorphus CABI-IMI 082348

Radio equipment
Aspergillus tamarii CABI-IMI 061268
Radio set
Aspergillus ustus CABI-IMI 089361
Penicillium pinophilum CABI-IMI 087160
Penicillium pinophilum CABI-IMI 087160ii
Rhizopus stolonifer CABI-IMI 061269
Rubber
Aspergillus fischeri var. *glaber* CABI-IMI 061447ii
Aspergillus fischeri var. *glaber* CABI-IMI 061447
Paecilomyces lilacinus CABI-IMI 117109
Sawdust
Thermoascus auranticus CABI-IMI 067936
Seed -Organo mercury treated
Syncephalastrum racemosum CABI-IMI 154276
Shoe leather
Cephaliophora tropica CABI-IMI 045555
Aspergillus unguis CABI-IMI 136526
Silcoset test panel
Cladosporium sphaerospermum CABI-IMI 135301
Silk screen of duplicating machine
Penicillium corylophilum CABI-IMI 095275
Skin lotion
Methylobacterium fujisawaense NCIMB 11272
Soap *Arthrobacter* sp. NCIMB 10798
Arthrobacter sp. NCIMB 10796
Arthrobacter sp. NCIMB 10800
Arthrobacter sp.NCIMB 10795
Arthrobacter sp.NCIMB 10799
Arthrobacter sp. NCIMB 10797
Stone
Oidiodendron rhodogenum CABI-IMI 235256
Sugar sack
Aspergillus mangini CABI-IMI 072050
Synthetic rubber
Paecilomyces variotii CABI-IMI 108007
Talcum powder
Aspergillus versicolor CABI-IMI 180556
Penicillium chrysogenum CABI-IMI 185483
Penicillium chrysogenum CABI-IMI 180550
Penicillium griseofulvum CABI-IMI 180551
Stephanosporim cereale CABI-IMI 180559
Syncephalastrum racemosum CABI-IMI 180560
Tanning liquor
Penicillium spinulosum CABI-IMI 167427
Tent canvas
Chaetomium brasiliense CABI-IMI 045557
Chaetomium brasiliense CABI-IMI 044212
Tent canvas
Memnoniella echinata CABI-IMI 045547ii
Memnoniella echinata CABI-IMI 045547
Trichoderma longibrachiatum CABI-IMI 045548
Penicillium duclauxii CABI-IMI 040210
Myrothecium verrucaria CABI-IMI 140591
Termites
Paecilomyces variotii CABI-IMI 242891
Test block
Fusarium melanochlorum CABI-IMI 112906
Textile exposure test sample
Myrothecium verrucaria CABI-IMI 140594
Myrothecium gramineum CABI-IMI 140595
Textile in soil
Trichoderma viride CABI-IMI 110145
Trichoderma viride CABI-IMI 110138
Textiles
Penicillium aculeatum CABI-IMI 040588
Penicillium sclerotiorum CABI-IMI 040574
Vinyl pyridine latex
Graphium penicillioides CABI-IMI 280035
Vinyl wall covering
Penicillium spinulosum CABI-IMI 341998
Penicillium pupurogenum CABI-IMI 341997
Wallpaper
Aspergillus tamarii CABI-IMI 318157
Cladosporium sphaerospermum CABI-IMI 317350
Penicillium aurantiogriseum CABI-IMI 317752
Penicillium chrysogenum CABI-IMI 318158
Ulocladium chartarum CABI-IMI 183940
Water bag
Memnoniella echinata CABI-IMI 015377
Wine bottle cork
Oidiodendron rhodogenum CABI-IMI 291885
Wood
Amyloporia xantha NCWRF 62C
*Antrodia juniperina*NCWRF 136
Antrodia sinosa NCWRF 303A
Apoxona nitida NCWRF 232
Armillaria mellea NCWRF 6H
Armillaria tabescens NCWRF 203A
Armillaria tabescens NCWRF 203
*Bjerkandera adusta*NCWRF 35B
Bondarzewia montana NCWRF 356
Boreostereum radiatum NCWRF 414
Buglossoporous pulvinus NCWRF 313
Cephaloascus fragrans CABI-IMI 046868
Cereporiopsis NCWRF 127
Chondrostereum purpureum NCWRF 88
Climacocystis borealis NCWRF 101
Coniophora puteana NCWRF 11A
Coriolopsis polyzina NCWRF 230
Coriolus hirstus NCWRF 1

Wood

Coriolus versicolor NCWRF 28C
Coriolus zonatus NCWRF 199
Crepidotus mollis NCWRF 400
Cyathus striatus NCWRF 22
Cylindrocarpon destructans CABI-IMI 313222
Daedaleopsis confragosa NCWRF 235
Daldinia concentrica NCWRF 26C
Datronia mollis NCWRF 154
Dichomitus squalens NCWRF 70A
Dictyopanus rhipidium NCWRF 260
Ditiola radicata NCWRF 364
Donkioporia expansa NCWRF 178E
Eschinodontium tinctorium NCWRF 78
Exophiala mansonii CABI-IMI 170820
Exophiala mansonii CABI-IMI 173394
Fibroporia radiculosa NCWRF 346
Fibroporia vaillantii NCWRF 14
Flammulina veloptipes NCWRF 54
Flavodon flavus NCWRF 292
Fomes fomentarius NCWRF 50C
Fomes fraxinophilus NCWRF 80
Fomes lividus NCWRF 293
Fomes melanosporus NCWRF 283
Fomes sclerodermeus NCWRF 223
Fomitopsis cajanderi NCWRF 106
Fomitopsis pinicola NCWRF 98
Fomitopsis rosea NCWRF 140
Funalia trogii NCWRF 247
Ganoderma lucideum NCWRF 89B
Ganoderma oregonense NCWRF 111
Ganoderma valesiacum NCWRF 189
Gleophyllum abietinum NCWRF 77C
Gleophyllum odoratum NCWRF 123B
Gleophyllum protractum NCWRF 314
Gleophyllum sepiarium NCWRF 10C
Gleophyllum trabeum NCWRF 108B
Gleosporus dichrous NCWRF 225
Grifola frondosa NCWRF 117
Gymnopilus spectabillis NCWRF 126
Gyrodontium versicolor NCWRF 339
Hapalopilus croceus NCWRF 145
Hericium coralloides NCWRF 60
Heteroporus biennis NCWRF 84C
Heteroporus biennis NCWRF 84D
Hirschioporus abietinus NCWRF 2
Hymenochaete rubiginosa NCWRF 243
Hypholoma fasciculare NCWRF 32
Hypoderma tenue NCWRF 420
Hypoxylon fragiforme NCWRF 209A
Incrustoporia nivea NCWRF 131B
Inonotus andersonnii NCWRF 288
Inonotus cuticularis NCWRF 278
Inonotus glomeratus NCWRF 284
Inonotus hispidus NCWRF 43
Inonotus mikadoi NCWRF 226
Inonotus nothofagi NCWRF 332
Inonotus obliquus NCWRF 298C
Inonotus radiatus var nodulosus NCWRF 37B
Inonotus rickii NCWRF 289
Inonotus weirii NCWRF 286
Irpex lacteus NCWRF 191A
Ischnoderma benzonium NCWRF 137B
Kuehneromyces mutabalis NCWRF 49A
Laetiporus sulpureus NCWRF 29

Wood

Laricifomes officinalis (syn. *Fomes*) NCWRF 81
Lentinellus cochleatus (syn *Lentinus*) NCWRF 171A
Lentinula deodes(syn *Lentinus*) NCWRF 335
Lentinus cyathiformis NCWRF 153B
Lentinus lepideus NCWRF 7
Lenzites betulina NCWRF 76
Lenzites palisotii NCWRF 231B
Lenzites subferruginea NCWRF 245
Leptoporus mollis NCWRF 253
Leucogyrophana mollusca (syn *Merulius*) NCWRF 94A
Leucogyrophana pinastri NCWRF 141B
Lopharia spadicea NCWRF 69A
Marasmius alliaceus NCWRF 275
Merulius tremellosus NCWRF 13
Merulopsis corium NCWRF 30C
Onnia circinata NCWRF 115
Oudemansiella radicata (syn *Collybia*) NCWRF 244
Oxysporus late-marginatus (syn *Poria*) NCWRF 375C
Oxysporus obduscens (syn *Poria*) NCWRF 51B
Panus tigrinus (syn *Lentinus*) NCWRF 68A
Panus torulosus NCWRF 64A
Paxillus atromentosus NCWRF 211
Paxillus panuoides NCWRF 8B
Peniphora gigantea NCWRF 175A
Peniphora quercinia NCWRF 152A
Phellinus conchatus *(*syn. *Fomes*) NCWRF 142
Phellinus contiguus (syn. *Poria*) NCWRF 376
Phellinus demidoffii (syn *Fomes*) NCWRF 158
Phellinus fastuosus (syn *Fomes*) NCWRF 132
Phellinus ferruginosus (syn *Fomes*) NCWRF 47C
Phellinus gilvus (syn *polyporus*) NCWRF 133a
Phellinus igniarius (syn *Fomes*) NCWRF 97B
Phellinus laevigatus (syn. *Poria*) NCWRF 147
Phellinus pini (syn. *Trametes*) NCWRF 45B
Phellinus pini (syn. *Trametes*) NCWRF 45A
Phellinus senex (syn *Fomes*) NCWRF 389
Phellinus torulosus (syn *Fomes*) NCWRF 222
Phlebia radiata NCWRF 19B
Phlebia rufa syn. (*Merulius*) NCWRF 330
Pholiota adiposa NCWRF 52B
Pholiota aurivella NCWRF 349
Piptoporus betulinus NCWRF 4B
Pleurotus eumosus NCWRF 66
Pleurotus cystidiosus (syn. *P.*corticats) NCWRF 212A
Pleurotus olearius NCWRF 325

Wood
- *Pleurotus osteatus* NCWRF 40B
- *Polyporus rugulosus* NCWRF 214
- *Polyporus anthracophilus* NCWRF 327
- *Polyporus brumalis* NCWRF 174A
- *Polyporus galactinus* (syn. *Spongipellis*) NCWRF 355
- *Polyporus lentus* NCWRF 337
- *Polyporus ochroleucus* NCWRF 261
- *Polyporus scopulosus* NCWRF 318
- *Polyporus spraguei* (syn. *Tyromyces*) NCWRF 227
- *Polyporus tumulosus* (var. *westralensis*) NCWRF 256A
- *Polyporus xanthopus* (syn. *Microporus*) NCWRF 365
- *Polystictus holsteii* NCWRF 366
- *Polystictus leoninus* (syn. *Coriolus leoninus*) NCWRF 251
- *Polystictus proteus* NCWRF 295
- *Poria aurea* (syn. *Auriporia*) NCWRF 367
- *Poria carbonacea* NCWRF 374
- *Poria clelandi* (syn. *Fomes*) NCWRF 259
- *Poria hypobrunnea* NCWRF 354
- *Poria incrassata* (syn. *Merulioporia*) NCWRF 71
- *Poria placenta* NCWRF 280
- *Poria sericeo-mollis (*syn. *Leptoporu)s* NCWRF 358
- *Poria subacida* (syn. *Perenniporia*) NCWRF 104
- *Pulcherricium coeruleum* (syn. *Corticum*) NCWRF 371
- *Pycnoporus cinnabranus* (syn. *Polystictus*) NCWRF 176
- *Pycnoporus sanguineus* (syn. *Polystictus*) NCWRF 150A
- *Rigidosporus lineatus* (syn. *Polyporus*) NCWRF 216
- *Rigidosporus microporus* (syn. Fomes) NCWRF 202
- *Rigidosporus ulmarius* (syn. *Fomes*) NCWRF 241
- *Rigidosporus vitreus* syn. *Polyporus adiposus* NCWRF 279
- *Scizopora* (syn *Irpex paradoxa*) NCWRF 16B
- *Serpula himantoides* NCWRF 73B
- *Serpula lacrymans* NCWRF 12
- *Spongipellis delectans* (syn. Polyporus) NCWRF 360
- *Spongipellis spumeus* (syn. *Polyporus*) NCWRF 166A
- *Spongipellis unicolor* (syn. *Polyporus*) NCWRF 198
- *Stereum lacteus* NCWRF 418
- *Stereum lobatum* NCWRF 263
- *Stereum necator* NCWRF 268
- *Stereum ostrea* NCWRF 105
- *Stereum rameale* NCWRF 265
- *Stereum rugosum* NCWRF 61A
- *Stereum sanguinolentum* (syn. *Haematostereum*) NCWRF 27
- *Stereum vellereum* NCWRF 269
- *Sterum hirsutum* NCWRF 3

Wood
- *Trametes feei* NCWRF 201
- *Trametes lactinea* NCWRF 329
- *Trametes lilacino-gilva* NCWRF 172A
- *Trametes ochro-leuca* NCWRF 328
- *Trechispora mollusca* (syn. *Poria*) NCWRF 234
- *Tyromyces immitis* (syn. *Polyporus*) NCWRF 359
- *Tyromyces undosus* (syn. *Polyporus*) NCWRF 361
- *Wolfiporia extensa* (syn *Poria*) NCWRF 344
- *Xylaria polymorpha* NCWRF 18
- *Xylobolus frustulatus* (syn. *Stereum* NCWRF 83
- *Xylobolus subpileatus* (syn. *Stereum*) NCWRF

Wood (from mine)
- *Stigmina longispora* CABI-IMI 139632
- *Tritirachium heimii* CABI-IMI 149642

Wood pulp chips
- *Scytalidium album* CABI-IMI 212120

Wood pulp chips of Pinus sylvestris
- *Xylogone sphaerospora* CABI-IMI 212121

Wood (treated with preservatives)
- *Trichoderma viride* CABI-IMI 153416

Wood rot (*Abies alba*)
- *Ischnoderma benzonium* NCWRF 137A

Wood - dry rot
- *Serpula lacrymans* NCWRF 12C

Wood rot (*Abies balsamea*)
- *Gleophyllum trabeum* NCWRF 108F

Wood rot (*Abies balsamea*)
- *Tyromyces guttulatus* (syn. *Polyporus*) NCWRF 185
- *Xeromphalina campanella* (syn. *Omphalia*) NCWRF 319

Wood rot (*Abies concolor*)
- *Ganoderma oregonense* NCWRF 111A

Wood rot (*Abies* sp.)
- *Columnocystis abietina* NCWRF 177A
- *Gleophyllum sepiarium* NCWRF 10E
- *Hericium abitis* NCWRF 300A
- *Hericium abitis* NCWRF 300B
- *Heterobasidion annosum* NCWRF 41F
- *Poria placenta* NCWRF 304G
- *Poria placenta* NCWRF 304E
- *Trametes lilacino-gilva* NCWRF 291

Wood rot (*Acer negundo*)
- *Pholiota formosa* NCWRF 342

Wood rot (*Acer* sp.)
- *Climacodon septentrionalis* NCWRF 237B

Wood rot (*Albies balsamea*)
- *Poria subacida* (syn. *Perenniporia*) NCWRF 104A

Wood rot (*Alnus glutinosa*)
- *Daedaleopsis confragosa* NCWRF 59A

Wood rot (*Alnus incana*)
- *Tyromyces chioneus* (syn. *Polyporus*) NCWRF 350C

Wood rot (*Alnus* sp.)
- *Inonotus radiatus var. radiatus* NCWRF 37C

Wood rot (*Alnus* sp.)
Plicatura sp. (syn. *Merulius*) NCWRF 240
Wood rot (*Araucaria* sp.)
Climacocystis borealis NCWRF 101A
Wood rot (*Astronium balansae*)
Fomes sclerodermeus NCWRF 223A
Wood rot (*Betula lutea*)
Daedaleopsis confragosa NCWRF 82A
Wood rot (*Betula papyrifera*)
Cerrena unicolor NCWRF 110A
Daedaleopsis confragosa NCWRF 82B
Hypochnicium vellereum NCWRF 321
Wood rot (*Betula* sp.)
Hypoxylon multiforme NCWRF 384
Lentinus lepideus NCWRF 7F
Piptoporus betulinus NCWRF 4C
Tyromyces fissilis (syn. *Polyporus*) NCWRF 85B
Wood rot (brickwork)
Coniophora marmorata NCWRF 410A
Coniophora marmorata NCWRF 410B
Wood rot (*Carya aquatica*)
Rigidosporus ulmarius (syn. *Fomes*) NCWRF 241B
Wood rot (*Carya* sp.)
Globiformes graveolens NCWRF 118
Wood rot (*Cassia siamea*)
Phaeolus manihotis (syn *Ganoderma)* NCWRF 338
Wood rot (*Cassipourea elliottii*)
Coriolopsis polyzina NCWRF 230A
Wood rot (*Cassipourea elliottii*)
Trametes cingulata NCWRF 250B
Wood rot (*Castanea sativa*)
Coriolus versicolor NCWRF 28A
Fistulina hepatica NCWRF 67
Laetiporus sulpureus NCWRF 29A
Wood rot (*Chamearops* sp.)
Podoscypha elegans (syn. *Stereum*) NCWRF 341
Wood rot (chipboard)
Pleurotus osteatus NCWRF 40C
Wood rot (*Citrus* sp.)
Trametes cervina NCWRF 167
Wood rot (*Cocus mucifera* leaves)
Grammothele fuligo NCWRF 390
Wood rot (conifer)
Amyloporia crassa NCWRF 373A
Amyloporia xantha NCWRF 62D
Gymnopilus sapineus NCWRF 155
Junghuhnia collabens NCWRF 336
Leptoporus mollis NCWRF 253B
Rigidosporus nigrescens (syn. *Poria*) NCWRF 315
Tyromyces chioneus (syn. *Polyporus*) NCWRF 350
Tyromyces stipticus (syn. *Polyporus*) NCWRF 208
Wood rot (*Corylus avellana*)
Dichomitus campestris NCWRF 393
Incrustoporia nivea NCWRF 131C
Wood rot (*Crataegus* sp.)
Phellinus ribis (syn *Fomes*) NCWRF 42
Wood rot (*Cupressus* sp.)
Coniophora puteana NCWRF 11E
Gleophyllum trabeum NCWRF 108E
Wood rot (*Dalbergia* sp.)
Pycnoporus sanguineus (syn. *Polystictus*) NCWRF 150D
Wood rot (*Elais guineensis*)
Ganoderma lucideum NCWRF 89C
Wood rot (*Erigthrina crista-galli*)
Polyporus platensis NCWRF 200
Wood rot (*Eucalyptus marginata*)
Fistulina hepatica NCWRF 67C
Polyporus eucalyptorum NCWRF 257
Wood rot (*Eucalyptus paniculata*)
Fomes lividus NCWRF 293A
Wood rot (*Eugenia uniflora*)
Hexagonia variegata NCWRF 340
Wood rot (*Fagus* sp.)
Antrodia serpens NCWRF 156
Aurantiporus alborobescens NCWRF 368
Chondrostereum purpureum NCWRF 88A
Ganoderma applanatum NCWRF 20C
Ganoderma applanatum NCWRF 50E
Ganoderma laccatum NCWRF 409
Grifola frondosa NCWRF 36A
Grifola frondosa NCWRF 117B
Hericium erinaceus NCWRF 277
Incrustoporia nivea NCWRF 131E
Kuehneromyces mutabalis NCWRF 49B
Meripilus giganteus NCWRF 407A
Ossicaulis lignatilis (syn *Pleurotus)* NCWRF 128
Oudemansiella mucida (syn *Armillaria)* NCWRF 5
Oxysporus late-marginatus (syn *Poria*) NCWRF 375
Phaeolus schweinitzii NCWRF 102B
Podoscypha multizonata (syn. *Stereum*) NCWRF 391
Pseudotrametes gibbosa (syn. Tramete) NCWRF 25B
Stereum insignitum NCWRF 369
Tyromyces floriformis (syn. *Polyporus*) NCWRF 351
Wood rot (*Fagus sylvatica*)
Fomes fomentarius NCWRF 50F
Wood rot (fibre board)
Amyloporia xantha NCWRF 62G
Sistotrema brinkmannii NCWRF 386D
Wood rot (*Fragus* sp.)
Hypoxylon fragiforme NCWRF 209B
Wood rot (*Fraxinus excelsior*)
Daldinia concentrica NCWRF 26D
Wood rot (*Fraxinus* sp.)
Fomitopsis cytisina NCWRF 17A
Gleophyllum striatum NCWRF 204
Wood rot (hardwood)
Odontia nothofagi NCWRF 403
Wood rot (*Hevea brasiliensis*)
Trametes incana NCWRF 299
Wood rot (*Hevea* sp.)
Ganoderma philippii NCWRF 388A
Wood rot (*Hevea* sp.)
Phellinus fastuosus (syn *Fomes*) NCWRF 132A
Phellinus lamaensis (syn *Fomes*) NCWRF 383

Wood rot (*Hevea* sp.)
- *Phellinus noxius* (syn *Fomes*) NCWRF 408
- *Polystictus vittas* NCWRF 348A
- *Polystictus vittas* NCWRF 348
- *Rigidosporus microporus* NCWRF 202B
- *Trametes badia* NCWRF 345A
- *Trametes meyenii* NCWRF 353
- *Trametes scarbrosa* NCWRF 347

Wood rot (*Juniperus bermudiana*)
- *Fomitopsis cajanderi* NCWRF 301

Wood rot (*Juniperus procera*)
- *Antrodia juniperina*NCWRF 136a
- *Phellinus demidoffii* (syn *Fomes*) NCWRF 158A

Wood rot (*Khaya* sp.)
- *Poria placenta* NCWRF 304H
- *Schizophyllum commune* NCWRF 9

Wood rot (*Larix decidua*)
- *Stereum sanguinolentum* (syn. *Haematostereum*) NCWRF 27B

Wood rot (*Larix kaempferi*)
- *Stereum sanguinolentum* (syn. *Haematostereum*) NCWRF 27C

Wood rot (*Larix laricina*)
- *Fomitopsis pinicola*NCWRF 98D

Wood rot (*Larix* sp)
- *Tyromyces lacteus* (syn. *Polyporus*) NCWRF 124C
- *Bjerkandera fumosa* NCWRF 24B
- *Laricifomes officinalis* (syn Fomes) NCWRF 81A
- *Laricifomes officinalis*(syn *Fomes*) NCWRF 81B
- *Resinicium bicolor* (syn. *Odontia*) NCWRF 165A
- *Serpula himantoides* NCWRF 233A

Wood rot (*Magnolia* sp.)
- *Rigidosporus ulmarius* (syn. *Fomes*) NCWRF 241A

Wood rot (*Malus* sp.)
- *Inonotus hispidus* NCWRF 43A
- *Pleurotus cornucopiae* NCWRF 167B
- *Sarcodontia setosa* (syn. *Acia*) NCWRF 362
- *Tyromyces fissilis* (syn. *Polyporus*) NCWRF 85D
- *Tyromyces fissilis* (syn. *Polyporus)* NCWRF 85A
- *Tyromyces fissilis* (syn. *Polyporus*) NCWRF 85

Wood rot (*Mitragnya* sp.)
- *Gleophyllum striatum* NCWRF 204A

Wood rot (*Ocotea rodiaeri*)
- *Pycnoporus sanguineus* (syn. *Polysticts*) NCWRF 150B

Wood rot (*Olex aquifolium*)
- *Stereum rugosum* NCWRF 61

Wood rot (*Peltophorum dubium*)
- *Fomes pseudosenex* NCWRF 190

Wood rot (*Picea excelsia*)
- *Pycnoporellus albo-luteus* (syn. *Hapalopilus*) NCWRF 399

Wood rot (*Picea abies*)
- *Gleophyllum sepiarium* NCWRF 10A

Wood rot (*Picea albeis*)
- *Resinicium bicolor* (syn. *Odontia*) NCWRF 165B

Wood rot (*Picea canadensis*)
- *Phellinus ferrugineo-fuscus* (syn *Poria*) NCWRF 193

Wood rot (*Picea excelsa*)
- *Amyloporia crassa* NCWRF 373B
- *Hapalopilus ochraceo-lateritius* NCWRF 397
- *Incrustoporia stellae* NCWRF 398
- *Phellinus ferrugineo-fuscus* (syn. *Phellinus*) NCWRF 193A

Wood rot (*Picea glauca*)
- *Peniphora septentrionalis* NCWRF 372
- *Peniphora septentrionalis* NCWRF 372A

Wood rot (*Picea mariana*)
- *Tyromyces balsameus* (syn. *Leptoporus*) NCWRF 114

Wood rot (*Picea sitchensis*)
- *Armillaria mellea* NCWRF 6F

Wood rot (*Picea sitchensis*)
- *Bondarzewia berkelyi* NCWRF 100

Wood rot (*Picea sitchensis*)
- *Columnocystis abietina* NCWRF 177
- *Heterobasidion annosum* NCWRF 41E

Wood rot (*Picea sithensis*)
- *Phaeolus schweinitzii* NCWRF 102A

Wood rot (*Picea* sp.)
- *Amylostereum areolatum* NCWRF 404A
- *Amylostereum chaillettii* NCWRF 318D
- *Antrodia seriales* NCWRF 107C
- *Coniophora marmorata* NCWRF 410
- *Fomitopsis pinicola*NCWRF 98B
- *Gleophyllum abietinum* NCWRF 77B
- *Gleophyllum odoratum* NCWRF 123C
- *Lentinus kauffmanii*NCWRF 309A
- *Peniphora gigantea*NCWRF 175C
- *Phellinus chrysoloma* (syn Fomes) NCWRF 246
- *Phellinus contiguus* (syn. *Poria*) NCWRF 376B
- *Poria sericeo-mollis* (syn. *Leptoporus*) NCWRF 296A
- *Stereum sanguinolentum* (syn. *Haematostereum*) NCWRF 27D
- *Tyromyces caesius* (syn. *Polyporus*) NCWRF 91F
- *Tyromyces revolutus* (syn. *Polyporus*) NCWRF 392
- *Phellinus nigrolIMI tatus* (syn *Fomes*) NCWRF 320

Wood rot (Pinus)
- *Ceratocystis pilfera* CABI-IMI 025316-

Wood rot (*Pinus caribaea*)
- *Lentinus pallidus* NCWRF 406

Wood rot (*Pinus nigra* subsp. *larico*)
- *Sistotrema brinkmannii* NCWRF 386A

Wood rot (*Pinus nigra* subsp. *larico*)
- *Sparassis crispa* NCWRF 312
- *Sphaerobolus stellatus* NCWRF 169

Wood rot (*Pinus resinosa*)
- *Sctinostroma galactinum* (syn. *Corticum*) NCWRF 324B

Wood rot (*Pinus* sp)
- *Tyromyces lacteus* (syn. *Polyporus*) NCWRF 124A
- *Tyromyces stipticus* (syn. *Polyporus*) NCWRF 208B
- *Tyromyces stipticus* (syn. *Polyporus*) NCWRF 208C
- *Armillaria mellea* NCWRF 6G
- *Boletopsis subsqamosa* NCWRF 402
- *Coniophora arida* NCWRF 411
- *Gleophyllum abietinum* NCWRF 77D
- *Kuehneromyces mutabalis* NCWRF 49
- *Lentinus lepideus* NCWRF 7E
- *Paxillus panuoides* NCWRF 8D
- *Phaeolus schweinitzii* NCWRF 102
- *Phanerochaete sanguinea* NCWRF 161A
- *Polyporus palustris* NCWRF 307A
- *Ptychogaster albus* NCWRF 333
- *Resinicium bicolor* (syn. *Odontia*) NCWRF 165D
- *Serpula himantoides* NCWRF 233B
- *Stereum sanguinolentum* (syn. *Haematostereum*) NCWRF 27E
- *Tyromyces balsameus* (syn. *Leptoporus*) NCWRF 311B
- *Tyromyces fragilis* (syn. *Polyporus*) NCWRF 173A

Wood rot (*Pinus strobus*)
- *Amyloporia xantha* NCWRF 62F
- *Sctinostroma galactinum* (syn. *Corticum*) NCWRF 324

Wood rot (*Pinus sylvestris*)
- *Amylostereum chaillettii* NCWRF 318E
- *Cartilosoma subsinosa* NCWRF 394
- *Dacromyces stillatus* NCWRF 412A
- *Fibroporia vaillantii* NCWRF 14F
- *Gleophyllum sepiarium* NCWRF 10D
- *Gloeocystidiellum lactescens* NCWRF 379
- *Heterobasidion annosum* NCWRF 41G
- *Hypoderma puberum* NCWRF 419A
- *Merulopsis taxicola* NCWRF 401
- *Peniphora pinus* NCWRF 267A
- *Phaeolus schweinitzii* NCWRF 102D
- *Phellinus contiguus* (syn. *Poria*) NCWRF 376A
- *Piloporia albobrunnea* (syn. Leptoporus*)* NCWRF 378
- *Resinicium bicolor* (syn. *Odontia*) NCWRF 165
- *Sistotrema brinkmannii* NCWRF 368F
- *Sistotrema brinkmannii* NCWRF 368E
- *Sistotrema brinkmannii* NCWRF 386C
- *Steccherinium fimbriatum* (syn. *Mycoleptodon)* NCWRF 395
- *Tyromyces fragilis* (syn. *Polyporus*) NCWRF 173E
- *Poria placenta* NCWRF 304B

Wood rot (*Podocarpus* sp.)
- *Gleophyllum trabeum* NCWRF 108D

Wood rot (*Populus* sp.)
- *Inonotus rheades var rheades* NCWRF 183
- *Lentinus cyathiformis* NCWRF 153E

Wood rot (*Populus tremuloides*)
- *Wolfiporia extensa* (syn *Poria*) NCWRF 344B

Wood rot (*Populus tremuloides*)
- *Wolfiporia extensa* (syn *Poria*) NCWRF 344A

Wood rot (*Prunus avium*)
- *Pycnoporus sanguineus* (syn. *Polystictus*) NCWRF 150C

Wood rot (*Prunus* sp.)
- *Armillaria mellea* NCWRF 6E
- *Daedaleopsis confragosa* NCWRF 59C
- *Ganoderma applanatum* NCWRF 20F
- *Phellinus pomaceus* (syn *Fomes*) NCWRF 33A
- *Phellinus pomaceus* (syn *Fomes*) NCWRF 33B
- *Pholiota lucifera* NCWRF 113
- *Tyromyces fissilis* (syn. Polyporus) NCWRF 85C
- *Xylaria hypoxylon* NCWRF 65B

Wood rot (*Pseudotsuga menziesii*)
- *Poria carbonica* NCWRF 308B
- *Poria carbonica* NCWRF 308C
- *Poria carbonica* NCWRF 308D
- *Fomitopsis pinicola* NCWRF 98E
- *Poria placenta* NCWRF 304C
- *Poria placenta* NCWRF 304D

Wood rot (*Pseudotsuga* sp.)
- *Poria rivulosa* NCWRF 316

Wood rot (*Pycnanthus angolensis*)
- *Lentinus squarrosolus* NCWRF 413

Wood rot (*Quercus albus*)
- *Phellinus gilvus* (syn *polyporus*) NCWRF 133

Wood rot (*Quercus ilex*)
- *Inonotus dryadeus* NCWRF 96A

Wood rot (*Quercus* sp.)
- *Antrodia oleracea* NCWRF 196
- *Bjerkandera fumosa* NCWRF 24
- *Daedalea quercina* NCWRF 38D
- *Daedalea quercina* NCWRF 38
- *Daedalea quercina* NCWRF 38E
- *Fistulina hepatica* NCWRF 67B
- *Ganoderma resinaceum* NCWRF 144A
- *Grifola frondosa* NCWRF 117A
- *Inonotus dryadeus* NCWRF 96
- *Lopharia spadicea* NCWRF 69C
- *Panus tigrinus* (syn *Lentinus*) NCWRF 68
- *Peniphora quercinia* NCWRF 152B
- *Polyporus durus* (syn *Fomes*) NCWRF 205
- *Poria medulla-panis* (syn. *Perenniporisa*) NCWRF 258B
- *Tyromyces chioneus* (syn. *Polyporus*) NCWRF 350A
- *Xylobolus frustulatus* (syn. *Stereum*) NCWRF 83A
- *Xylobolus subpileatus* (syn. *Stereum*) NCWRF
- *Phellinus ferrus* (syn *Poria*) NCWRF 387

Wood rot (*Robinia pseudoacacia*)
- *Fomitopsis cytisina* NCWRF 17
- *Phellinus robinae* (syn *Fomes*) NCWRF 180

Wood rot (*Rubus odoratus*)
- *Peniphora incarnata* NCWRF 86A

Wood rot (*Salix* sp.)
- *Oxysporus late-marginatus* (syn *Poria*) NCWRF 375A
- *Phellinus igniarius* (syn *Fomes*) NCWRF 97D
- *Phellinus igniarius* (syn *Fomes*) NCWRF 97E
- *Pholiota destruens* NCWRF 159

Wood rot (*Sapindus* sp.)
- *Oxysporus late-marginatus* (syn *Poria*) NCWRF 375B

Wood rot (*Schinopsis balansae*)
- *Fomes chaquensis* NCWRF 417

Wood rot (*Sequoia sempervirens*)
- *Poria sequoniae* NCWRF 306

Wood rot (softwood)
- *Hygrophoropsis aurantiaca* NCWRF 207
- *Hypoderma puberum* NCWRF 419

Wood rot (*Sorbus americana*)
- *Irpex lacteus* NCWRF 191

Wood rot (*Swietenia*)
- *Trametes hydnoides* NCWRF 377

Wood rot (*Thuja plicata*)
- *Poria sericeo-mollis* (syn. *Leptoporus*) NCWRF 296
- *Poria sericeo-mollis* (syn. *Leptoporus*) NCWRF 296B

Wood rot (*Thuja* sp.)
- *Amylostereum chaillettii* NCWRF 318

Wood rot (*Trachylobium verrucosum*)
- *Polystictus fulvo-cinereus* NCWRF 385

Wood rot (tree)
- *Polystictus fimbriatus* NCWRF 343

Wood rot (*Tsuga heterophylla*)
- *Coriolus versicolor* NCWRF 28B
- *Fomitopsis pinicola*NCWRF 98C
- *Phellinus contiguus* (syn. *Poria*) NCWRF 376C
- *Poria placenta* NCWRF 304J
- *Haematostereum*) NCWRF 27F

Wood rot (*Tsuga* sp.)
- *Columnocystis abietina* NCWRF 177C
- *Dacromyces stillatus* NCWRF 412
- *Hericium abitis* NCWRF 300C
- *Phellinus punctatus* (syn. *Poria*) NCWRF 194
- *Poria placenta* NCWRF 304F

Wood rot (*Uinas* sp.)
- *Fomitopsis cytisina* NCWRF 17B

Wood rot (*Ulmus* sp)
- *Agrocybe aererita* NCWRF 302
- *Flammulina veloptipes* NCWRF 54A
- *Lentinus adhaerens* NCWRF 415
- *Pleurotus osteatus* NCWRF 40A
- *Phellinus ferruginosus* (syn *Fomes*) NCWRF 47B
- *Pleurotus cornucopiae* NCWRF 167A
- *Polyporus squamosus* (syn. *Cerioporus*) NCWRF 103B
- *Poronidulus conchifer* (syn. *Polyporus*) NCWRF 116B
- *Rhodotus palmatus* (syn. *Pleurotus*) NCWRF 170

Wood rot (*Ulmus americana*)
- *Climacodon septentrionalis* NCWRF 237A

Wood rot (yellow straw of *Eucalyptus*)
- *Poria healeyi* NCWRF 380

Wooden storage bin
- *Malbranchea pulchella* CABI-IMI 096741

Wood (chemically treated)
- *Penicillium arenicola* CABI-IMI 367261*

Wood blocks in sea water
- *Piricauda articoceanorum* CABI-IMI 081625

Wood blocks in sea water
- *Zalerion maritima* CABI-IMI 081619
- *Zalerion maritima* CABI-IMI 081620
- *Zalerion maritima* CABI-IMI 081624

Wood desyroying (patent strain)
- *Aspergillus candidus* CABI-IMI 127260

Wood pulp
- *Penicillium roquefortii* CABI-IMI 057201
- *Trichoderma harzianum* CABI-IMI 055217

Wood pulp (Scandinavian)
- *Penicillium melinii* CABI-IMI 060041

Zinc based oil paint
- *Penicillium chrysogenum* CABI-IMI 178521

Food spoilage strains

See also: Biodeteriogens, Biodegraders and utilisers

Animal feed
- *Aspergillus repens* CABI-IMI 321288

Animal feed nuts
- *Penicillium solitum* CABI-IMI 291543

Apple
- *Phytohpthora cactorum* CABI-IMI 381060*
- *Penicillium expansum* CABI-IMI 378779*
- *Penicillium expansum* CABI-IMI 378781*
- *Penicillium expansum* CABI-IMI 378782*
- *Penicillium expansum* CABI-IMI 378783*
- *Penicillium expansum* CABI-IMI 378785*
- *Penicillium expansum* CABI-IMI 378786*
- *Penicillium expansum* CABI-IMI 378787*
- *Penicillium expansum* CABI-IMI 379324*
- *Penicillium expansum* CABI-IMI 379325*
- *Penicillium expansum* CABI-IMI 379326*
- *Penicillium expansum* CABI-IMI 379327*
- *Penicillium expansum* CABI-IMI 379328*
- *Penicillium pinophilum* CABI-IMI 378780
- *Penicillium pinophilum* CABI-IMI 379323

Apricot
- *Penicillium ulaiense* CABI-IMI 380701*

Australian apple
- *Monilinia fructicola* CABI-IMI 158424

Bakers yeast (mouldy)
- *Penicillium echinulatum* CABI-IMI 293180
- *Penicillium roquefortii* CABI-IMI 285518

Beer
- *Acinetobacter pasteurianus* NCIMB 6249
- *Acinetobacter pasteurianus* NCIMB 6248
- *Zymomonas mobis subspmobilis* NCIMB 8227

Bird feed
- *Penicillium griseofulvum* CABI-IMI 293195

Blackcurrent
- *Penicillium crustosum* CABI-IMI 113137

Blue mold (food)
- *Penicillium spp.* CABI-IMI Many

Blue peas (dried)
- *Penicillium phoeniceum* CABI-IMI 089286

Bran (Toxic)
- *Aspergillus flavus* CABI-IMI 092875

Bread
- *Penicillium crustosum* CABI-IMI 191240

Bread (India)
- *Aspergillus terricola* var. *indicus* CABI-IMI 172295

Butter
- *Paecilomyces fumosoroseus* CABI-IMI 058413

Cake
- *Penicillium crustosum* CABI-IMI 028040
- *Wallemia sebi* CABI-IMI 037338

Canned blueberries
- *Penicillium lapidosum* CABI-IMI 039743

Canned peaches
- *Penicillium viridicatum* CABI-IMI 297971

Cashew nuts
- *Penicillium miczynskii* CABI-IMI 191323

Cattle feed
- *Cladosporium tenuissimum* CABI-IMI 224833

Cheese
- *Lactobacillus brevis* NCIMB 4617
- *Mucor racemosus* f. *sphaerosporus* CABI-IMI 318800
- *Penicillium atramentosum* CABI-IMI 039752ii
- *Penicillium commune* CABI-IMI 039812ii
- *Penicillium chrysogenum* CABI-IMI 024314
- *Penicillium crustosum* CABI-IMI 089385
- *Penicillium crustosum* CABI-IMI 214071
- *Penicillium palitans* CABI-IMI 092276
- *Penicillium palitans* CABI-IMI 089378
- *Penicillium palitans* CABI-IMI 089379
- *Penicillium solitum* CABI-IMI 293210

Cheese (blue)
- *Penicillium roquefortii* CABI-IMI 337464

Cheese (Brie)
- *Penicillium commune* CABI-IMI 092215

Cheese (Camembert)
- *Penicillium atramentosum* CABI-IMI 039752

Cheese (Danish blue)
- *Penicillium roquefortii* CABI-IMI 129207
- *Penicillium roquefortii* CABI-IMI 173224

Cheese (Ellischauer)
- *Penicillium jensenii*CABI-IMI 039804

Cheese (Gorgonzola)
- *Penicillium roquefortii* CABI-IMI 092261

Cheese (mouldy Danbo)
- *Penicillium verrucosum* CABI-IMI 285523

Cheese (Parmesan
- *Penicillium solitum* CABI-IMI 297969

Cheese (Roquefort)
- *Penicillium roquefortii* CABI-IMI 024313

Cheese (Stilton blue)
- *Penicillium roquefortii* CABI-IMI 148775

Chocolate
- *Aspergillus mangini* CABI-IMI 068766

Chocolate covered sponge biscuit
- *Xeromyces bisporus* CABI-IMI 179743

Chocolate sponge biscuit
Penicillium viridicatum CABI-IMI 154731
Xeromyces bisporus CABI-IMI 154732
Chopped chinese dates
Xeromyces bisporus CABI-IMI 317902
Cider
Zymomonas mobilis subsp. *pomacii* NCIMB 8777
Citrus sinensis
Penicillium ulaiense CABI-IMI 380875*
Penicillium ulaiense CABI-IMI 380876*
Penicillium ulaiense CABI-IMI 380877*
Penicillium italicum CABI-IMI 380880*
Citrus xparidisi
Penicillium ulaiense CABI-IMI 380878*
Citrus xparidisi
Penicillium ulaiense CABI-IMI 380879*
Cocconut matting
Penicillium variabile CABI-IMI 040040
Coconut (dessicated
Aspergillus repens CABI-IMI 298307
Cooked meat
Penicillium aurantiogriseum CABI-IMI 089375
Corn (mouldy field)
Penicillium rubrum CABI-IMI 136127
Penicillium rubrum CABI-IMI 136128
Penicillium rubrum CABI-IMI 136126
Corn and rice cake
Penicillium herquei CABI-IMI 007651
Corn, barley and sorghum grain
Penicillium islandicum CABI-IMI 105272
Cottage cheese
Geotrichum candidum CABI-IMI 29023
Crumpets
Penicillium solitum CABI-IMI 297997
Dairy products
Fennellia flavipes CABI-IMI 135422
Dried fish
Penicillium chalybeum CABI-IMI 288722
Penicillium chalybeum CABI-IMI 288721
Polypaecilium pisce CABI-IMI 288727ii
Polypaecilium pisce CABI-IMI 288720ii
Dried prunes
Bettsia alvei CABI-IMI 151276
Bettsia alvei CABI-IMI 126288
Eggs
Pseudomonas taetrolens NCIMB 8396
Pseudomonas mucidolens NCIMB 9394
Fermented coccoa
Aspergillus tamarii CABI-IMI 016129
Fermented rice grains
Monascus purpueus CABI-IMI 210765
Foodstuffs
Aspergillus tonophilus CABI-IMI 226077
Bettsia alvei CABI-IMI 226074
Fruit based gel (baby food)
Penicillium oblatum CABI-IMI 288718
Fruit sauce (sweeet)
Monilinia acetobutans CABI-IMI 159918
Gelatine
Geomyces pannorum CABI-IMI 059628
Gelatine
Penicillium atramentosum CABI-IMI 089373
Grape
Penicillium crustosum CABI-IMI 293182
Grapes (stored)
Penicillium crustosum CABI-IMI 174717
Groundnuts
Penicillium aurantiogriseum CABI-IMI 091020
Hazelnut
Penicillium solitum CABI-IMI 321495
Aspergillus ruber CABI-IMI 071762
Aspergillus repens CABI-IMI 298305
Hops (stored)
Penicillium carneolutescens CABI-IMI 040218
Iron Bru drink
Brettanomyces sp. CABI-IMI 357902
Jam
Wallemia sebi CABI-IMI 037338
Lamb carcasses
Pseudomonas taetrolens NCIMB 11024
Lemon ice tea
Paecilomyces variotii CABI-IMI 371343*
Lemon juice
Penicillium brevicompactum CABI-IMI 194127
Lime flavour carton drink
Trichoderma viride CABI-IMI 296843
Liquorice
Xeromyces bisporus CABI-IMI 317904
Madeira cake
Penicillium expansum CABI-IMI 297965
Maize (stored)
Penicillium brevicompactum CABI-IMI 092034
Malt extract
Geotrichum candidum CABI-IMI 042074
Margarine
Penicillium sclerotiorum CABI-IMI 304282
Penicillium sclerotiorum CABI-IMI 304281
Meat
Cladosporium sphaerospermum CABI-IMI 091964
Geomyces pannorum CABI-IMI 023281i
Mucor racemosus CABI-IMI 017313
Pseudomonas sp. NCIMB 11652
Brochothrix thermosphacta NCIMB 10018
Milk
Enterobacter aerogenes NCIMB 96
Geotrichum candidum CABI-IMI 283163
Penicillium crustosum CABI-IMI 089386
Pseudomonas synxantha NCIMB 8178
Milk (pasteurised)
Dichotomomycetes cepji CABI-IMI 151074
Milled rice
Microascus trigonosorus CABI-IMI 086912
Millet alcoholic beverage
Mucor circinelloides forma *circinelloides* CABI-IMI 375454*
Nigerian groundnuts
Aspergillus flavus CABI-IMI 091020
Orange
Penicillium digitatum CABI-IMI 091956

Orange
Penicillium ulaiense CABI-IMI 380702*
Pecans (weevil damaged)
Penicillium solitum CABI-IMI 321496
Pepper
Stemphylium majusculum CABI-IMI 191067
Pistachio nuts
Aspergillus flavus CABI-IMI 190443
Plum juice
Aspergillus itaconicus CABI-IMI 016119
Poultry feed
Microdochium dimerum CABI-IMI 117087
Cheatomium cupreum CABI-IMI 299545
Rye bread
Penicillium roquefortii CABI-IMI 293204
Penicillium roquefortii CABI-IMI 293189
Salami sausage
Paecilomyces variotii CABI-IMI 131711
Penicillium nagliovense CABI-IMI 376118*
Salt fish
Wallemia sebi CABI-IMI 074824
Wallemia sebi CABI-IMI 076804
Salted anchovy paste
Wallemia sebi CABI-IMI 086292
Sausage product
Penicillium aurantiogriseum CABI-IMI 300381
Savoury preserve
Aspergillus amstelodami CABI-IMI 212944
Seafood
Alteromonas sp. NCIMB 2065
Shrimp
Alteromonas sp. NCIMB 2063
Soya products
Aspergillus tamarii CABI-IMI 321319
Aspergillus tamarii CABI-IMI 091888
Stick liquorice
Xeromyces bisporus CABI-IMI 063718
Stored food
Penicillium purpurescens CABI-IMI 141658
Curvularia borreriae CABI-IMI 141697
Sugar
Aspergillus terreus CABI-IMI 016044
Table jelly
Xeromyces bisporus CABI-IMI 076589
Bettsia alvei CABI-IMI 307744
Tobacco
Aspergillus versicolor CABI-IMI 091883
Aspergillus versicolor CABI-IMI 016041
Myrothecium leucotrichum CABI-IMI 001495ii
Myrothecium leucotrichum CABI-IMI 001495
Paecilomyces victoriae CABI-IMI 058412
Penicillium crustosum CABI-IMI 295697
Penicillium crustosum CABI-IMI 295696
Tobacco (cured)
Penicillium pseudostromaticum CABI-IMI 197491
Tobacco (pipe)
Fusarium culmorum CABI-IMI 291777
Walnut kernal
Penicillium spinulosum CABI-IMI 113139
Wheat bread
Penicillium italicum CABI-IMI 293196
Wine *Monascus pilosus* CABI-IMI 296424

Utilisers/biodegraders/bioremediators

See also: Biodeteriogens

Abietic acid
- *Flavobacterium resinovorum* NCIMB 8767

Acetate
- *Rhodococcus* sp. NCIMB 11316
- *Pseudomonas* sp. NCIMB 11318
- *Pseudomonas* sp. NCIMB 11317

N-Acetyl glucosamine
- *Lactobacillus helviticus* NCIMB 11493

Acetone
- *Corynebacterium* sp. NCIMB12633

Acetylsalicylic acid
- *Acinetobacter* sp. NCIMB 10553

Aconate
- Arthrobacter sp. NCIMB 9666

Alcohol
- *Amycolata hydrocarbonoxydans* NCIMB 9436
- *Mycobacterium flavum* NCIMB 9738
- *Mycobacterium flavum* NCIMB 9742
- *Rhodococcus* sp. NCIMB 9737
- *Rhodococcus* sp. NCIMB 9740
- *Rhodococcus* sp. NCIMB 9741
- *Rhodococcus* sp. NCIMB 9739

Aldehyde
- *Amycolata hydrocarbonoxydans* NCIMB 9436

Alginate
- *Aeromonas* sp. NCIMB 1139
- *Alteromonas* sp. NCIMB 1799
- *Bacillus alginolyticus* NCIMB 12517
- *Bacillus chondroitinus* NCIMB 12518
- *Deleya* sp. NCIMB 886
- *Vibrio alginolyticus*NCIMB 2047

Alkanes (also iso-alkanes)
- *Actinobacter* NCIMB 11507

Alkanes
- *Actinobacter* sp. NCIMB 11742
- *Bacillus cereus* NCIMB 10404
- *Mycobacterium* sp. NCIMB 11435
- *Pseudomonas aeruginosa* NCIMB 9571
- *Pseudomonas putida* NCIMB10408

N-alkanes C10-20
- *Acinobacter* sp. NCIMB 9205

N-alkanes
- *Bacterium* NCIMB 12697
- *Thermoleophilum minutum* NCIMB 12696

Alkanophosphate
- *Pseudomonas* sp. NCIMB 11753

N-Alkylcycloalkanes As consortium
- *Arthrobacter* sp. NCIMB 12631

Alkylamines
- *Paracoccus* sp. NCIMB 12140

Alkylamines
- *Methylobacterium extorquens*NCIMB 2879

p-Akylbenzenesulphonate
- *Alcaligenes xylosoxidians subsp. denitrificans* NCIMB 10771

Alkylbenzines
- *Rhodococcus* sp. NCIMB 11997

Alkylbenzines
- *Rhodococcus* sp. NCIMB 11998

Alkylethoxylate
- *Pseudomonas* sp. NCIMB 11750
- *Pseudomonas* sp. NCIMB 11751
- *Pseudomonas* sp. NCIMB 11753

Alkylethoxysulphae
- *Pseudomonas* sp. NCIMB 11751
- *Pseudomonas* sp. NCIMB 11750

Allantoine
- *Bacillus fastidiosis* NCIMB 11326
- *Bacillus fastidiosus* NCIMB 11326

alpha-Conidendrin
- *Unamed* NCIMB 10467

alpha-terpineol
- *Rhodococcus* sp. NCIMB 9784

Amino acids
- *Pseudomonas fluorescnes* NCIMB 12017

dl-1-Aminopropan2-ol
- *Pseudomonas* sp. NCIMB 10431

Amylose (soluble)
- *Cellulomonas mixtus* subsp. *Mixtus* NCIMB 8634

Aniline
- *Pseudomonas* sp. NCIMB 10426

Anthracene
- *Pseudomonas fluorescens* NCIMB 9815
- *Pseudomonas putida* NCIMB9816

Antipyrin
- *Phenylobacterium immobuke* NCIMB 12055

Aromatic compounds
- *Actinobacter* sp. NCIMB 850
- *Alcaligenes eutrophus* NCIMB 11842
- *Bacillus benzoevorans* NCIMB 12556
- *Bacillus benzoevorans* NCIMB 12555
- *Bacillus gordonae* NCIMB 12553
- *Pseudomonas putida* NCIMB 9876
- *Pseudomonas putida* NCIMB 8248
- *Pseudomonas putida* NCIMB 8249

Aromatic sulphonates
- *Alcaligenes xylosoxidans* subsp. *denitrificans* NCIMB 10771

Asphalt
- *Mycobacterium* sp. NCIMB 9573
- *Nocardia coeliaca* NCIMB 9574

Benzaldehyde
- *Acinetobacter* sp. NCIMB 9689
- *Pseudomonas* sp. NCIMB 9688

Benzene
- *Acinetobacter* sp. NCIMB 9689
- *Pseudomonas* sp. NCIMB 9688

Benzoate
- *Acinetobacter* sp. NCIMB 9689
- *Pseudomonas putida* NCIMB11086
- *Paracoccus* sp. NCIMB 10432
- *Pseudomonas* sp. NCIMB 9687
- *Pseudomonas* sp. NCIMB 9688

Benzyl alcohol
- *Acinetobacter* sp. NCIMB 9689
- *Pseudomonas* sp. NCIMB 9688

Betacyanin
- *Aspergillus foetidus*CABI-IMI 041871

Betaxanthin
- *Aspergillus foetidus*CABI-IMI 041871

Biphenyl
- *Flavobacterium* sp. NCIMB 10504
- *Pseudomonas* sp. NCIMB 10643

1-Bromobutane
- *Rhodococcus rhodochrous* NCIMB 13064
- *Rhodococcus erythropolis* NCIMB 13065

Butane
- *Arthrobacter* sp. NCIMB 11075

N-Butane
- *Graphium putredinis* CABI-IMI 151810

Caffeine
- *Pseudomonas* sp. NCIMB 11377

Camphor
- *Pseudomonas putida* NCIMB 10007
- *Pseudomonas* sp. NCIMB 11241
- *Rhodococcus* sp. NCIMB 9784
- *Rhodococcus* sp. NCIMB 8727

Carageenin
- *Alteromonas carragenovora* NCIMB 302

Carbaryl
- *Rhodococcus* sp. NCIMB 12038
- *Pseudomonas* sp. NCIMB 12042

Carbenicillin
- *Pseudomonas aeruginosa* NCIMB 10111
- *Pseudomonas aeruginosa* NCIMB 10110

Catechin (+)
- *Pseudomonas* sp. NCIMB 9940
- *Pseudomonas* sp. NCIMB 9941

Catechol
- *Pseudomonas putida* NCIMB 11198
- *Acinetobacter* sp. NCIMB 9689
- *Pseudomonas* sp. NCIMB 9688

Cattle waste (anearobic digestion)
- *Sporolactobacillus cellulosolvens* NCIMB 12173

Cellulose
- *Cellulomonas bibula* NCIMB 8142
- *Cellulomonas biazotea* NCIMB 8077
- *Sporocytophaga myxococcoides* NCIMB Many
- *Cellulomonas cellasea* NCIMB 8078
- *Cellulomonas cellulans* NCIMB 8868
- *Cytophaga aurantiaca* NCIMB 8628
- *Clostridium papyrosolvens* NCIMB 11394
- *Cellulomonas uda* NCIMB 11494
- *Cellvibrio* sp. NCIMB Many
- *Clostridium celerecrescens* NCIMB 12829
- *Cytophage hutchinsonnii* NCIMB 10782
- *Candida krusei* NCYC 872
- *Trichoderma viride* CABI-IMI 296458

Chitin
- *Aeromonas caviae* NCIMB 8500
- *Aeromonas caviae* NCIMB 8676
- *Alcaligenes* sp. NCIMB 9640
- *Arthrobacter* sp. NCIMB 9641
- *Bacillus* sp. NCIMB 9653
- *Bacillus* sp. NCIMB 9651
- *Bacillus* sp. NCIMB 9655
- *Cellulomonas cartae* NCIMB 11025
- *Cellulomonas cartae* NCIMB 9097
- *Cellulomonas cellulans* NCIMB 8868
- *Cellvibrio mixtus* NCIMB 12225
- *Cellvibrio mixtus* NCIMB 8634
- *Chitinpphaga pinensis* NCIMB 11801

Chitin
- *Chromobacterium violaceum* NCIMB 8182
- *Cytophaga johnsonae* NCIMB Any
- *Lysobacter* NCIMBAny
- *Photobacterium phosphoreum* NCIMB 395
- *Photobacterium phosphoreum* NCIMB 12839
- *Saccharomyces cerevisiae* NCYC 826
- *Serratia macerens* NCIMB 10428
- *Streptomyces alboflavus* NCIMB 9634
- *Streptomyces* sp. NCIMB 9637
- *Streptomyces violaceus* NCIMB 11194
- *Vibrio havreii* NCIMB 1280
- *Vibrio spendidum* NCIMB 1

Chloramphenicol
- *Flavobacterium* sp.. NCIMB 10082

Chloridazon
- *Phenylobacterium immobile* NCIMB 12055

Chlorophenols
- *Rhodococcus chlorophenolicus* NCIMB 12325

Choline
- *Achromobacter cholinophagum* NCIMB 1501
- *Desulfovibrio desulfuricans subsp. desulfuricans* NCIMB 9467

Chondroitin
- *Sphignobacterium heparinum*NCIMB 9290

Chondroitin (desulphated)
- *Bacillus chondroitinus* NCIMB 12518

Choroform
- *Methylosinus* sp. NCIMB 13214

1-Chlorobutane
- *Rhodococcus rhodochrous* NCIMB 13064
- *Rhodococcus erythropolis* NCIMB 13065
- *Rhodococcus erythropolis* NCIMB 13065
- *Rhodococcus rhodochrous* NCIMB 13064
- *Rhodococcus erythropolis* NCIMB 13065
- *Rhodococcus rhodochrous* NCIMB 13064
- *Rhodococcus erythropolis* NCIMB 13065
- *Rhodococcus rhodochrous* NCIMB 13064
- *Rhodococcus erythropolis* NCIMB 13065
- *Rhodococcus rhodochrous* NCIMB 13064
- *Rhodococcus rhodochrous* NCIMB 13064
- *Rhodococcus erythropolis* NCIMB 13065

Coal spoil
- *Calcarisporium thermophilium* CABI-IMI 144750

Collagen
- *Bacillus* sp. NCIMB 10937
- *Bacillus* sp.. NCIMB 10936
- *Bacillus* sp. NCIMB 10935
- *Flavobacterium* sp.. NCIMB 1314
- *Pseudomonas* sp. NCIMB 10927
- *Pseudomonas* sp.. NCIMB 10928
- *Pseudomonas* sp.. NCIMB 10929
- *Pseudomonas* sp. NCIMB 10930
- *Pseudomonas* sp. NCIMB 10932
- *Pseudomonas* sp. NCIMB 10934

Colophony
- *Flavobacterium resinovorum* NCIMB 8767

Creatine
Pseudomonas putida NCIMB 11978
Arthrobacter ureafaciens NCIMB 7811
Creatinine
Arthrobacter ureafaciens NCIMB 7811
m- Cresol
Pseudomonas crucivae NCIMB 9432
Acinetobacter sp. NCIMB 9689
Pseudomonas sp. NCIMB 9688
Comamonas terrigena NCIMB 2581
o- Cresol
Pseudomonas sp. NCIMB 9688
p- Cresol
Pseudomonas putida NCIMB 8251
Comamonas testosteroni NCIMB 8893
Cresol
Pseudomonas putida NCIMB 10015
Crude oil
Acinetobacter baumannii NCIMB 13260
Acinetobacter sp. NCIMB 11742
Pseudomonas sp. NCIMB 13261
Cyclohexane
Nocardia sp. sp. NCIMB 11399
Cyclohexane Acetic acid
Arthrobacter sp. NCIMB 12634
Cyclohexane carboxylic acid
Acinetobacter sp. NCIMB 9870
Cyclohexanol
Acinetobacter sp. NCIMB 9871
Cycloparaffins
Mycobacterium album NCIMB 11805
Mycobacterium vaccae NCIMB 11807
Cyclopentanol
Pseudomonas sp. NCIMB 9872
Cystine
Comamonas acidovorans NCIMB 4854
Decane
Pseudomonas proteofaciens NCIMB 10204
Acinetobacter baumanni NCIMB 13260
Detergent
Pseudomonas sp. NCIMB 10394
Pseudomonas sp. NCIMB 10393
Pseudomonas sp. NCIMB 10392
Pseudomonas sp. NCIMB 10390
Pseudomonas sp. NCIMB 10391
Pseudomonas sp. NCIMB 10770
Rhizobiaceae sp. NCIMB 10396
Pseudomonas sp. NCIMB 10397
Diesel oil
Rhodoturula glutinis NCYC 974
2-4 Dichlorophenoxyalkyl carboxylic acid
Flavobacterium sp. NCIMB 9776
Dihydrogossypetin
Pseudomonas sp. NCIMB 9940
Dimethylamine
Methylobacterium sp. NCIMB 11681
Aminobacter aminovorans NCIMB 11591
Dinitro-o-cresol
Pseudomonas sp. NCIMB 9772
Nocardioides jensenii NCIMB 9770
Pseudomonas putida NCIMB 9771
Dinitro-o-sec-butylphenol
Pseudomonas sp. NCIMB 9772
Dinitro-o-sec-butylphenol
Pseudomonas putida NCIMB 9771
2,4 Ditrophenol
Rhodococcus sp. NCIMB 9702
2,4-D Herbicide
Arthrobacter sp.. NCIMB 9777
Dodecane
Nocardioides fastidosa NCIMB 12713
Pseudomonas aeruginosa NCIMB 9904
Dodecyl sulphate
Pseudomonas sp NCIMB 11753
Dodecylbenzene sulphonate
Pseudomonas sp NCIMB 11753
Ethane
Pseudomonas sp. NCIMB 11318
Rhodococcus sp. NCIMB 11316
Mycobacterium para NCIMB 10420
Pseudomonas sp. NCIMB 11317
Ethyl alcohol
Rhodococcus sp. NCIMB 11275
Ethyl benzene
Acinetobacter sp. NCIMB 9689
Ethylene glycol
Xanthobacter autotrophicus NCIMB 11171
Fenchone
Rhodococcus sp. NCIMB 9784
Ferrichrome
Pseudomonas sp. NCIMB 9783
Flavonoids
Pseudomonas sp. NCIMB 9940
Flouroacetamide
Pseudomonas sp. NCIMB 9562
Formate
Methylobacterium extorquens NCIMB 2879
Corneyform sp. NCIMB 8167
Furan 2-carboxylic acid
Pseudomonas sp. NCIMB 12632
Galacturonic acid
Clostridium indolis NCIMB 9732
Clostridium indolis NCIMB 9731
Gas (natural)
Pseudomonas sp. NCIMB 11309
Pseudomonas sp. NCIMB 11310
Gas oil
Acetobacter calcoaeticus NCIMB 11742
Glutathione
Candida utilis NCYC 927
Glyphosate
Rhizobiaceae sp. NCIMB 12533
Gum arabic
Cellulomonas mixtus subsp.mixtus NCIMB 8634
Gum locust bean
Cellulomonas mixtus subsp.mixtus NCIMB 8634
Gum xanthan
Corneybacterium sp. NCIMB 11535
Cellulomonas mixtus subsp.mixtus NCIMB 8634
Halobenzoates
Corneyform sp. NCIMB 12617
Heavy metal removal
Chlorella emersonii CCAP 211/8A
Hemicellulose
Bacillus macerans NCIMB all strains

Heparin
- *Sphingobacterium heparinum* NCIMB 9292

Heparitin
- *Sphingobacterium heparinum* NCIMB 9292

Hexadecane
- *Acinetobacter* sp. NCIMB 11742
- *Acinetobacter baumannii* NCIMB 13260
- *Nocardioides fastidosa* NCIMB 12713

Hydrocarbon oxidation
- *Amycolata hydrocarbonoxydans* NCIMB 9436
- *Mycobacterium paraffinicum* NCIMB 10420
- *Mycobacterium* sp. NCIMB 9573
- *Nocardia petroleophila* NCIMB 9438
- *Nocardiodes fastidosa* NCIMB 12713
- *Rhodococcus erythropolis* NCIMB 9905
- *Rhodococcus rhodochrous* NCIMB 11273
- *Rhodococcus rhodochrous* NCIMB 11277
- *Rhodococcus* sp. NCIMB 11276
- *Rhodococcus sp.* sp. NCIMB 11160
- *Streptomyces coriofaciens* NCIMB 12966

Hydrocarbons
- *Candida tropicalis* NCYC 997
- *Yarrowia lipolytica* NCYC 825

m-Hydroxybenzoate
- *Pseudomonas* sp. NCIMB 9687

p-Hydroxybenzoate
- *Bacillus gordonae* NCIMB 12553
- *Pseudomonas* sp. NCIMB 9687

p-Hydroxybenzonitrile
- *Rhodococcus* sp. NCIMB 11215

Hydroxyethylcellulose
- *Bacillus sphaericus* NCIMB 10839
- *Bacillus sphaericus* NCIMB 10841
- *Providencia rettgeri* NCIMB 10842
- *Alcaligenes* sp. NCIMB 10840

Imidazolylpropionate
- *Comamonas testeroni* NCIMB 10808

Industrial waste
- *Chaetomium medusarum* CABI-IMI 317647
- *Chaetomium jodhpurense* CABI-IMI 317631

Insect moulting hormones
- *Rhizopus arrhizus* CABI-IMI 090340

Inulin
- *Erwina* sp. NCIMB 10130

Isoprenoids
- *Pseudomonas citronellolis* NCIMB 12783

Itaconate
- *Arthrobacter* sp. NCIMB 9666

Keratin
- *Bacillus licheniformis* NCIMB 10689
- *Lysobacter* sp. NCIMB 9497
- *Streptomyces fradiae* NCIMB11726

Kerosene
- *Pseudomonas aeruginosa* NCIMB 10434

Lactic acid
- *Kluyveromyces marxianus* NCYC 1429

Laminarin
- *Cellulomonas mixtus* subsp *mixtus* NCIMB 8634

Leucine
- *Acinetobacter* NCIMB 9542

Levan
- *Erwinia* sp. NCIMB 10120
- *Erwinia* sp. NCIMB 10121

Levulinic acid
- *Brevibacterium linens* NCIMB 11439

Lichenin
- *Cellulomonas mixtus subsp. miixtus* NCIMB 8634

Lignans
- Unnamed NCIMB 10467
- Unnamed NCIMB 10468
- Unnamed NCIMB 10469

Lignin
- *Phanerochaete chrysosporium* CABI-IMI 232175

Lignocellulose
- *Thermomonospora* NCIMB 11185

Limnonin
- *Pseudomonas aeruginosa* NCIMB 12049
- *Pseudomonas aeruginosa* NCIMB 12050

Limonene
- *Pseudomonas aeruginosa* NCIMB 12050
- *Pseudomonas aeruginosa* NCIMB 12049

Ls-alloisocitrate
- *Alcaligenes xylosoxidians subsp. denitrificans* NCIMB 9387

Metal working fluids
- *Alcaligenes faecialis* subsp. *faecialis* NCIMB 13104

Methane
- *Methylobacter agilis* NCIMB 11124
- *Methylobacter albus* NCIMB 11123
- *Methylobacter luteus* NCIMB11914
- *Methylobacter whittenburyi* NCIMB 11128
- *Methylococcus capsulatus* NCIMB 12141
- *Methylococcus capsulatus* NCIMB 11132
- *Methylococcus* sp. NCIMB 11083
- *Methylococcus ucrainius* NCIMB 11915
- *Methylocystis echinoides* NCIMB 13100
- *Methylocystis methanolicus* NCIMB 13101
- *Methylocystis minmus* NCIMB 13099
- *Methylocystis parvus* NCIMB11129
- *Methylocystis pyreformis* NCIMB 13102

Methane
- *Methylocystis sp* NCIMB 11107
- *Methylomonas gracilis* NCIMB 11912
- *Methylomonas methanica* NCIMB 11130
- *Methylomonas rubrum* NCIMB 11913
- *Methylomonas* sp. NCIMB 13224
- *Methylomonas* sp. NCIMB 12340
- *Methylomonas* sp. NCIMB 11695
- *Methylophilus methylotrophus* NCIMB 11809
- *Methylosinus* sp. NCIMB 13214
- *Methylosinus sporium* NCIMB 11126
- *Methylosinus trichosporum* NCIMB 11131
- *Soehngenia thermomethanica* NCIMB 11757

Methanol
- *Altermonas* sp. NCIMB 2161
- *Alteronomas thalssomethanolica* NCIMB 2159
- *Aminomonas animovorus* NCIMB 11268
- *Amylocolapsis methalonica* NCIMB11946

Methanol
- *Ancycobacter* sp. NCIMB 1200
- *Bacillus methanolicus* NCIMB 13113
- *Coryneform* sp. NCIMB 11344
- *Hyphomicrobium* sp. NCIMB 11706
- *Methylobacillus glycogenes* NCIMB 10593
- *Methylobacillus glycogenes* NCIMB 10509
- *Methylobacillus glycogenes* NCIMB 11673
- *Methylobacterium extorquens* NCIMB 10409
- *Methylobacterium fujisawaense* NCIMB 12417
- *Methylobacterium mesophilicum* NCIMB 11561
- *Methylobacterium organophilum* NCIMB 11278
- *Methylobacterium radiotolerans* NCIMB 10815
- *Methylobacterium rhodesianum* NCIMB 12440
- *Methylobacterium rhodium* NCIMB 12249
- *Methylobacterium* sp. NCIMB 12476
- *Methylobacterium zatmanii* NCIMB 12243
- *Methylophaga marina* NCIMB 2244
- *Methylophilus methyloptophus* NCIMB 12627
- *Methylophilus* sp. NCIMB 2242
- *Methylovorus glucosotrophus* NCIMB 13222
- *Mycobacterium gastri* NCIMB 12940
- *Paracoccus alcaliphilus* NCIMB 13180
- *Paracoccus denitrificans* NCIMB 11627
- *Paracoccus* sp. NCIMB 12140
- *Protoaminobacter thiaminophaga* NCIMB 12106
- *Pseudomonas insueta* NCIMB 12105
- *Xanthobacter autotrophicus* NCIMB 11171

Methionine
- *Alcaligenes faecialis* NCIMB 10688

Methyl cyanide
- *Pseudomonas* sp. NCIMB 10477

Methylamine
- *Aminobacter aminovorans* NCIMB 11591
- *Aminobacter aminovorans* NCIMB 11590
- *Aminomonas aminovorus* NCIMB 11268
- *Arthrobacter* sp. NCIMB 11625
- *Bacillus* sp. NCIMB 10492
- *Bacillus* sp. NCIMB 11343
- *Corynebacterium* sp. NCIMB 11344
- *Enterobacter cloacae* NCIMB 12432
- *Hyphomicrobium* sp. NCIMB 12162
- *Methylobacterium extorquens* NCIMB 10409
- *Methylobacterium mesophilicum* NCIMB 11561
- *Methylobacterium organophilum* NCIMB 11278
- *Methylobacterium radiotolerans* NCIMB 9142
- *Methylobacterium rhodesianum* NCIMB 12440

Methylamine
- *Methylobacterium rhodium* NCIMB 9421
- *Methylobacterium* sp. NCIMB 9141
- *Methylobacterium zatmanii* NCIMB 12243
- *Pseudomonas* sp NCIMB 12431
- *Rhodococcus* sp. NCIMB 12038

3- Methylcatechol
- *Acinetobacter* sp. NCIMB 9689
- *Pseudomonas* sp. NCIMB 9688

2- Methyl-4-chlorophenoxyacetic acid (MCPA)
- *Pseudomonas* sp. NCIMB 9340

2-Methylnaphthalene Phage
- *Pseudomonas putida* NCIMB 12199

Monomethyl sulphate
- *Agrobacterium* sp. NCIMB 13265

Monomethylamine
- *Methylobacterium* sp. NCIMB 11681
- *Pseudomonas* sp. NCIMB 12042

Monoterpenes
- *Pseudomonas* sp. NCIMB 12693

1-Napthol
- *Rhodococcus* sp. NCIMB 12038
- *Pseudomonas* sp. NCIMB 12042

2-Napthol
- *Pseudomonas* sp. NCIMB 9687

Napthol
- *Pseudomonas putida* NCIMB 8383

Nicotine
- *Arthrobacter nicotianae* NCIMB 9458
- *Pseudomonas fluorescens* NCIMB 9397

Nicotinic acid
- *Pseudomonas* sp. NCIMB 8226
- *Bacillus niacini* NCIMB 13159

Nitriles
- *Rhodococcus* sp. NCIMB 11215
- *Rhodococcus* sp. NCIMB 11216

o- Hydroxybenzoate
- *Pseudomonas* sp. NCIMB 9687

o-Nitrobenzoic acid
- *Pseudomonas putida* NCIMB 9726

p-Nitrobenzoic acid
- *Pseudomonas putida* NCIMB 9727

o-Nitrophenol
- *Pseudomonas putida* NCIMB 9723
- *Pseudomonas putida* NCIMB 9773

m-Nitrophenol
- *Pseudomonas putida* NCIMB 9724

p-Nitrophenol
- *Pseudomonas* sp. NCIMB 9774
- *Pseudomonas putida* NCIMB 9725

Oil
- *Pseudomonas fluorescens* NCIMB 11712

Oleoresins
- *Flavobacterium resinovorum* NCIMB 8767

Oxalate
- *Alcaligens* sp. NCIMB 8543
- *Alcaligens* sp. NCIMB 8643
- *Alcaligens* sp. NCIMB 8642
- *Alcaligens* sp. NCIMB 8544
- *Methylobacterium extorquens* NCIMB 2879
- *Methylobacterium extorquens* NCIMB 9399
- *Pseudomonas* sp. NCIMB 8737
- *Pseudomonas* sp. NCIMB 8736

Oxalate
- *Pseudomonas* sp. NCIMB 8735

Oxamate
- *Desulfovibrio vulgaris subsp. oxamicus* NCIMB 9442

Paint (bathroom)
- *Phoma violacea* CABI-IMI 090179

Paint (PVA emulsion)
- *Phoma epicoccina* CABI-IMI 178513
- *Phoma violacea* CABI-IMI 178508
- *Phoma violacea* CABI-IMI 178507

Paint (white lead)
- *Phoma violacea* CABI-IMI 049948
- *Phoma violacea* CABI-IMI 049948ii

Pantothenic acid
- *Variovorax paradoxus* NCIMB 9899
- *Variovorax paradoxus* NCIMB 11474

Paper
- *Phoma violacea* CABI-IMI 129267

Paper mill slime
- *Mucor plumbeus* CABI-IMI 014781

Paper Mill waste
- *Klebsiella pneumonia subsp. Pneumoniiae* NCIMB 10104

Paraquat
- *Lipomyces starkeyi* NCYC 682

Paraffins
- *Mycobacterium flavus subsp. methanicum* NCIMB 9738
- *Mycobacterium flavus subsp. methanicum* NCIMB 9742
- *Rhodococcus* sp. NCIMB 9740
- *Rhodococcus* sp. NCIMB 9741
- *Rhodococcus* sp. NCIMB 9737
- *Rhodococcus* sp. NCIMB 9739

Pectic acid
- *Clostridium indolis* NCIMB 9732
- *Clostridium indolis* NCIMB 9731

Pectic substances
- *Aureobacterium barkeri* NCIMB 9658
- *Bacillus polymyxa* NCIMB 11088
- *Bacillus polymyxa* NCIMB 8158
- *Bacillus subtilis* NCIMB 11034
- *Cellulomonas cellulans* NCIMB 8868
- *Cellvibrio mixtus* subsp. *dextranolyticus* NCIMB 12226
- *Cellvibrio mixtus* subsp. *mixtus* NCIMB 8634
- *Cellvibrio mixtus* subsp. *mixtus* NCIMB 12225
- *Cellvibrio mixtus* subsp. *mixtus* NCIMB 8633
- *Clostridium aurantibutyricum* NCIMB 10659
- *Clostridium felsineum* NCIMB 10690
- *Clostridium indolis* NCIMB 9732
- *Clostridium indolis* NCIMB 9731
- *Clostridium puniceum* NCIMB 11596
- *Clostridium* sp. NCIMB 11900
- *Clostridium* sp. NCIMB 11903
- *Clostridium* sp. NCIMB 11901
- *Clostridium* sp. NCIMB 11899
- *Clostridium* sp. NCIMB 11902
- *Cytophaga johnsonae* NCIMB All
- *Cytophaga* sp. NCIMB 11076
- *Cytophaga* sp. NCIMB 9488
- *Flavobacterium* sp. NCIMB 10414

Pectic substances
- *Flavobacterium* sp. NCIMB 10021

Pectin
- *Kluyveromyces marxianus* NCYC 587

Pentachlorophenol
- *Rhodococcus chlorophenolicus* NCIMB 12325
- *Flavobacterium* sp. NCIMB 12722

Phenacetin
- *Corynebacterium pseudoliphtheriticum* NCIMB 10803

Phenanthrene
- *Pseudomonas putida* NCIMB 9816
- *Pseudomonas fluorescens* NCIMB 9815

Phenol
- *Acinetobacter* sp. NCIMB 8250
- *Acinetobacter* sp. NCIMB 5178
- *Bacillus benzoevorans* NCIMB 12555
- *Bacillus benzoevorans* NCIMB 12556
- *Candida tropicalis* NCYC 1503
- *Comamonas terrigena* NCIMB 2581
- *Mycoplana bullata* NCIMB 9440
- *Mycoplana dimorpha* NCIMB 9439
- *Ochromonas dunica* CCAP 933/23
- *Pseudomonas cruciviae* NCIMB 9432
- *Pseudomonas pictorum* NCIMB 9152
- *Pseudomonas putida* NCIMB 8858
- *Pseudomonas putida* NCIMB 10015
- *Pseudomonas putida* NCIMB 78860
- *Pseudomonas putida* NCIMB 8859
- *Pseudomonas* sp. NCIMB 9688
- *Pseudomonas* sp. NCIMB 9687

Phenylacetate Phage
- *Pseudomonas* sp. NCIMB 12185

2-Phenylethanol
- *Pseudomonas putida* NCIMB 10015

β-Phenylpropionic acid
- *Acinetobacter* sp. NCIMB 9781

Phloroglucinol
- *Pseudomonas fluorescens* NCIMB 11142

Phthalate
- *Bacillus gordonae* NCIMB 12553

α-Pinene
- *Pseudomonas putida* NCIMB 10684
- *Pseudomonas* sp. NCIMB 10687

α-Pinene
- *Pseudomonas* sp. NCIMB 10686
- *Pseudomonas* sp. NCIMB 10685

Pipecolic acid
- *Pseudomonas putida* NCIMB 12237

poly-beta-Hydroxybutyrate
- *Variovorax paradoxus* NCIMB 11964
- *Pseudomopnas lemoignei* NCIMB 9947

Polycaprolactone polyurethane
- *Trichoderma harzianum* CABI-IMI 286366

Polyester PU
- *Penicillium simplicissium* CABI-IMI 313950

Polyether PU
- *Penicillium simplicissium*CABI-IMI 313950

Polygalactans (plant)
- *Alteromonas* sp. NCIMB 2062

Polypectate
- *Cellulomonas mixtus subsp.mixtus* NCIMB 8634

Polysaccharide of type 3 pneumococcus
Bacillus circulans NCIMB 10002
Bacillus circulans NCIMB 10001
Polysaccharide of type 8 pneumococcus
Bacillus circulans NCIMB 12846
Bacillus sp. NCIMB 10003
Polyurethane sheets
Alternaria alternata NCIMB 299022
Porphyrins
Cytophaga sp. NCIMB 1327
Propane
Mycobacterium album NCIMB 11806
Mycobacterium album NCIMB 11805
Mycobacterium vaccae NCIMB 11807
Rhodococcus sp. NCIMB 10810
Xanthobacter autotrophicus NCIMB 10809
Propane-1-2-diol
Xanthobacter autotrophicus NCIMB 11171
Propionate
Mycobacterium album NCIMB 11805
Mycobacterium album NCIMB 11806
Mycobacterium vaccae NCIMB 11807
N-Propylamine
Mycobacterium album NCIMB 11805
Mycobacterium vaccae NCIMB 11807
Mycobacterium album NCIMB 11806
Purines
Clostridium cylindosporum NCIMB 12822
Clostridium sticklandii NCIMB 10654
Puromycin aminonucleoside
Arthrobacter sp. NCIMB 9471
Pustulan
Cellulomonas mixtus subsp. *mixtus* NCIMB 8634
PVC
Penicillium simplicissium CABI-IMI 313950
Pseudomonas sp. NCIMB 10750
Aplosporella sp. CABI-IMI 361388*
Aureobasidium pullans CABI-IMI 361381*
Aureobasidium pullans CABI-IMI 361386*
Aureobasidium pullans CABI-IMI 361387*
Bacillus megaterium CABI-IMI 361382*
Cladosporium cladosporoides CABI-IMI 361389*
Coryneform sp. CABI-IMI 361383*
Curtobacterium sp. CABI-IMI 361384
Enterobacter aerogenes NCIMB 10102
Phoma sorghina CABI-IMI 361393*
Rhodosporidium sp. CABI-IMI 361385*
Escherichia coli CABI-IMI 362054*
Pyrimidines
Clostridium sticklandii NCIMB 10654
Quinic acid
Bacillus gordonae NCIMB 12553
Ribonucleic acid
Candida utilis NCYC 707
Ribonucleic acid
Kloeckera apiculata NCYC 245
Rubber
Nocardia sp. NCIMB 12811
Rubber
Nocardia sp. NCIMB 12814
Salicylic acid
Acinetobacter sp. NCIMB 10553
Salicylic acid hydroxylation to gentisic acid
Azotobacter chroococcum NCIMB 10427
Saline soil
Phoma ostiolata CABI-IMI 113691
Phoma ostiolata var brunnea CABI-IMI 113692
Starch
Amorpotheca resinae CABI-IMI 344050
Styrene
Rhodococcus rhodochrous NCIMB 13259
Succinoglycan
Cytophaga arvensicola NCIMB 11855
Taurine
Alcaligenes xylosoxidians subsp. denitrificans NCIMB 10751
Terpine hydrate
Rhodococcus sp. NCIMB 9784
Thiocyanate
Thiobacillus thioparus NCIMB 8349
Pseudomonas sp. NCIMB 10331
Unnamed NCIMB 9903
Thiocyanate with phenol simmultaneous
Pseudomonas sp. NCIMB 10331
Pseudomonas putida NCIMB 11198
Thiosulphate
Pseudomonas putida NCIMB 11198
Thiobacillus thioparus NCIMB 8370
Threonine
Arthrobacter globiformis NCIMB 9759
Arthrobacter sp. NCIMB 11092
Arthrobacter sp. NCIMB 11095
Arthrobacter sp. NCIMB 11094
Arthrobacter sp. NCIMB 11100
Arthrobacter sp. NCIMB 11101
Coryneform sp. NCIMB 11102
Coryneform sp. NCIMB 11103
Thymol
Pseudomonas putida NCIMB 10014
Toluates
Pseudomonas putida NCIMB 10432
Toluene Phage
Pseudomonas putida NCIMB 12184
Pseudomonas putida NCIMB 12182
Pseudomonas putida NCIMB 12186
Pseudomonas sp. NCIMB 12183
Pseudomonas putida NCIMB 12187
Toluene
Acinetobacter sp. NCIMB 9682
Pseudomonas sp. NCIMB 9688
Pseudomonas putida NCIMB 10432
Trichloroethylene
Methylosinus sp. NCIMB 13241
Trifluoralin
Pseudomonas sp. NCIMB 10329
Trimethylamine
Aminobacter aminovorans NCIMB 11590
Aminobacter aminovorans NCIMB 11591
Coryneform sp. NCIMB 11344
Hyphomicrobium sp. NCIMB 12162
Methylobacterium methylotrophus NCIMB 11348
Methylobacterium organophilum NCIMB 11278

Trimethylamine
- *Methylobacterium* sp. NCIMB 11681
- *Methylobacterium zatmanii* NCIMB 10610

Trimethylamine oxide
- *Arthrobacter* sp. NCIMB 11625

Trimethylsulphonium chloride
- *Aminobacter aminovorans* NCIMB 11591

Urea
- *Arthrobacter* sp. NCIMB 9862
- *Brachybacterium faecium* NCIMB 9861

Urea herbicides
- *Bacillus sphaericus* NCIMB 10489

Uric acid
- *Arthrobacter* sp. NCIMB 9864
- *Bacillus fastidiosus* NCIMB 11326
- *Brachybacterium faecium* NCIMB 9859
- *Candida tropicalis* NCYC 997
- *Clostridium cylindosporum* NCIMB 12822
- *Comamonas acidovorans* NCIMB 10013
- *Pichia farinosa* NCYC 937
- *Rhodoturula glutinis* NCYC 974

Veratyrl-glycerolb-coniferyl ether
- *Unnamed* NCIMB 10467

Waste water
- *Fusarium merismoides* CABI-IMI 297028
- *Fusarium merismoides* CABI-IMI 297027
- *Fusarium merismoides* CABI-IMI 297032

Wood shavings
- *Mucor racemosus* CABI-IMI 296421

Wool digestion
- *Bacillus licheniformis* NCIMB 10689

Xanthine
- *Cellulomonas cartae* NCIMB 11025
- *Vibrio tubiashii* NCIMB 2164

Xylan
- *Bacillus subtilis* NCIMB 11034
- *Cellulomonas cellulans* NCIMB 8868
- *Cellulomonas flavigena* NCIMB 8073

Xylene
- *Pseudomonas putida* NCIMB 10432
- *Pseudomonas* sp. NCIMB 12183

Xylene (Phage strain)
- *Pseudomonas putida* NCIMB 12182
- *Pseudomonas putida* NCIMB 12184
- *Pseudomonas putida* NCIMB 12186
- *Pseudomonas putida* NCIMB 12187

2,3-Xylenol
- *Pseudomonas putida* NCIMB 9685

2,4-Xylenol
- *Pseudomonas putida* NCIMB 9866

2,5-Xylenol
- *Pseudomonas putida* NCIMB 9867

3,4-Xylenol
- *Pseudomonas putida* NCIMB 9868

3,5-Xylenol
- *Pseudomonas putida* NCIMB 9869

Bioremediators

See also:Bioconverters, Biodegraders, Biodeteriogens

Activated sludge
- *Corynebacterium nitrilophilus* NCIMB 11594

Benzonitrile bioconversion to benzoic acid
- *Rhodococcus* sp. NCIMB 11216

Cellulosic wastes (to single cell protein)
- *Thermomonospora fusca* NCIMB 11185

Cyanide
- *Pseudomonas fluorescens* NCIMB 11764

Industrial waste (detoxification)
- *Rhodococcus* sp. NCIMB 11216
- *Rhodococcus* sp. NCIMB 11215

Mill sawdust Acid production
- *Lactobacillus pentosus* NCIMB 8026

Silage fermentation
- *Thielavia terrrestris*

Silage fermentation
- *Thielavia terrrestris*

Sulfite waste Fermentation liquor
- *Lactobacillus pentosus* NCIMB 8026

Tolerant Strains

See also: Environmental strains
Strains tolerant to chemical and environmental parameters

Acid (from sulphur springs)
- *Stichococcus bacillaris* CCAP 379/16
- *Stichococcus bacillaris* CCAP 379/15
- *Stichococcus bacillaris* CCAP 379/21
- *Stichococcus bacillaris* CCAP 379/23

Acidophile
- *Alicyclobacillus acidoterrestris* NCIMB 13137
- *Scytalidium acidophilum* CABI-IMI 183518
- *Sulfolobus* NCIMB 11770
- *Scytalidium acidophilum* CABI-IMI 173066ii
- *Alicyclobacillus acidocaldarius* NCIMB 11725
- *Micrasterias thomasiana* var. *notata* CCAP 649/5

Acidophile (acetic acid)
- *Paecilomyces dactylethromorphus* CABI-IMI 065752
- *Paecilomyces dactylethromorphus* CABI-IMI 065752
- *Lactobacillus actolerans* NCIMB12799

Acidophile (acetic preserve)
- *Moniliella acetoabutans* CABI-IMI 180570

Acidophile (grows in acid)
- *Thiobacillus* NCIMB 8455

Acidophile (grows on acetic acid media)
- *Penicillium solitum* CABI-IMI 300728
- *Penicillium simplicissium* CABI-IMI 296073

Acidophile (isolated from pH5.2)
- *Fottea pyrenoidosa* CCAP 326/1

Acidophile: Low pH tolerant
- *Coccomyxa minor* CCAP 216/23

Alcohol
- *Saccharomyces cerevisiae* NCYC 975
- *Saccharomyces cerevisiae* NCYC 478
- *Saccharomyces cerevisiae* NCYC 479
- *Saccharomyces cerevisiae* NCYC 1236

Alkali (from pulp manufacture)
- *Trichoderma viride* CABI-IMI 295977

Alkalophile
- *Bacillus alcalophilus* NCIMB 10438
- *Bacillus firmus* NCIMB 9366
- *Bacillus pasteurii* NCIMB 8221
- *Exiguobacterium aurantiacum* NCIMB 11798
- *Acremonium restrictum* CABI-IMI 206958
- *Acremonium implicatum* CABI-IMI 59628
- *Acremonium curvulum* CABI-IMI 297016
- *Geomyces pannorum* CABI-IMI 059628
- *Natronobacterium gregoryi* NCIMB 2189
- *Natronobacterium magadii* NCIMB 2190
- *Natronobacterium vacuolata* NCIMB 13189
- *Paracoccus alcaliphilus* NCIMB 13180
- *Trichoderma harzianum* CABI-IMI 297033

Alkalophile
- *Trichoderma harzianum* CABI-IMI 295979
- *Coprinus amphibius* CABI-IMI 133855
- *Fusarium merismoides* CABI-IMI 297028
- *Fusarium merismoides* CABI-IMI 297032
- *Fusarium merismoides* CABI-IMI 297027
- *Gliocladium virens* CABI-IMI 295989
- *Trichoderma viride* CABI-IMI 295977

Alkalophilic (from alkaline water)
- *Cyanophora paradoxa* CCAP981/1

Benzimidazole
- *Venturia inaequalis*CABI-IMI 223845

Benzoic acid (300ppm)
- *Candida kefyr* NCYC 744
- *Candida krusei* NCYC 1398
- *Candida valida* NCYC 335
- *Pichia anomala* NCYC 375
- *Pichia heedi* NCYC 1489
- *Pichia membranaefaciens* NCYC 55
- *Saccharomyces exiguus* NCYC 1478
- *Saccharomyces ludwigii* NCYC 734
- *Schizosaccharomyces pombe* NCYC 936
- *Saccharomyces unispous* NCYC 971
- *Torulaspora delbrueckii* NCYC 492
- *Yarrowia lipolytica* NCYC 1421
- *Zygosaccharomyces bailii* NCYC 1766
- *Zygosaccharomyces bisporus* NCYC 1496

Chasmoendolithic (Antartica isolate)
- *Desmococcus endolithicum* CCAP 417/2

Copper (tolerant to high concentrations)
- *Scytalidium acidophilum* CABI-IMI 173066ii

Creosote
- *Vibrio alginolyticus* NCIMB 1737

Glucose (50%)
- *Candida guilliermondii* NCYC 946
- *Debaryomyces poymorphus* NCYC 948
- *Pichia farinosa* NCYC 937
- *Schizosaccharomyces pombe* NCYC 1351
- *Torulaspora delbrueckii* NCYC 492
- *Zygosaccharomyces bailii* NCYC 1557
- *Zygosaccharomyces bisporus* NCYC 171
- *Zygosaccharomyces rouxii* NCYC 583

Glucose (60%)
- *Candida magnoliae*NCYC 765
- *Zygosaccharomyces rouxii* NCYC 568

Halophile
- *Chromatium* sp. NCIMB 8379
- *Desulfovibrio desulfuricans* subsp. *aestuarii* NCIMB 9335
- *Halobacterium* all strains
- *Halococcus* all strains
- *Haloferax* all strains
- *Halomonas* all strains
- *Halovibrio* all strains
- *Marinococcus halophilus* NCIMB 2178
- *Natronobacterium* all strains
- *Natronococcus* all strains
- *Paracoccus halodenitrificans* NCIMB 700
- *Paracoccus* sp. NCIMB 8669

Halophile
- *Planococcus* sp. NCIMB 2179
- *Pseudomonas* sp. NCIMB 8668
- *Sporosarcina halophila* NCIMB 2269
- Unnamed NCIMB 2067
- Unnamed NCIMB 2126
- *Vibrio costicola* NCIMB 701
- *Vibrio costicola* NCIMB 788

Halophile (photosynthetic)
- *Rhodospirillum salinarum* NCIMB 2243

Halotolerant
- *Planctomyces brasiliensis* NCIMB 13185

Halotolerant (Sodium chloride 15%)
- *Candida etchellsii* NCYC 407
- *Candida colliculosa* NCYC 140
- *Candida famata* NCYC 611
- *Candida haemulonii* NCYC 787
- *Candida parapsilosis* NCYC 458
- *Candida valida* NCYC 327
- *Debaryomyces hansenii* NCYC 103
- *Guilliermondella selenospora* NCYC 40
- *Kluyveromyces marxianus* NCYC 151
- *Pichia anomala* NCYC 20
- *Pichia subpelliculosa* NCYC 1653
- *Schwanniomyces occidentalis* NCYC 133
- *Zygosaccharomyces cidri* NCYC 1567
- *Zygosaccharomyces rouxii* NCYC 1682

Halotolerant (Sodium chloride 20%)
- *Candida famata* NCYC 71
- *Candida tropicalis* NCYC 1525
- *Candida versatilis* NCYC 1433
- *Debaryomyces hansenii* NCYC 102
- *Schwanniomyces occidentalis* NCYC 133
- *Zygosaccharomyces rouxii* NCYC 1522

Heavy metal
- *Coccomyxa minor* CCAP 216/23

High altitude
- *Colletotrichum kahawe* CABI-IMI

High temperature: see thermophiles

Low temperature: see psychrophiles

Psychrophile
- *Aquaspirillum arcticum* NCIMB 13152
- *Bacillus psychrosaccharolyticus* NCIMB 11729
- *Cladosporium sphaerospermum* CABI-IMI 091964
- *Clostridium estertheticum* NCIMB 12511
- *Clostridium* sp. NCIMB 11903
- *Clostridium* sp. NCIMB 11902
- *Clostridium* sp. NCIMB 11901
- *Clostridium* sp. NCIMB 11900
- *Clostridium* sp. NCIMB 11899
- *Colwellia psychroerythrus* NCIMB 2079
- *Curtobacterium psychrophilum* NCIMB 2068
- *Cytophaga xantha* NCIMB 2069
- *Mucor hiemalis* CABI-IMI 289880
- *Mucor racemosus* CABI-IMI 017313
- *Psychrobacter immobilis* NCIMB 2071
- *Rhodococcus martinonascens* NCIMB 2246
- *Spirillium pleomorphum* NCIMB 2070
- *Vibrio logei* NCIMB 1143
- *Vibrio marinus* NCIMB 1144

Psychrophile (Antarctica isolate)
- *Coccomyxa gloeobotrydiformis* CCAP 216/21

Psychrophile (Antarctica isolate)
- *Coccomyxa gloeobotrydiformis* CCAP 216/20
- *Desmococcus endolithicum* CCAP 417/2
- *Desmococcus olivaceus* CCAP 417/1
- *Dilabifilum prostratum* CCAP 415/4
- *Gloeobotrys* sp. CCAP 830/2
- *Gloeobotrys terrestres* CCAP 830/3
- *Gloeobotrys terrestres* CCAP 830/1
- *Hemichloris antartica* CCAP 239/1
- *Hemichloris antartica* CCAP 239/2
- *Hemichloris antartica* CCAP 239/3
- *Heteromita globosa* CCAP 1961/2
- *Heterothrix antartica* CCAP 836/6
- *Heterothrix* sp. CCAP 836/9
- *Heterotrichella gracilis* CCAP 881/1
- *Microthamnion kuetzingianum* CCAP 450/2
- *Muriella terrestries* var. *reticulatus* CCAP 249/4
- *Muriellopsis sphaericum* CCAP 256/1
- *Neospongiococcum* sp. CCAP 255/3
- *Oocystis minuta var. ellipsoidea* CCAP 257/5
- *Oocystis minuta var. ellipsoidea* CCAP 257/5
- *Penicillium rugulosum* CABI-IMI 291021
- *Planophila* sp. CCAP 462/1
- *Pseudochlorella subsphaerica var antartica* CCAP 164/3
- *Pseudococcomyxa aimplex* CCAP 812/3
- *Pseudococcomyxa aimplex* CCAP 812/4
- *Pseudococcomyxa aimplex* CCAP 848/2
- *Pseudodictyochloris multinucleata* CCAP 177/1
- *Pseudoschimeris mucosa* CCAP 363/1
- *Raphidonema pyrenoidifera var. alongata* CCAP 470/5
- *Raphidonema sempervirens* CCAP 470/6
- *Raphidonemopsis sessilis* CCAP 470/7
- *Stichococcus* sp. CCAP 379/29
- *Tribonema vulgare* CCAP 880/4
- *Diplosphaeria mucosa* CCAP 379/26
- *Alternaria alternaria* CABI-IMI 378423*
- Unamed CABI-IMI 378656*
- Unamed CABI-IMI 378657*
- Leptodontidium elatus CABI-IMI 369788*
- *Cladosporum* sp. CABI-IMI 377828*

Psychrophile (Alaskan-snow)
- *Klebsormidium subtilissimum* CCAP 384/1

Psychrophile (Alpine tundra)
- *Thielavia peruviana* CABI-IMI 135024

Psychrophile (Artic isolate (soil)
- *Phlyctochytrium* CABI-IMI 143636

Psychrophile (below 5^{o}C)
- *Candida succiphila* NCYC 1403
- *Cryptococcus laurentii* NCYC 1462

Psychrophile (below 5^{o}C)
- *Cryptococcus vishniacii* NCYC 1405
- *Phaffia rhodozyma* NCYC 874
- *Rhodotorula glutinis* NCYC 974
- *Rhodotorula graminis* NCYC 1401
- *Rhodotorula mucilaginosa* NCYC 797
- *Sporobolomyces roseus* NCYC 1464

Psychrophile (between 5-8^{o}C)
- *Bullera alba* NCYC 425
- *Candida boidinii* NCYC 1513
- *Candida diddensiae* NCYC 1535
- *Candida sake* NCYC 976
- *Candida utilis* NCYC 737
- *Cryptococccus albidus* NCYC 445
- *Cryptococcus luteolus* NCYC 1501
- *Debaryomyces hansenii* NCYC 793
- *Pichia farinosa* NCYC 937
- *Pichia opuntiae* NCYC 1479
- *Pichia pini* NCYC 1419
- *Rhodotorula glutinus* NCYC 377
- *Rhodotorula mucilaginosa* NCYC 158
- *Saccharomyces cerevisiae* NCYC 1499
- *Saturnospora zaruensis* NCYC 1531
- *Sporidiobolus ruineniae* NCYC 980
- *Sporobolomyces roseus* NCYC 1463
- *Torulaspora delbrueckii* NCYC 408
- *Yarrowia lipolytica* NCYC 825
- *Zygosaccharomyces rouxii* NCYC 1522

Psychrophile (from snow)
- *Chromulina chionophila* CCAP 909/9
- *Stichococcus bacillaris* CCAP 379/6

Psychrophile (from glacier pool)
- *Chlorellidium* sp. CCAP 811/2

Thermophile
- *Bacillus stearothermophilus* NCIMB 701094
- *Bacillus stearothermophilus* NCIMB 702010
- *Calcarisporium thermophilium* CABI-IMI 144750
- *Chaetomium thermophilium* CABI-IMI 289313
- *Desulfotomaculum nigrificans* NCIMB 8395
- *Desulfotomaculum nigrificans* NCIMB 8355
- *Desulfotomaculum nigrificans* NCIMB 8354
- *Bacillus thermocloaceae* NCIMB 13138
- *Desulfotomaculum nigrificans* NCIMB 8351
- *Desulfotomaculum nigrificans* NCIMB 8353
- *Humicola grisea* var. *themoidea* CABI-IMI 369788
- *Streptomyces macrosporus* NCIMB 12473
- *Streptomyces megasporus* NCIMB 12472
- *Penicillium lapidosum* CABI-IMI 039743

Thermophile (45^{o}C and above)
- *Aspergllu fumigatus* CABI-IMI 361368
- *Kluyveromyces marxianus* NCYC 1429
- *Candida kefyr* NCYC 744

Thermophile (45^{o}C and above)
- *Kluyveromyces marxianus* NCYC 587
- *Kluyveromyces marxianus* NCYC 827
- *Pichia angusta* NCYC 1458

Thermophile (between 40- 45^{o}C)
- *Candida albicans* NCYC 1471
- *Candida krusei* NCYC 1398
- *Candida lusitaniae* NCYC 1420
- *Candida rugosa* NCYC 391
- *Candida tropicalis* NCYC 1525
- *Candida utilis* NCYC 927
- *Kluyveromyces marxianus* NCYC 1425

Thermophile (between 40- 45^{o}C)
- *Pichia angusta* NCYC 1455
- *Pichia farinosa* NCYC 937
- *Pichia memranaefaciens* NCYC 938
- *Pichia mississippiensis* NCYC 1447

Thermophile (from fermenter system at elevated temperature)
- *Aspergillus fumigatus* CABI-IMI 293740

Thermophile (hot spring)
- *Naegleria clarki* CCAP 1518/15
- *Naegleria clarki* CCAP 1518/15
- *Sulfolobus acidocaldarius* NCIMB 11770
- *Naegleria clarki* CCAP 1506/1

Thermotolerant (Hot spring)
- *Naegleria clarki* CCAP 1518/15
- *Alicyclobacillus acidorterrestris* NCIMB 13137
- *Bacillus methanolicus* NCIMB 13113
- *Sulfolobus acidocaldarius* NCIMB 11770

Resistant strains

Strains resistant to Antibiotics, chemicals, radiation, environmental parameters etc.

5-Methyltrypophan
- *Bacillus subtilis* NCIMB 10485

Actinoglobolin
- *Enterococcus durans* NCIMB11077

Actinophage
- *Steptomyces griseus* NCIMB 9001

Aminoglycoside
- *Escherichia coli* NCIMB 11710

Ampicillin (phage)
- *Escherichia coli* NCIMB 12004
- *Escherichia coli* NCIMB 12230
- *Escherichia coli* NCIMB 12207
- *Escherichia coli* NCIMB 11908
- *Escherichia coli* NCIMB 12232
- *Escherichia coli* NCIMB 11967
- *Escherichia coli* NCIMB 11963
- *Escherichia coli* NCIMB 11702
- *Escherichia coli* NCIMB 11960
- *Klebsiella oxytoca* NCIMB 10417
- *Staphylococcus aureus* NCIMB 11722

Bacitracin
- *Escherichia coli* NCIMB 10072
- *Provodencia aeruginosa* NCIMB 9570

Benomyl Fungicide
- *Botrytis cinerea* CABI-IMI 313860
- *Cercospora api* CABI-IMI 186563
- *Colletotrichum Musae* CABI-IMI 165753
- *Penicillium hirsutum* CABI-IMI 170486

Carbenicillin
- *Pseudomonas mendocina* NCIMB 11688
- *Pseudomonas mendocina* NCIMB 11689
- *Pseudomonas mendocina* NCIMB 11687

Chloramphenicol
- *Lactobacillus casei* subsp. *rhamnosus* NCIMB 10463
- *Lactobacillus casei* subsp. *rhamnosus* NCIMB 11295
- *Pediococcus acidiactici* NCIMB 11432
- *Providencia rettgeri* NCIMB 9570

Chloramphenicol (phage)
- *Escherichia coli* NCIMB 11987
- *Escherichia coli* NCIMB 11988
- *Escherichia coli* NCIMB 11866

Chlorine
- *Flavobacterium* sp. NCIMB 10741
- *Pseudomonas deva* NCIMB 10747

Colicin Phage4
- *Escherichia coli* NCIMB 11929

Dihydrostreptomycin
- *Klebsiella pnumoniae subsp pneumoniae* NCIMB 10417
- *Micrococcus luteus* NCIMB 9660
- *Micrococcus luteus* NCIMB 10419
- *Micrococcus luteus* NCIMB 10818

Erythromycin
- *Micrococcus luteus* NCIMB 12874

Erythromycin
- *Mutans* NCIMB 11516

Formaldehyde
- *Pseudomonas putida* NCIMB 12772

Gamma ray
- *Rubrobacter radiotolerans* NCIMB 12766

Gentamycin
- *Escherichia coli* NCTC 11186

Germicides
- *Pseudomonas aeruginosa* NCIMB 10421

Heat resistant
- *Penicillium lapidosum* CABI-IMI 039743

Heavy metal ions
- *Thermus flavus* NCIMB 13085

Kanamycin (resistant phage)
- *Escherichia coli* NCIMB 11988
- *Escherichia coli* NCIMB 11721
- *Escherichia coli* NCIMB 11948
- *Escherichia coli* NCIMB 12002
- *Escherichia coli* NCIMB 11987
- *Escherichia coli* NCIMB 11963
- *Escherichia coli* NCIMB 11962
- *Escherichia coli* NCIMB 11907
- *Escherichia coli* NCIMB 11968
- *Escherichia coli* NCIMB 11715
- *Pseudomonas putida* NCIMB 11950

Lancefield grouping of streptococci
- *Streptomyces griseus* NCTC 7807

Lead
- STA p I258 NCTC 50581
- STA p I524 NCTC 50599

Mercury (resistant phage)
- *Pseudomonas* sp. NCIMB 12185

Mercury
- ECO R478 NCTC 50038
- ECO R828 NCTC 50346
- ECO R222Jap NCTC 50019
- PSA RIP64 NCTC 50075
- PSA FP2 NCTC 50121
- STA p I258 NCTC 50581
- STA p I524 NCTC 50599

Metal ion accumulation
- *Rhizobiaceae* sp NCIMB 10340
- *Zooglea ramigera* NCIMB 11941

Methicillin
- *Staphylococcus aureus* NCTC 10442
- *Staphylococcus epidermidis* NCTC 11964

Metronidazole
- *Bacteriodes melaninogenicus* NCTC 11559

Neomycin
- *Bordetella bronchiseptica* NCIMB 9936
- *Micrococcus luteus* NCIMB 8994
- *Micrococcus luteus* NCIMB 10418

Neomycin (phage)
- *Bacillus subtilis* NCIMB 11624

Novobiocin
- *Staphylococcus aureus* NCIMB 10819
- *Providencia rettgeri* NCIMB 9570

Oleandomycin
- *Providencia rettgeri* NCIMB 9570

Oxolinic acid
- *Aeromonas salmonicida* subsp. *salmonicida* NCIMB 2261

Penicillin
- *Staphylococcus aureus* NCTC 10443
- *Staphylococcus aureus* NCIMB 9968
- *Staphylococcus aureus* NCIMB 9969
- *Staphylococcus aureus* NCIMB 9971
- *Staphylococcus aureus* NCIMB 9972
- *Staphylococcus aureus* NCIMB 12864

Phenol mercury
- *Cladosporium cladosporioides* CABI-IMI 154279
- *Cladosporium cladosporioides* CABI-IMI 154278

Phenol mercury 2.5ppm
- *Penicillium crustosum* CABI-IMI 154282
- *Penicillium crustosum* CABI-IMI 154283

Polymyxin B
- *Providencia rettgeri* NCIMB 9570

Radiation
- *Deinococcus radiodurans* NCIMB 13156
- *Methylobacterium radiotolerans* NCIMB 10815
- *Deinococcus radiodurans* NCIMB 9279
- *Deinococcus radiophilus* NCIMB 10648
- *Escherichia coli* NCIMB 9485
- *Deinococcus radiopugnans* NCIMB 13155
- *Acinetobacter radioresistens* NCIMB 12753
- *Bacillus pumulis* NCIMB 10692

Rifampcin
- *Staphylococcus aureus* NCTC 10703
- *Staphylococcus aureus* NCTC 11150

Rifampicin (phage)
- *Escherichia coli* NCIMB 11667

Silver (toleration and bioaccumulate)
- *Stenotrophomas maltophila* NCIMB 11534

Silver resistant
- ECO p MG101 NCTC 50110

Streptomycin
- *Micrococcus luteus* NCIMB 9660
- *Staphylococcus aureus* NCIMB 9972
- *Micrococcus luteus* NCIMB 10419
- *Micrococcus luteus* NCIMB 10818
- *Providencia rettgeri* NCIMB 9570
- *Staphylococcus aureus* NCIMB 9968
- *Staphylococcus aureus* NCIMB 9970
- *Streptococcus mutans* NCIMB 11516
- *Staphylococcus aureus* NCIMB 9969

Streptomycin (phage)
- *Escherichia coli* NCIMB 11791
- *Escherichia coli* NCIMB 11949
- *Escherichia coli* NCIMB 11947
- *Escherichia coli* NCIMB 11967
- *Escherichia coli* NCIMB 11950
- *Escherichia coli* NCIMB 11715
- *Escherichia coli* NCIMB 11790
- *Escherichia coli* NCIMB 12002

Sulphonamide phage
- *Escherichia coli* NCIMB 11947

Tellurite resistant
- ECO R478 NCTC 50038
- ECO p HH1508a NCTC 50101
- ECO R828 NCTC 50346
- PSA p MG1 NCTC 50108

Tellurite resistant
- PSA R 931 NCTC 50066

Tetracycline
- *Bacillus cereus* NCIMB 11183
- *Staphylococcus aureus* NCIMB 9969
- *Staphylococcus aureus* NCIMB 9968

Tetracycline (phage)
- *Escherichia coli* NCIMB 11670
- *Escherichia coli* NCIMB 11721
- *Escherichia coli* NCIMB 11864
- *Escherichia coli* NCIMB 11866
- *Escherichia coli* NCIMB 12207
- *Escherichia coli* NCIMB 11911
- *Bacillus subtilis* NCIMB 11622
- *Escherichia coli* NCIMB 12004
- *Escherichia coli* NCIMB 11968
- *Escherichia coli* NCIMB 12205
- *Escherichia coli* NCIMB 11906
- *Escherichia coli* NCIMB 11905
- Escherichia coli NCIMB 12206
- *Escherichia coli* NCIMB 11963
- *Escherichia coli* NCIMB 11962
- *Escherichia coli* NCIMB 11918

Thiabendazole
- *Colletotrichum musae* CABI-IMI 165753

Tobramycin
- *Escherichia coli* NCTC 11186

UV
- *Escherichia coli* NCIMB 11187
- *Escherichia coli* NCIMB 9485
- *Deinococcus radiodurans* NCIMB 9276

UV (phage)
- *Escherichia coli* NCIMB 11702

Sensitive strains

4-Quinulone antibiotics
Yersinia ruckeri NCIMB 13282
Benzimidazole
Venturia inaequalis CABI-IMI 223844
Catabolite repression
Escherichia coli NCIMB 9999
Gentamycin
Pseudomonas aeruginosa NCIMB 13063
Killer toxin
Saccharomyces cerevisiae NCYC 1006
Metronidazole
Clostridium perfringens NCTC 11229
Serum
Escherichia coli NCIMB 11888
Sulphonamide
Streptococcus pyogenes NCIMB 8884
Temperature (Phage)
Escherichia coli NCIMB 12001
Temperature (Phage)
Escherichia coli NCIMB 12001
Escherichia coli NCIMB 11987
Lactobacillus sp. NCIMB 12118
Escherichia coli NCIMB 12191
Escherichia coli NCIMB 12192

Test strains

For cited specifications

Advanced pathogens subset (NCTC Code G)
- *Campylobacter coli* NCTC 11366
- *Campylobacter jejuni* NCTC 11322
- *Clostridium bifermentans* NCTC 506
- *Clostridium difficilie* NCTC 11204
- *Escherichia coli* 0157 VTEC NCTC 12079
- *Salminarium typhimurium* NCTC 12023
- *Vibrio cholerae Non O:1* NCTC 11348
- *Vibrio furnissi* NCTC 11218
- *Vibrio parahaemolyticus* NCTC 10885
- *Yersinia enterocolitica* NCTC 10460

Aerosols
- *Chaetomium globosum* CABI-IMI 045550
- *Chaetomium globosum* CABI-IMI 045550iv
- *Chaetomium globosum* CABI-IMI 045550iii
- *Chaetomium globosum* CABI-IMI 045550ii

Aerosols (aerial bactericides)
- *Staphylococcus epidermidis* NCTC 7944

Ames test
- *Salmonella typhimurium* NCTC 12115
- *Salmonella typhimurium* NCTC 11881
- *Salmonella typhimurium* NCTC 12116
- *Salmonella typhimurium* NCTC 12117

Antibacterial activity of enzyme preparations used as test
- *Bacillus cereus* NCIMB 11843
- *Bacillus circulans* NCIMB 11844
- *Escherichia coli* NCIMB 9517
- *Serratia marcescens* NCIMB 11851
- *Staphylococcus aureus* NCIMB 9581
- *Streptococcus pyogenes* NCIMB 11841

Antibiotic Sensitivity
- *Pseudomonas aeruginosa* NCIMB 13063

Antibiotic sensitivity (colistin and polymyxin) Control strain
- *Esscherichia coli* NCTC 10418

Antibiotic sensitivity (in serum and body fluids)
- *Staphylococcus aureus* NCTC 6571

Antibiotic susceptibility
- *Escherichia coli* NCIMB 12210
- *Pseudomonas aeruginosa* NCIMB 12469
- *Pseudomonas aeruginosa* NCIMB 8295
- *Staphylococcus aureus* NCIMB 6571

Antimicrobial susceptibility
- *Enterococcus* NCIMB 13280

Antimicrobial preservative testing
- *Aspergillus niger* CABI-IMI 149007

Antiseptics
- *Enterococcus hirae* NCIMB 8192
- *Mycobacterium hirae* NCIMB 13116
- *Pseudomonas aeruginosa* NCIMB 13056
- *Pseudomonas aeruginosa* NCIMB 10647

Antiserum-Aeromonas salmonicida diagnostic
- *Pseudomonas fluorescens* NCIMB 13041

AOAC phenol coeffecient
- *Salmonella typhi* NCTC 10787
- *Staphylococcus aureus* NCTC 10788

API 20 E Control strain
- *Pseudomonas aeruginosa* NCIMB 8295

ASTM G21
- *Aureobasidium pullans* CABI-IMI 145194
- *Aspergillus niger* CABI-IMI 091855
- *Penicillium pinophilum* CABI-IMI 087160
- *Chaetomium globosum* CABI-IMI 045550ii
- *Trichoderma viride* CABI-IMI 045553ii

Australian test mould proofing strain
- *Alternaria alternata* CABI-IMI 071749

Bacterocin reference set Strain BZB2
- 101 p CoIA-CA31 NCTC 50129
- 101 p CoIB-K260 NCTC 50130
- 103 p CoID-CA23 NCTC 50131
- 104 p CoIE1-K53 NCTC 50132
- 106 p CoIE3-CA38 NCTC 50134
- 107 p Co1E4-CT9 NCTC 50136
- 108 p CoIE5-099 NCTC 50137
- 110 p CoIE7-K317 NCTC 50139
- 114 p CoIIa-CA53 NCTC 50141
- 115 p Collb-P9drd NCTC 50142
- 116 p ColK-K235 NCTC 50143
- 123 p Co1N-284 NCTC 50145
- 125 p CoIE2-P9 NCTC 50133
- 149 p APBZ101 (ApTc)(co1E3) NCTC 50135
- 150 p APBZ102 (ApTc)(co1E6) NCTC 50138
- 283 p MM4 (mccB17) NCTC 50148
- 283 p RYC17::Tn10) NCTC 50148

Bacterocin reference set strains
- CA46 CoIG NCTC 50152
- CA58 CoIH NCTC 50153
- JF246 CoIL NCTC 50151
- PAP1 p CHAP1(coIM) NCTC 50144
- PAP1370 ColM NCTC 50150
- PAP2 p CHAP2(co1S4) NCTC 50146
- PAP222 p CoIV-K270 NCTC 50147
- PAP247 p PC101(co1E8-J) NCTC 50140
- RYC492 MCCe492 NCTC 50149

Baird Parker selective media (evaluatuion)
- *Staphylococcus aureus* NCIMB 12820

Biocide testing (paint)
- *Anacystis montana* CCAP 1430/1
- *Chlorella emersonii* CCAP 211/8G
- *C. fusca* CCAP 211/23
- *Gloeocapsa* sp. CCAP 6430/2
- *Nostoc commune* CCAP 1453/24
- *Nostoc commune* CCAP 1453/29
- *Nostoc muscorum* CCAP 1453/8
- *Nostoc muscorum* CCAP 1453/12
- *Nostoc muscorum* CCAP 1453/20
- *Oscillatoria tenuis* CCAP 1459/4
- *Pleurococcus* sp. CCAP 464/1
- *Stichococcus bacillaris* CCAP 379/1A
- *Stigeoclonium tenue* CCAP 477/11A
- *Trentopholia aurea* CCAP 483/1
- *T. odorata* CCAP 483/4

Camp test
- *Rhodococcus equii* NCIMB 6571
- *Rhodococcus equii* NCIMB 12702
- *Rhodococcus equii* NCIMB 12828

Camp test (Listeria)
- *Rhodococcus equi* NCTC 1621
- *Staphylococcus aureus* NCTC 1803

Camp test (Streptococci)
- *Staphylococcus aureus* NCTC 7428

Carbenicillin (control strain)
- *Pseudomonas aeruginosa* NCTC 10662

Chick Martin (disinfectant)
- *Salmonella typhi* NCTC 3390

Chlortetracycin (sensitivity discs)
- *Escherichia coli* NCIMB 8020

Cholera enterotoxin
- *Vibrio cholerae* non 0:1 NCTC 11195

Colicin (indicator for production)
- *Escherichia coli* NCIMB 11300

Crown Agents Test (disinfectant)
- *Salmonella typhi* NCTC 160

Cutting fluids (to bacterial attack)
- *Citrobacter freundii* NCIMB 12203
- *Corneyform* sp. NCIMB 12200
- *Pseudomonas fluorescens* NCIMB 12201
- *Shewanella putrefaciens* NCIMB 12202

Diptheria toxin test (Elek plate control) negative
- *Corynebacterium diphtheriae var. mitis* NCTC 10356

Diptheria toxin test (Elek plate control) strong
- *Corynebacterium diphtheriae var. gravis* NCTC 10648

Diptheria toxin test (Elek plate control) weak
- *Corynebacterium diphtheriae var. gravis* NCTC 3984

Disinfectant
- *Bacillus cereus* NCIMB 11925
- *Enterococcus faecium* NCIMB 12672
- *Enterococcus hirae* NCIMB 8192
- *Escherichia coli* NCIMB 9132
- *Escherichia coli* NCIMB 86
- *Escherichia coli* NCIMB 8277
- *Escherichia coli* NCIMB 9517
- *Mycobacterium fortuitum* NCIMB 10384
- *Mycobacterium smegmatis* NCIMB 13116
- *Proteus mirabilis* NCIMB 12596
- *Proteus vulgaris* NCIMB 11833
- *Pseudomonas aeruginosa* NCIMB 10421
- *Pseudomonas aeruginosa* NCIMB 13056
- *Staphylococcus aureus* NCIMB 11832
- *Staphylococcus aureus* NCIMB 9518

DNAse test (Campylobacters) Positive
- *Campylobacter jejuni* NCTC 12145
- *Campylobacter lari* NCTC 12144

DNAse test (Campylobacters) Weak positive
- *Campylobacter coli* NCTC 12143

Ecotoxicity testing (marine)
- *Phaeodactylum tricornutum* CCAP 1052/1a
- *Skeletonema costatum* CCAP 1077/5
- *Skeletonema costatum* CCAP 1077/3

Ecotoxicity testing (freshwater)
- *Chlorella vulgaris* CCAP 211/11b
- *Chlorella vulgaris fo.viridis* CCAP 211/12
- *Scenedesmus subspicatus* CCAP 276/20
- *Scenedesmus subspicatus* CCAP 276/22
- *Selenastrum capricornutum* CCAP 278/4

EEC meat strain (detection of inhibitors in meat)
- *Bacillus subtilis* EEC strain NCIMB 11232

Eijkman test-control
- *Escherichia coli* NCIMB 9132

Emulsion paint (Bacterial resistance to)
- *Aeromonas hydrophila subsp. hydrophila* NCIMB 9233
- *Enterobacter aerogenes* NCIMB 10102
- *Escherichia coli* NCIMB 8114
- *Micrococcus luteus* NCIMB 8166
- *Morganella morgani* NCIMB 9525
- *Proteus vulgaris* NCIMB 67
- *Pseudomonas aeruginosa* NCIMB 8295
- *Pseudomonas putida* NCIMB 9494

Enterobacteriaceae set (NCTC Code E)
- *Acinetobacter lwoffi* NCTC 5866
- *Citrobacter freundii* NCTC 9750
- *Edwardsiella tarda* NCTC 11934
- *Enterobacter cloacae* NCTC 11936
- *Serratia macerens* NCTC 11935
- *Shigella sonnei* NCTC 8574

Enterotoxin D (Staphylococci)
- *Staphylococcus aureus* NCTC 10656

Enterotoxin (E.coli)
- *Escherichia coli* NCTC 11601
- *Escherichia coli* NCTC 11602
- *Escherichia coli* NCTC 11603

Enterotoxin A (Staphylococci)
- *Staphylococcus aureus* NCTC 10652
- *Staphylococcus aureus* NCTC 11965
- *Staphylococcus aureus* NCTC 11962

Enterotoxin A/B (Staphylococci)
- *Staphylococcus aureus* NCTC 10657

Enterotoxin B (Staphylococci)
- *Staphylococcus aureus* NCTC 10654

Enterotoxin C (Staphylococci)
- *Staphylococcus aureus* NCTC 10655
- *Staphylococcus epidermidis* NCTC 12100

Ethylene oxide sterilizers (spore)
- *Bacillus macerans* NCTC 11491
- *Bacillus subtilis* NCTC 10073

European standard pr EN152 (I & II)
- *Sydowia polyspora* CABI-IMI 269217

Fabrics (antibacterial activity)
- *Klebsiella pnumoniae* subsp. *pnumoniae* NCIMB 10341

Fabrics (antibacterial activity)
- *Staphylococcus aureus* NCIMB 9518

FDA
- *Salmonella typhi* NCTC 779
- *Staphylococcus aureus* NCTC 4163

Food and dairy products set NCTC Code A/B
- *Aeromonas hydrophila* NCTC 8049
- *Bacillus cereus* NCTC 7464
- *Bacillus subtilis* NCTC 10400
- *Clostridium perfringes* NCTC 8237
- *Enterococcus faecalis* NCTC 775
- *Escherichia coli* NCTC 10418
- *Klebsiella aerogenes* NCTC 9528
- *Lactococcus lactis* NCTC 662
- *Listeria monocytogenes* NCTC 11994
- *Proteus mirabilis* NCTC 10975
- *Proteus rettgeri* NCTC 7475
- *Salmonella poona* NCTC 4840
- *Staphylococcus aureus* NCTC 6571
- *Staphylococcus epidermidis* NCTC 11047

Formaldehyde disinfection-Aerial
Mycobacterium avium NCTC 8551
Staphylococcus epidermidis NCTC 7944
Freeze drying work
Klebsiella pnumoniae subsp. *pnumoniae* NCIMB 10695
Fungal toxicity of alcohols and fatty acids
Mucor circinelloides CABI-IMI 078379
Fungus resistance test BS2011 PART 2J
Aspergillus niger CABI-IMI 017454
Aspergillus terreus CABI-IMI 045543
Aureobasidium pullans CABI-IMI 045533
Paecilomyces variotii CABI-IMI 108007
Penicillium pinophilum CABI-IMI 114933
Penicillium ochrochloron CABI-IMI 061271
Scopulariopsis brevicaulis CABI-IMI 049528
Trichoderma viride CABI-IMI 045553ii
Fungus resistance test DEF 133
Aspergillus amstelodami CABI-IMI 017455
Aspergillus niger CABI-IMI 017454
Chaetomium globosum CABI-IMI 016203
Paecilomyces variotii CABI-IMI 108007
Penicillium brevicompactum CABI-IMI 017456
Penicillium cyclopium CABI-IMI 019759
Stachybotrys atra CABI-IMI 082021
Fungus resistance test MIL 810D (MIL C 62122b)
Aspergillus niger CABI-IMI 091855
Aspergillus flavus CABI-IMI 091856
Aspergillus versicolor CABI-IMI 045554ii
Penicillium funiculosum CABI-IMI 211742
Penicillium pinophilum CABI-IMI 087160
Chaetomium globosum CABI-IMI 045550ii
Fungus resistance testing
Aureobasidium pullans CABI-IMI 145194
Paecilomyces lilacinus CABI-IMI 117109
Paecilomyces variotii CABI-IMI 108007
General inhibitor test (detection of biological residues)
Bacillus subtilis NCIMB 11232
Gentamycin Control strain
Kleibsiella edwardsii var. *attantae* NCTC 10896
Pseudomonas aeruginosa NCTC 10662
Germicidal activity in swimming pools
Enterococcus faecium NCIMB 13192
Government chemist- general purpose disinfectants
Salmonella choleraeusis var. *kuzendorf* NCTC 10653
Government chemist-against Mycobacterium tuber`s
Mycobacterium fortuitum NCTC 8573
Hops (estimation of antiseptic power)
Lactobacillus plantarum NCIMB 6461
Hyaluronidase
Acinetobacter sp. NCIMB 10454
Indicator
(btuB) BZB1030 NCTC 50156
(cir) BZB1022 NCTC 50157
Indicator
(fepA) PAP1372 NCTC 50155
(ompA) PAP702 NCTC 50163
(ompF) PAP308 NCTC 50161
(ompR::Tn5) PAP138b NCTC 50162
(semA::Tn5) PAP710 NCTC 50164
(tonA) BZB1191 NCTC 50159
(tonB) PAP1192 NCTC 50160
(tsx) PAP1360 NCTC 50158
tolQ TPS NCTC 50602
Indicators (sensitive indicator)
BZB1011 NCTC 50154
Isoniazid (sensitivity)
Mycobacterium tuberculosis NCTC 7417
Kanamycin (control strain)
Kleibsiella edwardsii var. *attantae* NCTC 10896
Kanamycin quality control
Providencia rettgeri NCIMB 9570
Kelsey and Sykes
Escherichia coli NCTC 8196
Proteus vulgaris NCTC 4335
Pseudomonas aeruginosa var *erythrogenes* NCTC 6749
Staphylococcus aureus NCTC 4163
Kelsey capacity
Escherichia coli NCTC 8196
Pseudomonas aeruginosa NCTC 6749
Salmonella typhi NCTC 3390
Salmonella typhi NCTC 786
Staphylococcus aureus NCTC 4163
Laundry disenfectant
Streptococcus faecalis var. *zymogenes* NCTC 10927
Listeria set NCTC Code F
Listeria innocua NCTC 1288
Listeria ivanovii NCTC 11846
Listeria monocytogenes NCTC 11994
Rhodococcus equi NCTC 1621
Staphylococcus aureus NCTC 1803
Long term effectiveness
Pseudomonas aeruginosa var. *erythrogenes* NCTC 6749
Lysozyme activity test
Micrococcus luteus NCIMB 10474
Micrococcus luteus NCIMB 9278
Macrolides
Bacillus cereus NCIMB 11183
Membrane filter control strain
Serratia marcescens NCIMB 12779
Metal working fluid -assay of antIMI crobials
Klebsiella pneumoniae NCIMB 13281
Milk (of antibiotics in)
Streptococcus salivsarius subsp. *thermophilus* NCIMB 10387
Mold proof
Aspergillus amstelodami CABI-IMI 017455
Aspergillus clavatus CABI-IMI 155703
Aspergillus flavus CABI-IMI 114929
Aspergillus flavus CABI-IMI 0901856
Aspergillus flavus CABI-IMI 091856
Aspergillus flavus CABI-IMI 091856iii
Aspergillus niger CABI-IMI 070102
Aspergillus niger CABI-IMI 017454
Aspergillus niger CABI-IMI 114927
Aspergillus niger CABI-IMI 059374

Mold proof
- *Aspergillus niger* CABI-IMI 091855iii
- *Aspergillus ustus* CABI-IMI 045552
- *Aureobasidium pullans* CABI-IMI 070103
- *Chaetomium globosum* CABI-IMI 045550iv
- *Chaetomium globosum* CABI-IMI 045550ii
- *Chaetomium globosum* CABI-IMI 016203
- *Chaetomium globosum* CABI-IMI 045550
- *Chaetomium globosum* CABI-IMI 045550iii
- *Fusarium moniliforme* CABI-IMI 061274
- *Paecilomyces variotii* CABI-IMI 017457
- *Penicillium brevicompactum* CABI-IMI 114932
- *Penicillium brevicompactum* CABI-IMI 017456
- *Penicillium citrinum* CABI-IMI 061272
- *Penicillium commune* CABI-IMI 039812ii
- *Penicillium ochrochloron* CABI-IMI 061271ii
- *Penicillium ochrochloron* CABI-IMI 061271
- *Penicillium pinophilum* CABI-IMI 087160
- *Penicillium pinophilum* CABI-IMI 114933
- *Penicillium pinophilum* CABI-IMI 087160ii
- *Rhizopus stolonifer* CABI-IMI 061269
- *Scopulariopsis brevicaulis* CABI-IMI 049528

Mould proofing strain (Australia)
- *Aspergillus niger* CABI-IMI 091855
- *Aspergillus niger* CABI-IMI 091855ii
- *Pestalotiopsis gracilis* CABI-IMI 089307

Mold proofing (USA)
- *Aspergillus niger* CABI-IMI 045551
- *Aspergillus tamarii* CABI-IMI 061268
- *Trichoderma viride* CABI-IMI 045553

Mould testing resistance
- *Aspergillus niger* CABI-IMI 091855iii

Mutagenicity Testing strain MP1 ATCC42131
- *Saccharomyces cerevisiae* NCYC 1454

Nisin-estimation and differentiation in processed Cheese
- *Lactococcus lactis* subsp. *lactis* NCIMB 8586
- *Micrococcus luteus* NCIMB 8166

Ornithine (est. using ornithine decarboxylase)
- *Escherichia coli* NCIMB 8571

Panthenol (determination in pharmaceutical preps)
- *Escherichia coli* NCIMB 9624

Parenteral opthalmic preparations (Preservation testing)
- *Staphylococcus aureus* NCIMB 9518

Penicillin (in erythromycin)
- *Micrococcus luteus* NCIMB 12874

Phenol coeffecient (Staph)
- *Salmonella typhi* NCTC 160
- *Staphylococcus aureus* NCTC 3750

Plastic deterioration testing
- *Pseudomonas aeruginosa* NCIMB 10938

Polymyxin (control strain)
- *Pseudomonas aeruginosa* NCTC 10662

Pullan 4-glucanhydrolase
- *Aspergillus niger* CABI-IMI 091855iii

Quaternary ammonium compounds (Cousins)
- *Escherichia coli* NCTC 5934

Radiation sterilizers
- *Bacillus pumulis* NCTC 10327

Rideal-Walker
- *Salmonella typhi* NCTC 786

Serological typing
- *Pseudomonas aeruginosa* NCIMB 10914
- *Pseudomonas aeruginosa* NCIMB 10907
- *Pseudomonas aeruginosa* NCIMB 10895
- *Pseudomonas aeruginosa* NCIMB 10897
- *Pseudomonas aeruginosa* NCIMB 10896
- *Pseudomonas aeruginosa* NCIMB 10908
- *Pseudomonas aeruginosa* NCIMB 10913
- *Pseudomonas aeruginosa* NCIMB 10912
- *Pseudomonas aeruginosa* NCIMB 10909
- *Pseudomonas aeruginosa* NCIMB 10893
- *Pseudomonas aeruginosa* NCIMB 10899
- *Pseudomonas aeruginosa* NCIMB 10894
- *Pseudomonas aeruginosa* NCIMB 10906
- *Pseudomonas aeruginosa* NCIMB 10905
- *Pseudomonas aeruginosa* NCIMB 10904
- *Pseudomonas aeruginosa* NCIMB 10903
- *Pseudomonas aeruginosa* NCIMB 10902
- *Pseudomonas aeruginosa* NCIMB 10901
- *Pseudomonas aeruginosa* NCIMB 10900
- *Pseudomonas aeruginosa* NCIMB 10910
- *Pseudomonas aeruginosa* NCIMB 10891
- *Pseudomonas aeruginosa* NCIMB 10890
- *Pseudomonas aeruginosa* NCIMB 10911
- *Pseudomonas aeruginosa* NCIMB 10892

Sewage (tracing of)
- *Serratia marcescens* NCIMB 8869

Spoilage set (NCTC Code D)
- *Aspergillus niger* NCTC 2275
- *Clostridium sporogenes* NCTC 532
- *Lactobacillus casei* NCTC 10302
- *Pseudomonas cepacia* NCTC 10661
- *Pseudomonas fluorescens* NCTC 10038
- *Saccharomyces cerevisiae* NCTC 3178
- *Zygosaccharomyces rouxii* NCPF 3879

Sporocidal testing
- *Bacillus subtilis* NCIMB 12900
- *Clostridium sporogenes* NCIMB 10696

Steam sterilizers
- *Bacillus stearothermophilus* NCTC 10003
- *Bacillus subtilis* NCTC 10452

Steam sterilizers (Low temp. and formaldehyde)
- *Bacillus stearothermophilus* NCTC 10003
- *Clostridium sporogenes* NCTC 276

Sterilisation control (ethylene oxide)
- *Bacillus subtilis* subsp. *niger* NCIMB 8649

Sterilisation control (hot air)
- *Bacillus subtilis* subsp. *niger* NCIMB 8058

Sterilisation control (steam))
- *Bacillus stearothermophilus* NCIMB 8157
- *Bacillus stearothermophilus* NCIMB 8919

Sterilisers
- *Bacillus licheniformis* NCIMB 7224

Sterility testing
- *Bacillus subtilis* NCIMB 8054

Sterility testing
- *Candida albicans* NCYC 854
- *Candida albicans* NCYC 1363
- *Clostridium sporogenes* NCIMB 12343
- *Clostridium sporogenes* NCIMB 532
- *Micrococcus luteus* NCIMB 8553
- *Staphylococcus aureus* NCIMB 8625

Sterility testing of sulphur compounds
- *Clostridium sporogenes* NCIMB 8053

T cell rosette test
- *Escherichia coli* NCIMB 11595

Toxic shock syndrome toxin 1
- *Staphylococcus aureus* NCTC 11965
- *Staphylococcus aureus* NCTC 11963
- *Staphylococcus aureus* NCTC 11962

Tryptone bile agar QC
- *Klebsiella oxytoca* NCIMB 12819

Typhus diagnosis
- *Proteus vulgaris* NCIMB 8259
- *Proteus vulgaris* NCIMB 8261

Use dilution confirmation
- *Salmonella choleraesuis* NCTC 10789

Verotoxin VT1/VT2
- *Escherichia coli* NCTC 12079

Verotoxin VT2
- *Escherichia coli* NCTC 12080

Vinyl (for resistance to mildew)
- *Streptoverticillium reticulum* NCIMB 12463
- *Streptoverticillium reticulum* NCIMB 12857

Wall covering adhesive
- *Aspergillus niger* CABI-IMI 017454
- *Chaetomium globosum* CABI-IMI 016203
- *Penicillium pinophilum* CABI-IMI 113730
- *Penicillium rubrum* CABI-IMI 113729
- *Stachybotrys atra* CABI-IMI 082021

Water (toxicity testing of)
- *Pseudomonas putida* NCIMB 12708

Water set (NCTC Code BC)
- *Aeromonas hydrophila* NCTC 8049
- *Clostridium perfringes* NCTC 8237
- *Enterobacter aerogenes* NCTC 10006
- *Enterococcus faecalis* NCTC 775
- *Escherichia coli* NCTC 10418
- *Klebsiella aerogenes* NCTC 9528
- *Legionella pneumophila* NCTC 12821
- *Proteus rettgeri* NCTC 7475
- *Pseudomonas aeruginosa* NCTC 10662
- *Staphylococcus aureus* NCTC 6571

Wood preservatives BS1982: Part 1 of contructional
- *Amyloporia xantha* NCWRF 62G
- *Coniophora puteana* NCWRF 11E
- *Coriolus versicolor* NCWRF 28A

Wood preservatives BS1982:Part 1 of contructional
- *Gleophyllum trabeum* NCWRF 108N
- *Pleurotus ostreatus* NCWRF 40C
- *Serpula lacrymans* NCWRF 12C

Wood preservatives BS6009:1982 Additional
- *Coniophora puteana* NCWRF 11E
- *Gleophyllum abietinum* NCWRF 77C
- *Gleophyllum sepiarium* NCWRF 10E
- *Paxillus panuoides* NCWRF 8D
- *Poria placenta* NCWRF 304K
- *Serpula himantiodes* NCWRF 233C
- *Serpula lacrymans* NCWRF 12E
- *Stereum hisutum* NCWRF 3B

Wood preservatives BS6009:1982 Creosotes etc.
- *Lantinus lepideus* NCWRF 7H
- *Lentinus cyathiformis* NCWRF 153E

Wood preservatives BS6009:1982 General
- *Coniophora puteana* NCWRF 11R
- *Coriolus versicolor* NCWRF 28G

Wood preservatives BS6009:1982 Other
- *Gleophyllum trabeum* NCWRF 108N
- *Poria placenta* NCWRF 280

Assay strains including assay of antibiotics and vitamins

6-Mercaptopurine
Lactobacillus casei subsp. *rhamnosus* NCIMB 6375
Actinomycin D
Bacillus subtilis NCIMB 8054
Aflatoxin
Bacillis megaterium NCIMB 10820
a-Ketoglutaric acid
Pseudomonas fluorescens NCIMB 9520
Alanine
Lactobacillus delbrueckii NCIMB 8130
Lactobacillus fermentum NCIMB 6991
Lactobacillus fermentum NCIMB 8028
Pediococcus pentosaceus NCIMB 8968
Pediococcus pentosaceus NCIMB 7837
Pediococcus pentosaceus NCIMB 8124
Amikacin
Escherichia coli NCIMB 12210
Psaeudomonas aeruginosa NCIMB 12469
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCIMB 12703
Amino acids
Clostridium perfringes NCIMB 8875
Lactobacillus acidophilus NCIMB 8880
Lactobacillus casei subsp. *rhamnosus* NCIMB 8608
Lactobacillus casei subsp. *Rhamnosus* NCIMB 8721
Lactobacillus casei subsp. *rhamnosus* NCIMB 8722
Pedicocus acidilactici NCIMB 11432
p-Aminobenzoic acid
Clostridium acetobutylicum NCIMB 6376
Gluconobacter oxydans NCIMB 621
Gluconobacter oxydans NCIMB 7069
Gluconobacter oxydans NCIMB 8035
Lactobacillus plantarum NCIMB 8014
Lactobacillus plantarum NCIMB 8016
Lactobacillus plantarum NCIMB 8030
Neurospora crassa CABI-IMI 031288
γ Aminobutyric acid
Pseudomonas fluorescens NCIMB 9520
Aminocidin E
Staphylococcus epidermis NCIMB 8853
Amino-methyl piperazine
Micrococcus luteus NCIMB 8553
6-Aminopenicillinic acid
Serratia marcescens NCIMB 12714
Amoxycillin
Bacillus subtilis NCIMB 8054
Micrococcus luteus NCIMB 8553
Amoxycillin (Clavulanic acid/Augmentin)
Escherichia coli NCTC 11560
Escherichia coli NCTC 11954
Staphylococcus aureus NCTC 11561
Amphomycin
Micrococcus luteus NCIMB 10418
Amphotericin
Saccharomyces cerevisiae NCTC 10716
Amphotericin B
Candida albicans NCYC 1363
Saccharomyces cerevisiae NCYC 853
Amphotericin B
Saccharomyces cerevisiae NCYC 87
Ampicillin
Bacillus subtilis NCIMB 8054
Enterococcus faecium NCIMB 13280
Escherichia coli NCIMB 12210
Escherichia coli NCTC 10418
Micrococcus luteus NCIMB 8553
Pseudomonas aeruginosa NCIMB 12469
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 12702
Anisomycin
Saccharomyces cerevisiae NCTC 10716
Saccharomyces cerevisiae NCYC 87
Antibacterial agents (In urine)
Bacillus subtilis NCIMB 3610
Antibiotic
Staphylococcus areus NCIMB 11182
Antifongine
Saccharomyces cerevisiae NCYC 87
Antimicrobial preservatives
Escherichia coli NCIMB 8545
Escherichia coli NCIMB 8879
Pseudomonas auruginosa NCIMB 8626
Staphyoccoccus aureus NCIMB 9518
Arginine
Citrobacter freundii NCIMB 7020
Escherichia coli NCIMB 8876
Escherichia coli NCIMB 86
Lactobacillus plantarum NCIMB 6376
Pedicoccus pentosaecus NCIMB 8968
Pedicoccus pentosaecus NCIMB 7837
Aspartic acid
Acinetobacter NCIMB 8862
Lactobacillus casei subsp. *rhamnosus* NCIMB 8019
Lactobacillus casei subsp. *rhamnosus* NCIMB 7483
Pedicococcus acidilactici NCIMB 6990
Pedicococcus acidilactici NCIMB 8018
Aspartocin
Staphylococcus epidermidis NCIMB 8853
Auromycin (Determination in body fluids)
Bacillus cereus NCIMB 8122
Ayfin
Corneybacterium xerosis NCIMB 10087
Azathioprine
Lactobacillus casei subsp. *rhamnosus* NCIMB 8010
Lactobacillus casei subsp. *rhamnosus* NCIMB 6375
B317 -9-9a Argenine 10
Neurospora crassa CABI-IMI 147008
B46004-Argenine 1
Neurospora crassa CABI-IMI 147007
Bacitracin
Corneybacterium xerosis NCIMB 10087
Escherichia coli NCIMB 12210
Micrococccus luteus NCTC 7743
Micrococcus luteus NCIMB 10419
Micrococcus luteus NCIMB 10818
Micrococcus luteus NCIMB 8994
Bacitracin

Micrococcus luteus NCIMB 8166
Staphylococcus aureus NCIMB 12702
Staphylococcus aureus NCIMB 8888
Staphylococcus aureus NCTC 8342
Staphylococcus equiscabilis NCIMB 8190

Benzalkonium chloride
Miccrococcus luteus NCIMB 8166

Benzylpenicillin
Escherichia coli NCIMB 8666
Staphylococcus aureus NCIMB 12702

Biotin
Lactobacillus casei subsp. *rhamnosus* NCIMB 8010
Lactobacillus casei subsp. *rhamnosus* NCIMB 6375
Lactobacillus plantarum NCIMB 6376
Lactobacillus plantarum NCIMB 8014
Lactobacillus plantarum NCIMB 8030
Micrococcus leteus NCIMB 8854
Ochromonas danica CCAP 933/2b
Saccharomyces cerevis CABI-IMI 019391
Saccharomyces cerevisiae NCYC 79
Saccharomyces cerevisiae NCYC 81
Saccharomyces cerevisiae NCYC 695
Saccharomyces cerevisiae NCYC 695
Saccharomyces cerevisiae NCYC 81
Saccharomyces cerevisiae NCYC 79

B-lactamase inhibition synergy assay
Klebsiella pneumoniae NCTC 11288

Candicidin
Saccharomyces cerevisiae NCYC 87

Capreomycin
Bacillus subtilis NCIMB 8054
Bacillus subtilis NCTC 10400
Klebsiella pneumoniae subsp *pneumoniae* NCIMB 9111

Carbenicillin
Enterococcus faecalis NCIMB 13280
Escherichia coli NCIMB 12210
Pseudomonas aeruginosa NCIMB 12469
Pseudomonas aeruginosa NCIMB 10817
Pseudomonas aeruginosa NCTC 10701

Carbomycin
Micrococcus leteus NCIMB 8553
Staphylococcus saprophyticus NCTC 8340

Cedphrapirin
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 8625

Cefamandole
Klebsiella pneumoniae subsp pneumoniae NCIMB 9111
Staphylococcus aureus NCIMB 8625

Cefazolin
Bacillus subtilis NCIMB 8054
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 8625

Cefotaxime
Proteus morganii NCTC 11354

Cefoxitin
Klebsiella pneumoniae subsp *pneumoniae* NCIMB 9111
Staphylococcus aureus NCIMB 8625

Cefradroxil
Staphylococcus aureus NCIMB 8625

Cephacetrile
Staphylococcus aureus NCIMB 8625

Cephalexin
Bacillus subtilis NCIMB 8054
Escherichia coli NCIMB 12110
Micrococcus leteus NCIMB 8553
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 9518
Staphylococcus aureus NCIMB 6751

Cephalexin monohydrate
Staphylococcus aureus NCIMB 12702

Cephaloglycin
Bacillus subtilis NCIMB 8054
Escherichia coli NCIMB 12210
Klebsiella pneumoniae subsp *pneumoniae* NCIMB 9111
Micrococcus leteus NCIMB 8553
Staphylococcus aureus NCIMB 6571
Staphylococcus aureus NCIMB 9518
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 9625

Cephaloglycin dihydrate
Staphylococcus aureus NCIMB 12702

Cephaloridine
Bacillus subtilis NCIMB 8054
Escherichia coli NCIMB 12210
Klebsiella pneumoniae subsp *pneumoniae* NCIMB 9111
Micrococcus leteus NCIMB 8553
Staphylococcus aureus NCTC 6571
Staphylococcus aureus NCIMB 6571
Staphylococcus aureus NCIMB 9518
Staphylococcus aureus NCIMB 9625
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCTC 7447

Cephaloridine dihydrate
Staphylococcus aureus NCIMB 12702

Cephalosporin C
Alcaligenes faecalis NCIMB 8156

Cephalothin
Bacillus subtilis NCIMB 8054
Enterococcus faecalis NCIMB 13280
Escherichia coli NCIMB 12210
Klebsiella pneumoniae subsp *pneumoniae* NCIMB 9111
Micrococcus leteus NCIMB 8553
Pseudomonas aeruginosa NCIMB 12469
Staphylococcus aureus NCIMB 12702
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCIMB 9518
Staphylococcus aureus NCIMB 12703

Cephradine
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCIMB 12703

Cetrimide
Bacillus subtilis NCIMB 8054
Staphylococcus aureus NCIMB 6571

Chelocardin
Bacillus subtilis NCIMB 8054
Micrococcus leteus NCIMB 8553

Chloramphenicol
Enterococcus faecalis NCIMB 13280
Escherichia coli NCTC 10418
Escherichia coli NCIMB 12210
Escherichia coli NCIMB 8879
Micrococcus leteus NCIMB 8553
Micrococcus leteus NCIMB 8942

Chloramphenicol
Pseudomonas aeruginosa NCIMB 12469

Staphylococcus aureus NCIMB 12702
Staphylococcus saprophyticus NCTC 8340

Chlorohexidine diacetate
Micrococcus leteus NCIMB 8166

Chlorohexidine gluconate
Micrococcus leteus NCIMB 8166

Chlortetracycin
Bacillus cereus NCIMB 8012
Bacillus cereus NCIMB 8849
Bacillus cereus NCIMB 8122
Bacillus pumilis NCIMB 8982
Bacillus subtilis NCIMB 8533
Escherichia coli NCIMB 8879
Proteus vulgaris NCIMB 8067
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCIMB 6571

Chlortetracycline
Bacillus cereus NCTC 8035
Bacillus cereus NCTC 10320
Bacillus pumulis NCTC 8241
Bacillus subtilis NCTC 10400
Escherichia coli NCTC 10418
Staphylococcus aureus NCTC 10988
Staphylococcus aureus NCTC 7447
Staphylococcus aureus NCTC 6571

Cholesterol
O*chrobactrum anthropi* NCIMB 8688
Pseudomonas pictorum NCIMB 9152
Rhodococcus sp. NCIMB 10555
Rhodococcus sp. NCIMB 10554

Choline
Neurospora crassa CABI-IMI 019419

Clavulanic acid
Klebsiella pneumoniae NCTC 11228
Kleibsiella pnumoniae subsp. *pnumoniae* NCIMB 418

Clindamycin
Enterococcus faecalis NCIMB 13280
Escherichia coli NCIMB 12210
Micrococcus luteus NCIMB 8553
Pseudomonas aeruginosa NCIMB 12469
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 12702
Staphylococcus aureus NCIMB 8625
Staphylococcus saprophyticus NCTC 8340

Cloxacillin
Bacillus subtilis NCIMB 8054
Staphylococcus aureus NCIMB 12703

Colicin indicator strain
Escherichia coli NCTC 10850

Colimycin
Escherichia coli NCIMB 12210

Colistimethate sodium
Bordetella bronchiseptica NCIMB 9935

Colistin
Bordetella bronchiseptica NCTC 8344
Bordetella bronchiseptica NCIMB 9936
Bordetella bronchiseptica NCIMB 9935
Enterobacter faecalis NCIMB 13280
Escherichia coli NCIMB 12210
Pseudomonas aeruginosa NCIMB 12469

Colistin sulphate
Bordetella bronchiseptica NCIMB 9935

Copper Mulders strain in soils
Aspergillus niger CABI-IMI 031283

Coumermycin
Staphylococcus aureus NCIMB 8625

Cycloserine
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 8625

Cystine
Lactobacillus leichmannii NCIMB 8118

Cytosine
Lactobacillus brevis NCIMB 947
Lactobacillus brevis NCIMB 8038
Lactobacillus brevis NCIMB 947

Cytosine arabinoside
Enterococcus durans NCIMB11077

Demeclocycline
Bacillus cereus NCTC 10320

Demethylchlortetracycline
Bacillus cereus NCIMB 8849
Bordetella bronchiseptica NCIMB 9935
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 6571

Desacetyl cephalothin
Bacillus subtilis NCIMB 8054

Desertomycin
Bacillus subtilis NCIMB 8054

Diaminopimelic acid
Bacillus megaterium NCIMB 11251

Dicloxacillin
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCIMB12703

Dihydrostreptomycin
Bacillus subtilis NCIMB 8054
Bacillus subtilis NCTC 10400
Klebsiella aerogenes NCTC 7427
Klebsiella pnumoniae subsp *pneumoniae* NCIMB 9111

Dimethylchloretetracycline
Bordetella bronchiseptica NCTC 8344

DNA
Lactobacillus johnsonii NCIMB 8795

Doxycycline
Bacillus cereus NCTC 10320
Bacillus cereus NCIMB 8849
Micrococcus luteus NCIMB 8553
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 8625

Enoxacin
Neisseria gonorrhoeae NCTC 12141

Erythromycin
Bacillus pumilis NCIMB 8982
Bacillus pumulis NCTC 8241
Bacillus subtilis NCIMB 8054
Enterococcus faecalis NCIMB 13280
Escherichia coli NCIMB 12210
Klebsiella pnumoniae subsp *pneumoniae* NCIMB 9111
Micrococcus luteus NCIMB 8553
Pseudomonas aeruginosa NCIMB 12469
Staphylococcus aureus NCTC 6571
Staphylococcus aureus NCIMB 6571
Staphylococcus aureus NCIMB 12702
Staphylococcus saprophyticus NCTC 8340

Everninomicins
Staphylococcus aureus NCIMB 8625

Folic acid group

Bacillus coagulans NCIMB 8870
Enterococcus hirae NCIMB 9191
Enterococcus hirae NCIMB 8123
Enterococcus hirae NCIMB 6459
Lactobacillus casei subsp. *rhamnosus* NCIMB 8010
Lactobacillus casei subsp. *rhamnosus* NCIMB 11295
Lactobacillus casei subsp. *rhamnosus* NCIMB 10023
Lactobacillus casei subsp. *rhamnosus* NCIMB 6375
Lactobacillus casei subsp. *rhamnosus* NCIMB 10463
Pediococcus pentosaceus NCIMB 8124
Pediococcus pentosaceus NCIMB 7837
Pediococcus pentosaceus NCIMB 8968

Framycetin
Bacillus pumilis NCIMB 8982
Bacillus pumulis NCTC 8241

Fructose
Lactobacillus fructosus NCIMB 10784

Fucidin
Corneybacterium xerosis NCTC 9755
Staphylococcus aureus NCTC 6571

Fusidic acid
Corneybacterium xerosis NCIMB 10087

Gentamycin
Bacillus pumilis NCIMB 8982
Bacillus pumulis NCTC 8241
Bacillus subtilis NCIMB 8054
Enterococcus faecalis NCIMB 13280
Escherichia coli NCIMB 12210

Gentamycin
Escherichia coli NCTC 10418
Proteus mirabilis NCIMB 10823
Proteus mirabilis NCTC 10975
Pseudomonas aeruginosa NCIMB 12469
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCTC 6571
Staphylococcus aureus NCIMB 12702
Staphylococcus epidermidis NCIMB 8853

Gentamycin with other antibiotics
Klebsiella edwarsii var. *attandae* NCTC 10896

Glutamic acid
Escherichia coli NCIMB 86
Lactobacillus casei subsp. *rhamnosus* NCIMB 8019
Lactobacillus casei subsp. *rhamnosus* NCIMB 6375
Lactobacillus casei subsp. rhamnosus NCIMB 8010
Lactobacillus casei subsp. *rhamnosus* NCIMB 7473

Gramicidin
Enterococcus hirae NCIMB 8192

Halomycin
Staphylococcus aureus subsp. *aureus* NCIMB 8625

Haloprogin
Candida albicans NCYC 1363

Hexachlorophene
Bacillus subtilis NCIMB 8054

Histidine
Enterococcus hirae NCIMB 8123
Enterococcus hirae NCIMB 8191
Enterococcus hirae NCIMB 6459
Escherichia coli NCIMB 86
Lactobacillus fermentum NCIMB 8028
Lactobacillus fermentum NCIMB 6991
Pedicoccus acidilacti NCIMB 6990
Pseudomonas putida NCIMB 10807

Hydroxamate siderophore iron chelators
Arthrobacter sp. NCIMB 9471

Hygromycin B
Bacillus subtilis NCIMB 8054
Staphylococcus aureus NCIMB 6571

Inositol
Kloeckera apiculata NCYC 245
Neurospora crassa CABI-IMI 147006
Neurospora crassa CABI-IMI 017836
Saccharomyces cerevisiae NCYC 86
Saccharomyces cerevisiae NCYC 81
Saccharomyces cerevisiae NCYC 74
Saccharomyces cerevisiae NCYC 354
Schizosaccharomyces pombe NCYC 535

Kanamycin
Bacillus pumilis NCIMB 8982
Bacillus pumulis NCTC 8241
Enterococcus faecalis NCIMB 13280
Escherichia coli NCTC 10418
Escherichia coli NCIMB 12210
Pseudomonas aeruginosa NCIMB 12469
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCTC 6571
Staphylococcus aureus NCIMB 12702
Staphylococcus aureus NCIMB 8625
Staphylococcus epidermidis NCIMB 8853

Kanamycin (and B)
Bacillus subtilis NCIMB 8054

Kanamycin with others
Klebsiella edwarsii var. *attandae* NCTC 10896

Kundramycin
Bacillus subtilis NCIMB 8054

Lasalocid
Bacillus subtilis NCIMB 8054

Laundry additives
Klebsiella pneumoniae subsp. *pnumoniae* NCIMB 10341

Uracil
Lactobacillus brevis NCIMB 947

L-Cystine
Pediococcus acidilactici NCIMB 7881
Pediococcus acidilactici NCIMB 6990
Pediococcus acidilactici NCIMB 8018
Pediococcus pentosaceus NCIMB 7837
Pediococcus pentosaceus NCIMB 8124
Pediococcus pentosaceus NCIMB 8968

Leucine
Proteus mirabilis NCIMB 60
Proteus mirabilis NCIMB 8268
Proteus mirabilis NCIMB 2100
Proteus mirabilis NCIMB 5887
Proteus vulgaris NCIMB 8261
Proteus vulgaris NCIMB 4175

Leucine A
Neurospora crassa CABI-IMI 019420ii

Lincomycin
Bacillus pumulis NCTC 8241
Micrococcus luteus NCIMB 8553
Staphylococcus aureus NCTC 6571

Lipoic acid
- *Enterococcus faecalis* NCIMB 8661
- *Escherichia coli* NCIMB 10440

L-lysine
- *Pediococcus acidilactici* NCIMB 8018
- *Pediococcus acidilactici* NCIMB 6990
- *Pediococcus acidilactici* NCIMB 7881

L-Serine
- *Pediococcus acidilactici* NCIMB 6990
- *Pediococcus acidilactici* NCIMB 8018
- *Pediococcus acidilactici* NCIMB 7881

L-Threonine
- *Pediococcus acidilactici* NCIMB 6990
- *Pediococcus acidilactici* NCIMB 7881
- *Pediococcus acidilactici* NCIMB 8018
- *Pediococcus pentosaceus* NCIMB 8968
- *Pediococcus pentosaceus* NCIMB 7837
- *Pediococcus pentosaceus* NCIMB 8124

L-tyrosine
- *Pediococcus acidilactici* NCIMB 7881
- *Pediococcus acidilactici* NCIMB 6990
- *Pediococcus acidilactici* NCIMB 8018
- *Pediococcus pentosaceus* NCIMB 8968
- *Pediococcus pentosaceus* NCIMB 8124
- *Pediococcus pentosaceus* NCIMB 7837

Lymecycline
- *Bacillus pumilis* NCIMB 8982
- *Bacillus pumulis* NCTC 8241

Lysine
- *Enterococcus faecium* NCIMB 8842
- *Enterococcus hirae* NCIMB 8123
- *Enterococcus hirae* NCIMB 8191
- *Enterococcus hirae* NCIMB 6459
- *Hafnia alvei* NCIMB 6578

Magnesium in soils
- *Aspergillus niger* (Mulders strain) CABI-IMI 031283

Methacycline
- *Bacillus cereus* NCTC 10320
- *Bacillus cereus* NCIMB 8849
- *Staphylococcus aureus* NCIMB 12703
- *Staphylococcus aureus* NCIMB 8625

Methionine
- *Enterococcus hirae* NCIMB 6459
- *Enterococcus hirae* NCIMB 8123
- *Enterococcus hirae* NCIMB 9191
- *Escherichia coli* NCIMB 8877
- *Lactobacillus plantarum* NCIMB 8030
- *Lactobacillus plantarum* NCIMB 6376
- *Lactobacillus plantarum* NCIMB 8014
- *Neurospora crassa* CABI-IMI 075723
- *Pedicoccus acidilactici* NCIMB 7881
- *Pedicoccus acidilactici* NCIMB 8018
- *Pedicoccus acidilactici* NCIMB 6990
- *Pedicoccus pentosaceus* NCIMB 7837
- *Pedicoccus pentosaceus* NCIMB 8968
- *Pedicoccus pentosaceus* NCIMB 8124

Metronidazole
- *Clostridium sporogenes* NCIMB 12148

Milk (assay of inhibitory substances)
- *Bacillus stearothermophilus* NCIMB 11780

Minocycline
- *Bacillus cereus* NCIMB 8849
- *Staphylococcus aureus* NCIMB 12703
- *Staphylococcus aureus* NCIMB 8625

Mithramycin
- *Staphylococcus aureus* NCIMB 8625
- *Bacillus subtilis* NCIMB 8054

Molybidium in soils
- *Aspergillus niger* (Mulders strain) CABI-IMI 031283

Monensin
- *Bacillus subtilis* NCIMB 8054
- *Enterococcus hirae* NCIMB 8123
- *Enterococcus hirae* NCIMB 8191
- *Enterococcus hirae* NCIMB 6459
- *Staphylococcus aureus* NCIMB 6571

Mycobactin
- *Areobacterium terregens* NCIMB 8909

Nafcillin
- *Staphylococcus aureus* NCIMB 8625
- *Staphylococcus aureus* NCIMB 12703

Nalidixic acid
- *Escherichia coli* NCIMB 12210
- *Escherichia coli* NCTC 10418
- *Klebsiella oxytoca* NCIMB 10417

Napthalene
- *Comamonas terrigena* NCIMB 2582
- *Pseudomonas fluorescens* NCIMB 9815
- *Pseudomonas putida* NCIMB 8860
- *Pseudomonas putida* NCIMB 9816
- *Pseudomonas putida* NCIMB 8859
- *Pseudomonas putida* NCIMB 9427
- *Pseudomonas putida* NCIMB 8858

Napthalene Phage
- *Pseudomonas putida* NCIMB 12198
- *Pseudomonas putida* NCIMB 12199

Natamycin
- *Saccharomyces cerevisiae* NCYC 87
- *Bacillus pumulis* NCIMB 8982
- *Bacillus pumulis* NCTC 8241
- *Bacillus subtilis* NCIMB 8054
- *Escherichia coli* NCIMB 12210
- *Escherichia coli* NCIMB 8879
- *Escherichia coli* NCIMB 10072
- *Escherichia coli* NCTC 10418
- *Klebsiella pnumoniae* subsp. *pneumonia* NCIMB 9111
- *Staphylococcus aureus* NCIMB 8625
- *Staphylococcus aureus* NCTC 7447
- *Staphylococcus aureus* NCIMB 12702
- *Staphylococcus epidermidis* NCIMB 8853

Neomycin with streptomycin
- *Staphylococcus aureus* NCIMB 9968
- *Staphylococcus aureus* NCIMB 9969
- *Staphylococcus aureus* NCIMB 9970
- *Staphylococcus aureus* NCIMB 9972

Nicotinamide
- *Lactobacillus fructosus* NCIMB 10784

Nicotinic acid
- *Gluconobacter oxydans* NCIMB 7069
- *Gluconobacter oxydans* NCIMB 621
- *Gluconobacter oxydans* NCIMB 8035
- *Lactobacillus casei* subsp. *rhamnosus* NCIMB 6375
- *Lactobacillus casei* subsp. *rhamnosus* NCIMB 8010

Nicotinic acid
- *Lactobacillus plantarum* NCIMB 8014
- *Lactobacillus plantarum* NCIMB 6376
- *Lactobacillus plantarum* NCIMB 8030

Nisin

Bacillus stearothermophilus NCIMB 8224
Streptococcus agalactiae NCIMB 8778
Nitrate reductase
Escherichia coli NCTC 11633
Nitrocefin
Haemophilus influenzae NCTC 11315
Nitrofurantoin
Enterococcus faecialis NCIMB 13280
Escherichia coli NCIMB 10416
Escherichia coli NCIMB 12210
Nitrofurantoin
Pseudomonas aeruginosa NCIMB 12469
Novobiocin
Bacillus subtilis NCTC 10315
Bacillus subtilis NCIMB 8993
Escherichia coli NCIMB 12210
Micrococcus luteus NCIMB 8553
Staphylococcus aureus NCIMB 12702
Staphylococcus aureus NCTC 6571
Staphylococcus epidermidis NCIMB 8853
Nyastatin
Candida albicans NCYC 1363
Candida tropicalis NCYC 1393
Saccharomyces cerevisiae NCYC 87
Saccharomyces cerevisiae NCYC 853
Saccharomyces cerevisiae NCTC 10716
Oleandomycin
Bacillus cereus NCTC 10989
Bacillus subtilis NCIMB 8054
Escherichia coli NCIMB 12210
Micrococcus leuteus NCIMB 8553
Staphylococcus aureus NCIMB 112702
Staphylococcus epidermidis NCIMB 8853
Staphylococcus saprophyticus NCTC 8340
Opsonin
Saccharomyces cerevisiae NCYC 873
Ornithine
Clostridium septicum NCIMB 547
Orotoc acid
Lactobacillus helveticus NCIMB 10552
Oxacillin
Micrococcus leuteus NCIMB 8553
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCIMB 12703
Oxytetracycline
Bacillus cereus NCTC 10320
Bacillus pumulis NCTC 8241
Escherichia coli NCTC 10418
Staphylococcus aureus NCTC 6571
Staphylococcus aureus NCTC 10988
Staphylococcus aureus NCTC 7447
Bacillus cereus NCIMB 8122
Bacillus cereus NCIMB 8849
Bacillus cereus NCIMB 8012
Bacillus pumilis NCIMB 8982
Escherichia coli NCIMB 8879
Staphylococcus aureus NCIMB 6571
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCIMB 12703
Streptomyces albofaciens NCIMB 10975
Pantethine
Lactobacillus helveticus NCIMB 8934
Lactobacillus helveticus NCIMB 8733
Panthenol
Gluconobacter oxydans NCIMB 8036
Pediococcus acidilactici NCIMB 7881
Pediococcus acidilactici NCIMB 6990
Pediococcus acidilactici NCIMB 8018
Pantothenic acid
Gluconobacter oxydans NCIMB 621
Gluconobacter oxydans NCIMB 7069
Gluconobacter oxydans NCIMB 8035
Lactobacillus casei subsp. *rhamnosus* NCIMB 8010
Lactobacillus casei subsp. *rhamnosus* NCIMB 6375
Lactobacillus plantarum NCIMB 8014
Lactobacillus plantarum NCIMB 8030
Lactobacillus plantarum NCIMB 6376
Morganella morganii NCIMB 10466
Pediococcus acidilactici NCIMB 7881
Pediococcus acidilactici NCIMB 8018
Pediococcus acidilactici NCIMB 6990
Saccharomyces cerevisiae NCYC 81
Saccharomyces cerevisiae NCYC 74
Saccharomyces cerevisiae NCYC 354
Paramomycin
Bacillus subtillis NCIMB 8054
Paronomycin sulphate
Bacillus subtilis NCTC 10400
Patulin
Escherichia coli NCIMB 8522
Penicillin
Bacillus stearothermus NCIMB 11780
Bacillus subtilis NCIMB 8057
Bacillus subtilis NCIMB 8054
Bacillus subtilis NCTC 10400
Bacillus subtilis NCTC 8236
Bacillus subtilis NCIMB 8739
Lactobacillus helveticus NCIMB 9949
Micrococcus luteus NCIMB 9660
Micrococcus luteus NCIMB 8553
Staphylococcus aureus NCIMB 10819
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCIMB 8588
Staphylococcus aureus NCTC 6571
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 8244
Staphylococcus aureus NCTC 7447
Staphylococcus saprophyticus NCTC 8340
Penicillin (in body fluids)
Streptococcuys pyogenes NCIMB 8884
Penicillin (in feeds)
Staphylococcus aureus NCIMB 8588
Penicillin (in milk)
Streptococcus salivarius subsp. *thermophilus* NCIMB 10387
Streptococcus salivarius subsp. *thermophilus* NCIMB 8779
Penicillin F
Bacillus subtilis NCIMB 8159
Penicillin G
Enterococcus faecalis NCIMB 13280
Escherichia coli NCIMB 12210
Micrococcus luteus NCIMB 8553
Pseudomonas aeruginosa NCIMB 12469
Penicillin G
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCIMB 9571
Staphylococcus aureus NCIMB 12703
Penicillin V

Staphylococcus aureus NCIMB 8625

Penicillin X
Bacillus subtilis NCIMB 9159

Penylmercuric acetate
Micrococcus luteus NCIMB 8166
Staphylococcus aureus NCIMB 6571

Penylmercuric nitrate
Micrococcus luteus NCIMB 8166

Phenethicillin
Bacillus subtilis NCIMB 8054
Micrococcus luteus NCIMB 8553

Phenoxymethylpenicillin
Bacillus subtilis NCIMB 8739
Bacillus subtilis NCIMB 8054
Staphylococcus aureus NCIMB 8625

Phenylalanine
Enterococcus hirae NCIMB 6459
Enterococcus hirae NCIMB 8191
Enterococcus hirae NCIMB 8123
Pediococcus acidilactici NCIMB 7881
Pediococcus acidilactici NCIMB 6990
Pediococcus acidilactici NCIMB 8018
Pediococcus pentosacceus NCIMB 7837
Pediococcus pentosacceus NCIMB 8968
Pediococcus pentosacceus NCIMB 8124

Phenylketonuria sreening assay
Bacillus subtilis NCIMB 3610
Bacillus subtilis NCIMB 8054

Phosphorous in soils
Aspergillus niger CABI-IMI 094145

Plicamycin
Staphylococcus aureus NCIMB 12703

Polymyxin
Bordetella bronchiaseptica NCTC 8344
Escherichia coli NCTC 10418
Escherichia coli NCIMB 8879
Staphylococcus aureus subsp.aureus NCIMB 12702

Polymyxin (in flluids, foods and pharm.l products)
Bordetella bronchiseptica NCIMB 9935

Polymyxin B
Bordetella bronchiseptica NCIMB 9935
Escherichia coli NCIMB 12210

Potassium in soils
Aspergillus niger CABI-IMI 094145

Proline
Neurospora crassa CABI-IMI 147005
Neurospora crassa CABI-IMI 044244

Proline (L-form)
Pediococcus acidilactici NCIMB 7881
Pediococcus acidilactici NCIMB 8018
Pediococcus acidilactici NCIMB 6990

Pyridoxal
Enterococcus hirae NCIMB 8191
Enterococcus hirae NCIMB 8123
Enterococcus hirae NCIMB 6459
Escherichia coli NCIMB 10083
Lactobacillus casei subsp. *rhamnosus* NCIMB 6375
Lactobacillus casei subsp. *rhamnosus* NCIMB 8010
Lactobacillus delbrueckii NCIMB 8130

Pyridoxal
Saccharomyces cerevisiae NCYC 74
Saccharomyces cerevisiae NCYC 74
Saccharomyces cerevisiae NCYC 81
Saccharomyces cerevisiae NCYC 81

Pyridoxamine
Enterococcus hirae NCIMB 8123
Enterococcus hirae NCIMB 8191
Enterococcus hirae NCIMB 6459
Saccharomyces cerevisiae NCYC 81
Saccharomyces cerevisiae NCYC 74

Pyridoxine
Saccharomyces cerevisiae NCYC 81
Saccharomyces cerevisiae NCYC 354
Saccharomyces cerevisiae NCYC 74
Saccharomyces cerevisiae NCYC 355
Saccharomyces cerevisiae NCYC 534

Riboflavin
Enterococcus faecalis NCIMB 7432
Enterococcus faecalis NCIMB 8644
Enterococcus hirae NCIMB 8191
Enterococcus hirae NCIMB 6459
Enterococcus hirae NCIMB 8123
Lactobacillus casei subsp. *rhamnosus* NCIMB 8963
Lactobacillus casei subsp. *rhamnosus* NCIMB 8651
Lactobacillus casei subsp. *rhamnosus* NCIMB 8010
Lactobacillus casei subsp. *rhamnosus* NCIMB 6375

Rifampicin
Bacillus subtilis NCIMB 8054
Staphylococcus aureus NCTC 10702

Rifamycin
Escherichia coli NCIMB 8879
Micrococcus luteus NCIMB 8553

Ristocetin
Bacillus subtilis NCIMB 8054
Bacillus subtilis NCIMB 8577
Staphylococcus aureus NCIMB 8625

Rolietracycline
Bacillus cereus NCIMB 8849
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 8625

Serine
Enterococcus hirae NCIMB 6459
Enterococcus hirae NCIMB 8191
Enterococcus hirae NCIMB 8123
Lactobacillus casei subsp. *rhamnosus* NCIMB 7473
Lactobacillus casei subsp. *rhamnosus* NCIMB 8019

Sisomycin
Staphylococcus aureus NCIMB 8625

Soap germicides
Staphylococcus warneri NCIMB 13144

Sodium cephalothin
Staphylococcus aureus NCIMB 12702

Spectinomycin
Escherichia coli NCIMB 8879
Klebsiella pneumoniae subsp. *pneumoniae* NCIMB 9111

Streptomycin
Bacillus amylolyticus NCIMB 8144
Bacillus megaterium NCIMB 8291
Bacillus subtilis NCTC 8236

Streptomycin
Bacillus subtilis NCIMB 8739
Bacillus subtilis NCIMB 8159
Bacillus subtilis NCIMB 8057
Bacillus subtilis NCIMB 8054

Bacillus subtilis NCIMB 8533
Bacillus subtilis NCTC 10400
Bordetella bronchiseptica NCTC 9344
Escherichia coli NCIMB 8666
Escherichia coli NCIMB 12210
Escherichia coli NCTC 10418
Escherichia coli NCTC 7360
Klebsiella aerogenes NCTC 7427
Klebsiella edwardsii var. *edwardsii* NCTC 7242
Klebsiella pneumoniae subsp. *pneumoniae* NCIMB 9111
Lactobacillus helviticus NCIMB 9949
Mycobacterium tuburculosis NCTC 7416
Serratia macetescens NCIMB 8889
Staphylococcus aureus NCIMB 12702
Staphylococcus aureus NCTC 6571
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 8625
Bacillus subtilis NCIMB 8054

Streptothricin
Bacillus subtilis NCIMB 8057
Escherichia coli NCIMB 8666

Subtilin
Arthrobacter citreus NCIMB 8915

Sulphomethate
Bordetella bronchiaspetica NCIMB 9935

Sulphomyxin
Bordetella bronchiseptica NCTC 8344

Sulphonamide
Escherichia coli NCIMB 10718

Sulphoyxin
Bordetella bronchiaspetica NCIMB 9935

Tetracycline
Bacillus cereus NCTC 10320
Bacillus cereus NCIMB 8849
Bacillus cereus NCIMB 8012
Bacillus pumulis NCIMB 8982
Bacillus pumulis NCTC 8241
Bacillus subtilis NCIMB 8739
Enterococcus faecalis NCIMB 13280
Escherichia coli NCIMB 12210
Escherichia coli NCTC 10418
Escherichia coli NCIMB 8879
Micrococcus luteus NCIMB 8553
Pseudomonas aeruginosa NCIMB 12469
Staphylococcus aureus NCTC 6571
Staphylococcus aureus NCTC 10988
Staphylococcus aureus NCTC 7447
Staphylococus aureus NCIMB 12703
Staphylococus aureus NCIMB 12702
Staphylococus aureus NCIMB 6571
Staphylococus aureus NCIMB 8625

Thiamin
Kloeckera apiculata NCYC 245
Kluyveromyces marxianus NCYC 243

Thiamine
Lactobacillus fermentum NCIMB 8028
Lactobacillus fermentum NCIMB 6991
Lactobacillus fermentum NCIMB 8962
Lactobacillus fermentum NCIMB 8961
Neurospora crassa CABI-IMI 024298

Thiamine
Ochromonas danica CCAP 933/2b
Streptococcus salivarius subsp. *salivarius* NCIMB 8883
Weissella viridescens NCIMB 8965

Thimerosal
Micrococcus luteus NCIMB 8166
Staphylococcus aureus NCIMB 6571

Thiostrepton
Enterococcus hirae NCIMB 8191
Staphylococcus aureus NCIMB 8625

Threonine
Enterococcus hirae NCIMB 8191
Enterococcus hirae NCIMB 6459
Enterococcus hirae NCIMB 8123

Thymine
Enterococcus hirae NCIMB 6459
Enterococcus hirae NCIMB 8123
Enterococcus hirae NCIMB 8191
Escherichia coli NCIMB 8583
Lactobacillus paracasei subsp. *paracasei* NCIMB 4113

Ticaricillin/Clavulonic acid (Timetin)
Escherichia coli NCTC 11954

Tobramycin
Bacillus subtilis NCIMB 8739
Enterococcus faecalis NCIMB 13280
Escherichia coli NCIMB 12210
Pseudomonas aeruginosa NCIMB 12469
Staphylococcus aureus NCIMB 6571
Staphylococcus aureus NCIMB 8625
Staphylococcus aureus NCIMB 12703
Staphylococcus aureus NCIMB 12702

Tomato juice factor
Leuconostoc oenos NCIMB 11648

Trimethoprimsulfamethoxazole
Enterococcus faecalis NCIMB 12756
Enterococcus faecalis NCIMB 13280
Escherichia coli NCIMB 12210
Pseudomonas aeruginosa NCIMB 12469

Trimethoprin
Bacillus pumulis NCIMB 10822

Troleandomycin
Klebsiella pneumoniae subsp. *pneumoniae* NCIMB 9111
Staphylococcus epidermidis NCIMB 8853

Tryptophan
Enterococcus hirae NCIMB 8191
Enterococcus hirae NCIMB 8123
Enterococcus hirae NCIMB 6459
Lactobacillus plantarum NCIMB 8030
Lactobacillus plantarum NCIMB 8014
Lactobacillus plantarum NCIMB 6376
Pediococcus acidilactici NCIMB 7881
Pediococcus acidilactici NCIMB 8018
Pediococcus acidilactici NCIMB 6990

Tylosin
Micrococcus luteus NCIMB 8553
Staphylococcus aureus NCIMB 6571

Tyrosine
Enterococcus faecalis NCIMB 6783
Enterococcus hirae NCIMB 8123
Enterococcus hirae NCIMB 6459
Enterococcus hirae NCIMB 8191

Tyrothricin
Enterococcus faecalis NCIMB 8886

Tyrothricin
Enterococcus hirae NCIMB 8192

UDP-N-acetlyglucosamine
Lactobacillus brevis NCIMB 8038
Lactobacillus brevis NCIMB 947

Lactobacillus paracasei subsp. *paracasei* NCIMB 4113

Valine

Enterococcus hirae NCIMB 6459
Enterococcus hirae NCIMB 8123
Enterococcus hirae NCIMB 8191
Proteus mirabilis NCIMB 2100
Proteus mirabilis NCIMB 60
Proteus mirabilis NCIMB 5887
Proteus mirabilis NCIMB 8268
Proteus vulgaris NCIMB 8261
Proteus vulgaris NCIMB 4175

Vancomycin

Bacillus cereus NCIMB 8849
Bacillus subtilis NCIMB 8739
Bacillus subtilis NCIMB 8054
Bacillus subtilis NCTC 8236
Escherichia coli NCIMB 12210
Pseudomonas aeruginosa NCIMB 10817
Staphylococcus aureus NCIMB 12702
Staphylococcus aureus NCTC 6571
Staphylococcus epidermidis NCIMB 8853

Viomcin

Bacillus subtilis NCTC 10400
Bacillus subtilis NCTC 8236
Klebsiella aerogenes NCTC 7427

Viomycin

Bacillus subtilis NCIMB 8739
Bacillus subtilis NCIMB 8850
Bacillus subtilis NCIMB 8054
Klebsiella pneumoniae subsp. *pneumoniae* NCIMB 9111

Virginiamycin

Corynebacterium xerosis NCIMB 10087
Enterococcus hirae NCIMB 8191
Enterococcus hirae NCIMB 6459
Enterococcus hirae NCIMB 8123

Vitamin B_{12}

Arthrobacter sp. NCIMB 10821
Bacillus cereus NCIMB 8012
Bacillus cereus NCIMB 8849
Escherichia coli NCIMB 8134
Escherichia coli NCIMB 9270
Euglena gracilis CCAP 1224/5z
Lactobacillus delbrueckii subsp. *lactis* NCIMB 7278
Lactobacillus delbrueckii subsp. *lactis* NCIMB 8170
Lactobacillus delbrueckii subsp. *lactis* NCIMB 8882
Lactobacillus leichmanni NCIMB 7854
Lactobacillus leichmanni NCIMB 8117
Lactobacillus leichmanni NCIMB 8118
Lactobacillus leichmanni NCIMB 12519
Lactobacillus leichmanni NCIMB 8964

Vitamin B_6

Saccharomyces cerevisiae NCYC 74
Saccharomyces cerevisiae NCYC 86
Saccharomyces cerevisiae NCYC 74

Vitamins

Lactobacillus casei subsp. rhamnosus NCIMB 8722

Wood smoke condensate

Staphylococcus aureus NCIMB 12702

Zinc in soils

Aspergillus niger (Mulders strain) CABI-IMI 031283

Special features: anatomical and morphological

Adiaspore
- *Chrysosporium merardium* CABI-IMI 201979

Akinete (resting cell)
- *Anabaena cylindrica* CCAP 1403/30
- *Nostoc* CCAP 1453/27

Amoebostome(sucking device)
- *Naegleria galeacystis* CCAP 1506/1

Apistonema
- *Pleurochrysis carterae* CCAP961/8

Apothecium
- *Scutellinia*
- *Aleuria*

Appresoria
- *Colletotrichium coccodes* CABI-IMI 078581

Appresorium
- *Ventura inaequalis* CABI-IMI 273208

Arthoconidia
- *Crinipellis periciosa* CABI-IMI 367909

Arthrospores (Yeast teaching strain)
- *Trichosporon beigelii* NCYC 1422
- *Trichosporon beigelii* NCYC 1432

Ascospore (dark discharge)
- *Daldinia concentrica* CABI-IMI 193348

Ascospore (enanescent ascii)
- *Ceratocystis* CABI-IMI 159574

Ascospore (perithecial)
- *Sordaria fIMI cola* CABI-IMI 146886

Ascospores
- *Aspergillus alliaceus* CABI-IMI 051982

Ascospores (conjugation) (Yeast)
- *Zygosaccharomyces bailii* NCYC 1427

Ascospores (hat shaped) (Yeast teaching strain)
- *Pichia angusta* NCYC 1456

Ascospores (reniform) (Yeast teaching strain)
- *Kluyveromyces lodderae* NCYC 1417

Ascospores (round) (Yeast teaching strain)
- *Saccharomyces cerevisiae* NCYC 78
- *Saccharomyces cerevisiae* NCYC 1370
- *Saccharomyces cerevisiae* NCYC 609

Ascospores (saturn shaped) Yeast (Teaching strain)
- *Williopsis saturnus* NCYC 23

Ascospores (saturn shaped) (Yeast teaching strain)
- *Williopsis saturnus* NCYC 22

Ballistospore
- *Sporobolomyces coprosmae* NCYC 2627
- *Itersonilia perplexans* CABI-IMI 264396

Ballistospores (yeast teaching strain)
- *Bullera alba* NCYC 425
- *Sporidiobolus pararoseus* NCYC 1428

Beige spored mating type A
- *Sordaria brevicollis* CABI-IMI 210764

Black yeasts
- *Aureobasidium pullans* CABI-IMI 269216

Chlamydospores (teaching)
- *Fusarium oxysporum* CABI-IMI 244439
- *Phoma glomerata* CABI-IMI 327750
- *Candida albicans* NCYC 1472
- *Phoma jolgana* CABI-IMI 361393

Cilliate protozoa (e.g.)
- *Tetrahymena thermophila* CCAP 1630/1p
- *Tetrahymena thermophila* CCAP1630/1q

Clamps
- *Crinipellis periciosa* CABI-IMI 367909*
- *Serpula lacrymans* NCWRF 12C

Endospore formation
- *Bacillus firmus* NCIMB 9366
- *Geotrichum capitatum* NCYC 473

Filamentous and heterocysts
- *Anabaena cylindrica* CCAP 1403/2B

Flagella forming Amoeba
- *Schizopyrenidia*
- *Hisyomonas*

Gas vacuoles
- *Anabaena flos-aquae* CCAP 1403/13F

Gliding bacteria
- *Saprospira grandis* NCIMB 1363
- *Myxococcus xanthus* NCIMB 9412

Myxospore
- *Myxococcus xanthus* NCIMB 9412

Mycrocysts
- *Myxococcus xanthus* NCIMB 9412

Helical filaments (mutant)
- *Anabaena flos-aquae* CCAP 1403/13e

Macrconidia
- *Fusarium oxysporum* CABI-IMI 244439

Macroconidia (5 septate)
- *Nectridiscophora* sp. CABI-IMI 370949

Microconida
- *Fusarium oxysporum* CABI-IMI 244439

Micoconidia (aseptate)
- *Periconiella sapienfumicola* CABI-IMI 380798

Mitichondrial plasmid containing
- *Neurospora crassa* CABI-IMI 317528
- *Neurospora intermedia* CABI-IMI 317527

Mitochondria present amoeba
- *Saccamoeba stagnicola* CCAP 1572/1

Monster and twin mutant
- *Chlamydomonas moeswusii* CCAP 11/16d

Monster mutant
- *Chlamydomonas moeswusii* CCAP 11/16b

Motionless mutant
- *Chlamydomonas moeswusii* CCAP 11/16a

Non-gas vacuolate mutant when exposed to UV light
- *Microcystis* sp. CCAP 1450/14

Non-sporulating in glass bottles
- *Aspergillus spathulatus* CABI-IMI 308593

Non-vacuolate
- *Anabaena* sp. CCAP 1403/13a

Ogival cells - Teaching strain
- Dekkera anomala NCYC 2

Oval cells - Teaching strain
- *Candida krusei* NCYC 1398

Palmelloid tendency
- *Chlamydomonas asymmetrica* CCAP 11/7

Pennate diatom Gliding motility
- *Amphora coffeaeformis* CCAP1001/1

Pycnidia production on peas
- *Sphaeropsis sapinea* CABI-IMI 057821

Rhapidosomes
- *Saprospira grandis* NCIMB 1414

Rhapidosomes
Saprospira grandis NCIMB 1409
Saprospira grandis NCIMB 1410
Saprospira grandis NCIMB 1447
Round cells (yeast teaching strain)
Debaryomyces hansenii NCYC 459
Smooth form without spicules
Trichosphaerium sieboldi CCAP 1585/2
Spore production
Anabaena variabilis CCAP 1403/12
Sporulates in culture
Cryptomonas sp. CCAP 979/63
Sporulation
Cochromonas tuberculata CCAP 933/2b
Strand formation (basidiomycete)
Serpula lacrymans NCWRF 12C
Streptomycete morphology and pigmentation
Streptomyces viridochromogenes
NCIMB 10168
Teliospores (yeast teaching strain)
Rhodosporodim toruloides NCYC 15776
True mycelium (yeast teaching strain)
Hyphopichia burtonii NCYC 1423
True mycelium (yeast teaching strain)
Trichosporon beigelii NCYC 1432
Triangular cells (yeast teaching strain) NCYC 378

Special features: physiological

Including nutrient requiring strains, fixators, teaching strains and strains exhibiting specific Physiological processess, and mutant strains.

L-threonine (utilisation)
Arthrobacter globiformis NCIMB 9759
Adenine
Neurospora crassa CABI-IMI 075724
Adjuvant effects
Gordona rubropertinctus NCIMB 9433
Aerotaxis
Esherichia coli NCIMB
Agar liquefaction
Cytophaga sp. NCIMB 1993
Alteromonas sp. NCIMB 2062
Albino strain
Neurospora crassa CABI-IMI 147004
Amixes (deviant sexual process)
Podospora arizonensis
Amoboidophagous
Arthrobotrys pyriformis CABI-IMI 056803
Antibacterial activity
Streptomyces albidoflavus NCIMB12787
Streptomyces albidoflavus NCIMB 12787
Antibiotic properties
Alteromonas sp. NCIMB 1892
Alteromonas citrea NCIMB 1889
Alteromonas rubea NCIMB 1890
Alteromonas aurantia NCIMB 2033
Antifungal activity
Streptomyces albidoflavus NCIMB 12787
Antimicrobial activity
Alteromonas luteoviolacaea NCIMB 2035
Alteromonas piscicida NCIMB 1938
Alteromonas luteoviolacaea NCIMB 1942
Armillaria mellea (inhibits)
Ascomycotina sp. CABI-IMI 381608*
Autoclaved wool (attacks)
Trichoderma longibrachiatum CABI-IMI 045548
Autotrophic mutant
Verticillium lamellicola CABI-IMI 093439
Acremonium fusidioides CABI-IMI 087758
b-Ketoglycoside (studies of)
Agrobacter radiobacter NCIMB 9162
Agrobacter radiobacter NCIMB 9161
Bacterial Adhesion (study of)
Pseudomonas sp. NCIMB 2021
Bacteriophage host: phages T1-T7
Escherichia coli NCTC 10537
Biotin biosynthesis (study of)
Bacillus sphaericus NCIMB 11935
Bunker oil odour
Microcystis aeruginosa CCAP 1450/8
Catabolite repression (insensitive to)
Escherichia coli NCIMB 10000
Escherichia coli NCIMB 9999
Cellulose Membranes (preparation of)
Acetobacter hansenii NCIMB 8246
Acetobacter hansenii NCIMB 1375
Chemotype 1
Penicillium aurantiogriseum CABI-IMI 293186
Cinnamon coloured mutant
Aspergillus cinnamomeus CABI-IMI 016148
Clumping factor: staphylococc (positive)
Staphylococcus aureus NCTC 10344
Clumping factor: staphylococci negative
Staphylococcus aureus NCTC 10345
Coccolithrophorid
Pleurochrysis carterae CCAP 961/8
Colonuial mutants
Escherichia coli NCIMB 11595
Colony forming
Microcystis aeruginosa CCAP 1450/11
Microcystis aeruginosa CCAP 1450/15
Colourless strain
Humicola hyalothermophila CABI-IMI 204250
Copiotroph
Staphylococcus aureus NCIMB 12795
Cryptic prophage
Escherichia coli NCIMB 11910
Cyanobacteria (antIMI crobial effect on)
Cellvibrio mixtus subsp. *mixtus* NCIMB 8634
Cyclohexane carboxylic acid (metabolic studies)
Acinetobacter sp NCIMB 10487
Cysteine requiring
Acremonium fusidioides CABI-IMI 087758
Cytochromes (study of)
Alcaligenes xylosoxidians subsp. *Xylosoxidians* NCIMB 11015
Paracoccus sp. NCIMB 8669
Dessication (resistant)
Aspergillus repens CABI-IMI 298307
DNA (hybridisation reference strain)
Proteus myxofaciens NCIMB 13273
DNA (Inhibition of synthesis)
Escherichia coli NCIMB 10430
DNA (recombinant research)
Escherichia coli NCIMB 12711
Escherichia coli NCIMB 11788
Dormancy studies
Micrococcus luteus NCIMB 13267
Endophyte
Diploidia salicorniar CABI-IMI 295925
Energy and Nutrient starvation (adaptive study of mechanism)
Vibrio sp.NCIMB 2290
Fission yeast (teaching strain)
Schizosaccharomyces pombe NCYC 132
Food for protozoans
Oscillatoria amoena CCAP 1459/39

Fungistatic (on Heterobasidion annosum)
Trichoderma harzianum CABI-IMI 206040
Trichoderma polysporum CABI-IMI 206039
Haemagluttinating properies
Renibacterium salmoninarum NCIMB 2235
Haploid isolate stable
Penicillium viridicatum CABI-IMI 039758ii
High Galactosamine content
Aspergillus niger CABI-IMI 146891
High salinity of wild sample 33 ppt
Prorocentrum minimum CCAP1136/10
Homothallic
Pythium splendens CABI-IMI 323145
Pythium splendens CABI-IMI 323148
Rhizomucor pusillus CABI-IMI 226166
Rhizomucor miehei CABI-IMI 089882
Rhizomucor pusillus CABI-IMI 226172
Hydrogen (chemolithotrophic growth with)
Xanthobacter autotrophicus NCIMB 10809
Xanthobacter autotrophicus NCIMB 10811
Ancylobacter sp. NCIMB 12000
Xanthobacter flavus NCIMB 10071
Xanthobacter autotrophicus NCIMB 12468
Hydrophobic properties
Renibacterium salmoninarum NCIMB 2235
Hygrophobic
Aspergillus fumigatus CABI-IMI 051984
Aspergillus glaucus CABI-IMI 053242
Hypoxanthine
Neurospora crassa CABI-IMI 075724
Immunological adjuvant
Corynebacterium rubrum NCTC 10391
Indole (study of biosynthesis)
Escherichia coli NCIMB 10200
Iron pyrite removal from coal
Thiobacillus ferrooxidans 9490 NCIMB 9490
Isolated from casein hydrolysate
Mucor racemosus CABI-IMI 114891
Jute retting
Bacillus sp. NCIMB 11043
Bacillus sp. NCIMB 11044
Bacillus sp. NCIMB 11048
Bacillus laterosporus NCIMB 11046
Bacillus sp. NCIMB 11041
Bacillus laterosporus NCIMB 11047
Bacillus sp. NCIMB 11042
Bacillus sp. NCIMB 11049
K antigens, study of
Escherichia coli NCIMB 8797
Keratinolytic
Chrysosporium tropicum CABI-IMI 094288
Koch's postulates
Listonella anguillarum NCIMB 6
Listonella anguillarum NCIMB 829
L-Histidine (study of degenerative pathway)
Comamonas testosteroni NCIMB 10808
L-Threonine (low activities of enzymes acting on)
Rhizobiaceae sp. NCIMB 11104
Lactose in dairy products-hydrolysis of
Bacillus stearothermus NCIMB 11413
Bacillus stearothermus NCIMB 11410
Bacillus stearothermus NCIMB 11409
Bacillus stearothermus NCIMB 11407
Bacillus stearothermus NCIMB 11411
Lipid studies
Kitasatoa diplospora NCIMB 11312
Streptomyces purpureus NCIMB 11311
Kitasatoa nagasakiensis NCIMB 11314
Kitasatoa antimyceticus NCIMB 11315
Kitasatoa kauaiensis NCIMB 11313
Low pH (tolerant)
Chloerella saccharophila var. *ellipsoidea* CCAP 211/1d
Luminescent bacteria
Photobacterium phosphoreum NCIMB 62
Photobacterium phosphoreum NCIMB 1275
Photobacterium phosphoreum NCIMB 1279
Photobacterium mandapamensis NCIMB 1198
Vibrio splendidus NCIMB 1
Vibrio harveyi NCIMB 2032
Photobacterium leiognathi NCIMB 2193
Photobacterium leiognathi NCIMB 1511
Vibrio harveyi NCIMB 1280
Vibrio harveyi NCIMB 393
Photobacterium mandapamensis NCIMB 841
Photobacterium phosphoreum NCIMB 65
Photobacterium phosphoreum NCIMB 64
Shewanella hanedai NCIMB 2157
Vibrio fischeri NCIMB 1281
Vibrio albensis NCIMB 41
Photobacterium phosphoreum NCIMB 1282
Vibrio fischeri NCIMB 1544
Lysogenic induction
Escherichia coli NCIMB 11976
Escherichia coli NCIMB 11977
Magnetotactic
Magnetospirillum magnetotacticum NCIMB 12542
Marine adapts to fresh water media
Acanthamoeba griffini CCAP 1501/4
Methionine requiring
Acremonium fusidioides CABI-IMI 087758
Microcopiotroph
Vibrio sp. NCIMB 12794
Vibrio sp. NCIMB 12794
Monokaryon culture (SS)
Ganoderma sp. CABI-IMI 380170*
Ganoderma sp. CABI-IMI 380171*
Ganoderma sp. CABI-IMI 380172*
Ganoderma sp. CABI-IMI 380173*
Ganoderma sp. CABI-IMI 380174*
Ganoderma sp. CABI-IMI 380175*
Morphological mutant
Fusarium coccineum CABI-IMI 087759

Mucilage with growth rings on agar
Chlamydomonas asymmetrica CCAP 11/41
Nematophagous
Arthrobotrys amerospora CABI-IMI 232238
Niacin requiring
Neurospora crassa CABI-IMI 075727ii
Nicotinamide (study of metabolism)
Pseudomonas putida NCIMB 10521
Nicotinic acid requiring
Neurospora crassa CABI-IMI 075726ii
Non aggressive waxy strain from *Ulmus* sp
Ceratocystis ulmi CABI-IMI 177907
Non-cyclopiazonic acid producer
Aspergillus oryzae CABI-IMI 309454
Non-nitrate utiliser
Trichoderma reesei CABI-IMI 192654
Non-pigmented
Blepharisma japonicum CCAP1607/4
Nutritional and structural studies
Alteromonas haloplanktis NCIMB 19
Oleic acid requiring mutant
Saccharomyces cerevisiae NCYC 812
Oligotroph
Agromonas oligotrophica NCIMB 12152
Agromonas oligotrophica NCIMB 12151
Pseudomonas fluorescens NCIMB 12797
Pseudomonas sp. NCIMB 12796
Paralysed sex mutant
Chlamydomonas moeswusii CCAP 11/16h
Pentose utilisation pathway (study of)
Klebsiella oxytoca NCIMB 9901
PEP carboxylase (lacks activity)
Escherichia coli NCIMB 11218
Pep synthase(lacks activity)
Escherichia coli NCIMB 11218
Perseulose preparation
Gluconobacter oxydans NCIMB 7069
Gluconobacter oxydans NCIMB 621
Gluconobacter oxydans NCIMB 8035
Phenazine (study of)
Pseudomonas phenazinium NCIMB 11724
Phenazine (study of)
Pseudomonas phenazinium NCIMB 11027
Phosphoglycerate Kinase mutantBC3
Saccharomyces cerevisiae NCYC 2266
Photosynthesis (study of)
Chromatium vinosum NCIMB 10441
Phytophthora (antagonist)
Penicillium expansum CABI-IMI 028619
Polyamine (study of degredation)
Pseudomonas sp. NCIMB 10889
Potential challenge organism
Scopulariopsis brevicaulis CABI-IMI 321976
Produces teleomorph crossed with IMI 43707 or 288002
Cochliobolus tuberculatus CABI-IMI 278848
Produces teleomorph 288003 crossed with IMI 43707
Cochliobolus tuberculatus CABI-IMI 286597
Produces teleomorph 288004 when crossed with IMI 43707
Cochliobolus tuberculatus CABI-IMI 287394
Protein (assay of values)
Enterococcus faecalis NCIMB 9695
Protein (identificaion from plasmids/plasmid insert)
Escherichia coli NCIMB 11867
Protein (used in production of labelled)
Coryneform sp. NCIMB 9216
Protein A (A-layer) deficient
Aeromonas salmonicida subsp. *salmonicida* NCIMB 13076
Proteolytic
Pseudomonas fluorescens NCIMB 1953
Protoplast preparation
Bacillus megaterium NCIMB 9521
Protoplast preparation
Bacillus megaterium NCIMB 8291
Protoplasts of Candida albicans
Streptomyces violaceus NCIMB 11194
Pyridoxal-dependent streptococcus
Streptococcus sp. NCTC 11664
Pyrogen (preparation of)
Pseudomonas aeruginosa NCIMB 8297
Pseudomonas aeruginosa NCIMB 8298
Quaternary ammonium compounds (evaluation of)
Escherichia coli NCIMB 9132
Radiation (study of damage)
Escherichia coli NCIMB 9485
Salt requiring strains
Desulfovibrio desulfuricans subsp. *desulfuricans* NCIMB 8393
Desulfovibrio salexigenes NCIMB 8329
Desulfovibrio desulfuricans subsp. *desulfuricans* NCIMB 8310
Desulfovibrio desulfuricans subsp. desulfuricans NCIMB 8318
Desulfovibrio desulfuricans subsp. *desulfuricans* NCIMB 8338
Desulfovibrio desulfuricans subsp. desulfuricans NCIMB 8374
Desulfovibrio salexigenes NCIMB 8365
Desulfovibrio desulfuricans subsp. *desulfuricans* NCIMB 8400
Desulfovibrio salexigenes NCIMB 8308
Desulfovibrio salexigenes NCIMB 8315
Desulfovibrio salexigenes NCIMB 8364
Desulfovibrio africanus NCIMB 8397
Desulfovibrio salexigenes NCIMB 8403
Desulfovibrio vulgaris subsp. *vulgaris* NCIMB 8399
Desulfovibrio desulfuricans subsp. *desulfuricans* NCIMB 8314
Desulfovibrio desulfuricans subsp. desulfuricans NCIMB 8368
Scotochromogenic
Mycobacterium poriferae NCIMB 12538

Slow growing with deformed plastid mutant
Chlamydomonas moeswusii CCAP 11/16e
Soil analyis
Cunninghamella blakesleena CABI-IMI 063877
Study of denitrification
Alcaligenes xylosoxidans NCIMB 11015
Sucrose plasmid ECO encoded p WR23
NCTC50115
Sucrose plasmid ECO encoded MIP233
NCTC 50123
Tape ketan production
Amylomycs rouxii CABI-IMI 283878
Tetrasporophyte of C.moniliforme
Chromastrum humile CCAP 1365/3
Toxic strain
Aspergillus versicolor CABI-IMI 173208
Toxic to termites
Aspergillus flavus CABI-IMI 102171
Tryptophan requiring
Neurospora crassa CABI-IMI 075727ii
Tyrosine (enzyme studies)
Escherichia coli NCIMB 10616
Virulance factors (research into)
Aeromonas salmonicida subsp. *salmonicida* NCIMB 13076
Aeromonas salmonicida subsp. *salmonicida* NCIMB 13077
Virus infected
Penicillium chrysogenum CABI-IMI 026211
Penicillium brevicompactum CABI-IMI 143520
Virus infected
Penicillium brevicompactum CABI-IMI 039824
Aspergillus niger CABI-IMI 146891
Virus infected
*Penicillium chrysogenum*CABI-IMI 026210
Aspergillus niger CABI-IMI 050566
Acremonium chrysogenium CABI-IMI 049137
*Aspergillus foetidus*CABI-IMI 104688
Weil-Felix antibiotic reaction
Proteus vulgaris NCIMB 7052
Proteus vulgaris NCIMB 67
White mutant
Aspergillus sp. CABI-IMI 378801*
White spores
Neurospora crassa CABI-IMI 142820
Wild strain
Penicillium funiculosum CABI-IMI 134755
Wild-type
Saccharomyces cerevisiae NCYC 956
Saccharomyces cerevisiae NCYC 957
Saccharomyces cerevisiae NCYC 857
Saccharomyces cerevisiae NCYC 1357
Saccharomyces cerevisiae NCYC 857
Saccharomyces cerevisiae NCYC 1356
Wild-type A
Neurospora crassa CABI-IMI 053240
Neurospora crassa CABI-IMI 053239ii
Neurospora crassa CABI-IMI 053238ii
Neurospora crassa CABI-IMI 147002

Mating strains

Achyla bisexualis CABI-IMI 141473
Female mates with CABI-IMI 141474
Achyla bisexualis CABI-IMI 141474
Male mates with CABI-IMI 141473
*Arthroderma multifidium*CABI-IMI 094206
Mates with CABI-IMI 094205
*Arthroderma multifidium*CABI-IMI 094205
Mates with CABI-IMI 094206
*Arthroderma cuniculi*CABI-IMI 096245
Mates with CABI-IMI 096244
*Arthroderma tuberculatum*CABI-IMI 086177
Mates with CABI-IMI 086178
*Arthroderma tuberculatum*CABI-IMI 086178
Mates with CABI-IMI 086177
*Arthroderma cuniculi*CABI-IMI 096244
Mates with CABI-IMI 096245
*Aspergillus heterothallicus*CABI-IMI 139278
Mating type A
*Aspergillus heterothallicus*CABI-IMI 139277
Mating type A
Blakeslea trispora CABI-IMI 179034
Mates(+) with CABI-IMI 179035
Blakeslea trispora CABI-IMI 179035
Mating strain (-)Mates with CABI-IMI 179034
*Cochliobolus tuberculatus*CABI-IMI 287394
Produces teleomorph CABI-IMI 288004 when crossed with IMI 43707
*Cochliobolus ativus*CABI-IMI 166173
Mating type A
*Cochliobolus ativus*CABI-IMI 166172
Mating type A
*Cochliobolus hawaiiensis*CABI-IMI 208337
Mates with CABI-IMI 208336
*Cochliobolus hawaiiensis*CABI-IMI 208336
Mates with CABI-IMI 208337
Coprinus cinereus CABI-IMI 140506
Type A5B6
Coprinus cinereus CABI-IMI 140505
Type A5B5
Crinella muscae CABI-IMI 224150
Mating strain (+)
Crinella muscae CABI-IMI 224149
Mating strain (-)
Crinella umbellata CABI-IMI 325631
Female mating strain
Cunninghamella blakesleena CABI-IMI 053586
(-)Mates with CABI-IMI 053585
Cunninghamella blakesleena CABI-IMI 053585
(+)Mates with CABI-IMI 053586
Cunninghamella elegans CABI-IMI 021198
(+)Mates with CABI-IMI 021199
*Cunninghamella elegans*CABI-IMI 021199
(-)Mates with CABI-IMI 021198
Dichotomocladium elegans CABI-IMI 211272
(-)Mates with CABI-IMI 211271
Dichotomocladium elegans CABI-IMI 211271
(+)Mates with CABI-IMI 211272
Dichotomocladium robustum CABI-IMI 211273
(+)Mates with CABI-IMI 211274
Dichotomocladium robustum CABI-IMI 211274
(-)Mates with CABI-IMI 211273
Fusarium solani f.sp. *cucurbitae* CABI-IMI 325431
Hermaphrodyte(+)
Fusarium solani f.sp. *cucurbitae* CABI-IMI 325432
Hermaphrodyte(-)
Fusarium solani f.sp. *cucurbitae* CABI-IMI 325434
Hermaphrodyte(+)
Fusarium solani f.sp. *cucurbitae* CABI-IMI 325433
Female(+)
Gongronella butleri CABI-IMI 038498
Mates with CABI-IMI 38499
Gongronella butleri CABI-IMI 038499
Mates with CABI-IMI 38498
Mucor circinelloides CABI-IMI 039478
Mates with CABI-IMI (-)25330
Mucor circinelloides CABI-IMI 025330
(+)Mates with CABI-IMI 39748
Mucor hiemalis CABI-IMI 021216
(+)Mates with CABI-IMI 21217
Mucor hiemalis CABI-IMI 138261
(+)Mates with CABI-IMI 138262
Mucor hiemalis CABI-IMI 089291
(+)Mates with CABI-IMI 089292
Mucor hiemalis f. *corticola* CABI-IMI 129976
Mating strain (-) strain
Mucor hiemalis f. *corticola* CABI-IMI 192541
(+) strain Mates with CABI-IMI 138261
Mucor hiemalis CABI-IMI 021217
(-)Mates with CABI-IMI 21216
Mucor hiemalis CABI-IMI 089292
(-)Mates with CABI-IMI 089291
Mucor hiemalis CABI-IMI 138262
(-)Mates with CABI-IMI 138261
Mucor mucedo CABI-IMI 133298
(-) strain Mates with CABI-IMI 133299 and 78407
Mucor mucedo CABI-IMI 078407
(+) strain Mates with CABI-IMI 133298
Mucor mucedo CABI-IMI 133299
(+) strain Mates with CABI-IMI 133298
Nectria fuckeliana CABI-IMI 277828
Mates with CABI-IMI 27827
Nectria penicillioides CABI-IMI 063372
Mates with CABI-IMI 063372ii
Neurospora crassa CABI-IMI 075721
Mates with CABI-IMI 075722
Neurospora crassa CABI-IMI 142820
Mates with CABI-IMI 142819
Neurospora sitophila CABI-IMI 063919
Mates with CABI-IMI 063920
Oedogonium cardiacum CCAP 575/1A
(+) strain
Oedogonium cardiacum CCAP 575/1B
(-) strain
Paramecium bursaria CCAP 1660/11
Mating strain with CCAP 1660/10
Paramecium bursaria CCAP 1660/10
Mating strain with CCAP 1660/11
Parasitella parasitica CABI-IMI 320579
Mating strain (-)
Parasitella parasitica CABI-IMI 041058(+)
Mates with CABI-IMI 014057(-)
Parasitella parasitica CABI-IMI 014058ii
Mating strain(-)
*Phycomyces blakesleeanus*CABI-IMI 118796(-)
Mates with CABI-IMI 118497(+)

Phycomyces blakesleeanus CABI-IMI 200162
Mating strain(+)
Phycomyces blakesleeanus CABI-IMI 200163
Mating strain(+)
*Phycomyces blakesleeanus*CABI-IMI 200164
Mating strain(+)
Phycomyces blakesleeanus CABI-IMI 200165
Mating strain(+)
Phycomyces nitens CABI-IMI 063923
Mating strain(+)
Phycomyces nitens CABI-IMI 044751
Mating strain(-)
Phycomyces nitens CABI-IMI 051077
Mating strain(+)
Phytophora meadii CABI-IMI 325862
Mating strain A1
Phytophthora cryptogea CABI-IMI 325908
Mating strain A2
Phytophthora cryptogea CABI-IMI 325907
Mating strain A1
Phytophthora botrysa CABI-IMI 136915
Mating strain(-)
Phytophthora botrysa CABI-IMI 136916
Compatibility(+) type A2
Phytophthora cambivora CABI-IMI 162672
Compatibility type A2
Phytophthora cambivora CABI-IMI 059156
Compatibility type A1
Phytophthora cambivora CABI-IMI 040505
Compatibility type A2
Phytophthora cambivora CABI-IMI 223987
Compatibility type A2
Phytophthora cambivora CABI-IMI 162671
Compatibility type A2
Phytophthora cinnamomi CABI-IMI 022938
Mates with CABI-IMI 21278
Phytophthora cinnamomi CABI-IMI 158786
Mating strain A1
Phytophthora cinnamomi CABI-IMI 136395
Mating strain A2
Phytophthora cinnamomi CABI-IMI 230381
Mating strain A1
Phytophthora cinnamomi CABI-IMI 283248
Mating strain A1
*Phytophthora cinnamomi*CABI-IMI 077377
Mating strain A2
Phytophthora cinnamomi CABI-IMI 077375
Mating strain A2
Phytophthora nicotianae CABI-IMI 035087
(+)Mates with CABI-IMI 22176
Phytophthora nicotianae CABI-IMI 329726
Compatibility A1
Phytophthora nicotianae CABI-IMI 136411
Mates with CABI-IMI 35087
Phytophthora nicotianae CABI-IMI 022176
(-)Mates with CABI-IMI 35087
Phytophthora nicotianae CABI-IMI 325902
Mating strain A2
Pilaria anomala CABI-IMI 228216
(+)Mates with CABI-IMI 228217
Pilaria anomala CABI-IMI 228217
(-)Mates with 228216
*Pirella circinans*CABI-IMI 085609
Mates with CABI-IMI 085608
Pirella circinans CABI-IMI 085608
Mates with CABI-IMI 085609
Poitrasia circinans CABI-IMI 078521ii
Mates with CABI-IMI 078522ii
Protomyces macrosporus CABI-IMI 102385
(-)Mates with CABI-IMI 102384
*Protomyces macrosporus*CABI-IMI 115296(-)
Mates with CABI-IMI 115295(+)
*Protomyces macrosporus*CABI-IMI 102384
(+)Mates with CABI-IMI 102385
Pythium flevoense CABI-IMI 323128
Female
Pythium flevoense CABI-IMI 323126
Female
Pythium flevoense CABI-IMI 323127
Female
Pythium flevoense CABI-IMI 176046
Female
Pythium flevoense CABI-IMI 176045
Male
Pythium flevoense CABI-IMI 176044
Male
Pythium intermedium CABI-IMI 323126
Mating strain(+)
Pythium intermedium CABI-IMI 323124
Mating strain(+)
Pythium intermedium CABI-IMI 308149
Mating strain(+)
Pythium macrosporum CABI-IMI 323141
Mating strain(+)
Pythium macrosporum CABI-IMI 323139
Mating strain(+)
Pythium splendens CABI-IMI 323150
Mating strain(+)
Pythium splendens CABI-IMI 323149
Mating strain(+)
Pythium intermedium CABI-IMI 323134
Mating strain(-)
Pythium intermedium CABI-IMI 323132
Mating strain(-)
Pythium intermedium CABI-IMI 323131
Mating strain(-)
Pythium intermedium CABI-IMI 323130
Mating strain(-)
Pythium intermedium CABI-IMI 308150
Mating strain(-)
Pythium intermedium CABI-IMI 323133
Mating strain(-)
Pythium macrosporum CABI-IMI 323140
Mating strain(-)
*Pythium macrosporum*CABI-IMI 323144
Mating strain(-)
*Pythium macrosporum*CABI-IMI 323142
Mating strain(-)
Pythium splendens CABI-IMI 323147
Mating strain(-)
Pythium splendens CABI-IMI 323349
Mating strain(-)
Pythium splendens CABI-IMI 323350
Mating strain(-)
Pythium splendens CABI-IMI 323146
Mating strain(-)
Pythium macrosporum CABI-IMI 323143
Mating strain
Pythium sylvatcum CABI-IMI 323156
Male strain
Pythium sylvatcum CABI-IMI 323151
Male strain

Pythium sylvatcum CABI-IMI 308298
Mates with CABI-IMI 308299
Pythium sylvatcum CABI-IMI 248395
Mates with CABI-IMI 248394
Pythium sylvatcum CABI-IMI 248394
Mates with CABI-IMI 248395
Pythium sylvatcum CABI-IMI 323153
Female strain
Pythium sylvatcum CABI-IMI 323155
Female strain
Pythium sylvatcum CABI-IMI 323154
Female strain
Pythium sylvatcum CABI-IMI 323352
Male strain
Pythium sylvatcum CABI-IMI 323152
Male strain
Pythium sylvatcum CABI-IMI 323353
Female strain
Renispora flavissima CABI-IMI 241795
Mating strain
Renispora flavissima CABI-IMI 241796
Mating strain
*Thamnostylum nigricans*CABI-IMI 208234
Mates with CABI-IMI 208235
Thamnostylum nigricans CABI-IMI 208235
Mates with CABI-IMI 208234
Tapesia acuformis CABI-IMI 369188
MAT1 mating type
Tapesia acuformis CABI-IMI 369189
MAT2 Mating type
Tetrahymena americanis CCAP 1630/7A
Mating type IV
Tetrahymena americanis CCAP 1630/7B
Mating type VIII
Tetrahymena borealis CCAP 1630/JA
Mating type II
Tetrahymena borealis CCAP 1630/5B
Mating type VI
Tetrahymena canadensis CCAP 1630/6B
Mating type III
Tetrahymena capricornis CCAP 1630/13A
Mating type I
Tetrahymena hyperangularis CCAP 1630/9A
Mating type I
Tetrahymena thermophila CCAP 1630/1M
Mating type I
Tetrahymena thermophila CCAP 1630/1N
Mating type II
Tetrahymena thermophila CCAP 1630/1P
Mating type III
Tetrahymena thermophila CCAP 1630/1Q
Mating type IV
Tetrahymena thermophila CCAP 1630/1V
Mating type II
Tetrahymena thermophila CCAP 1630/4A
Mating type II
Tetrahymena tropicalis CCAP 1630/11A
Mating type I
Thanatephorus cucumeris CABI-IMI 303158
Anastomosis group 3
Thanatephorus cucumeris CABI-IMI 305033
Anastomosis group 8
Thanatephorus cucumeris CABI-IMI 305038
Anastomosis group 8
Thanatephorus cucumeris CABI-IMI 305037
Anastomosis group 8
Thanatephorus cucumeris CABI-IMI 305034
Anastomosis group 8
Thanatephorus cucumeris CABI-IMI 305036
Anastomosis group 8
Thanatephorus cucumeris CABI-IMI 303155
Anastomosis group AG2 type1
Thanatephorus cucumeris CABI-IMI 303157
Anastomosis group AG2 type2
Thanatephorus cucumeris CABI-IMI 303156
Anastomosis group AG2 type2
Thanatephorus cucumeris CABI-IMI 303162
Anastomosis group AG4
Thanatephorus cucumeris CABI-IMI 303159
Anastomosis group AG5
Thanatephorus cucumeris CABI-IMI 303161
Anastomosis group AG7
Thanatephorus cucumeris CABI-IMI 303163
Anastomosis group AGB1
Thanatephorus cucumeris CABI-IMI 303152
Anastomosis group AGI culture type 1A
Thanatephorus cucumeris CABI-IMI 303153
Anastomosis group AGI cuture type 1B
Thanatephorus cucumeris CABI-IMI 303154
Anastomosis group AGI cuture type 1C
Thanatephorus cucumeris CABI-IMI 303160
Anastomosis group6

Special features: chemical transformation, bioconversion and bioaccumulation

11-beta (hydroxylation) of steroids
Cochliobolus lunatus CABI-IMI 312658
3,4- Fluorophenols (oxidation of)
Acinetobacter sp. NCIMB 8250
3-4 dichloroproprionanilide (metabolises)
Fusarium solani CABI-IMI 129056
6-alphapragane steroids to 11 alpha hydroxy analogues
*Aspergillus nidulans*CABI-IMI 227976
6-alphapragane steroids to 11 alpha hydroxy analogues
*Aspergillus nidulans*CABI-IMI 227976
Acronycine to 9-hydroxyacronycine (Bioconv.)
Aspergillus niger CABI-IMI 041874
Alpha-Carboxycephalosporins (Hydrolysis of)
Alcaligenes faecalis NCIMB 10109
Alcaligenes faecalis NCIMB 10109
Alpha-Carboxycephalosporins (Hydrolysis of)
Pseudomonas aeruginosa NCIMB 10111
Pseudomonas aeruginosa NCIMB 10110
Amylo (process)
Rhizopus oryzae CABI-IMI 309922
Rhizopus oryzae CABI-IMI 309923
Ang-kak (production)
Monascus purpueus CABI-IMI 283870
Anti tumour agents (transformation)
Penicillium brevicompactum CABI-IMI 040225
Aresenite (oxidation)
Pseudomonas putida NCIMB 8685
Ochrobactrum anthropi NCIMB 8688
Pseudomonas putida NCIMB 8689
Aromatic (biosynthesis)
Escherichia coli NCIMB 8741
Escherichia coli NCIMB 8744
Ascorbic acid (reduction)
Escherichia coli NCIMB 8557
Benzylpenicillin tetrazoles (bioconversion of)
Escherichia coli NCIMB 8666
Caproic acid (oxidation)
Bacillus sphaericus NCIMB 8867
Caproic acid (oxidation)
Pseudomonas fluorescens NCIMB 8866
Pseudomonas putida NCIMB 8865
Carbon dioxide (fixation)
Alteromonas sp. NCIMB 2083
Alcaligenes xylosoxidians subsp *xylosoxidiand* NCIMB 11475
Cellulosic wastes (bioconversion)
Thermomonospora fusca NCIMB 11185
Chlorine ions (from organic acids)
Pseudomonas dehalogenans NCIMB 9061
Chloropyriphos (Hydrolysis of)
Flavobacterium sp. NCIMB 11635
Citric acid (accumulation of)
Aspergillus niger CABI-IMI 309921
Dehydroascorbic acid (reduction to ascorbic acid)
Escherichia coli NCIMB 8545
Dehydroascorbic acid (reduction to ascorbic acid)
Staphylococcus epidermidis NCIMB 8558
Denitrification
Pseudomonas sp. NCIMB 1976
Pseudomonas sp. NCIMB 885
Diazinon (hydrolysis of)
Flavobacterium sp. NCIMB 11635
Ester (Stereo specific hydrolysis)
Bacillus subtilis NCIMB 11705
Fatty acids (oxidation)
Pseudomonas aeruginosa NCIMB 9571
Ferric ion (reduction)
Shewanella putrefaciens NCIMB 12578
Shewanella putrefaciens NCIMB 12582
Shewanella putrefaciens NCIMB 12580
Shewanella putrefaciens NCIMB 12577
Shewanella putrefaciens NCIMB 12581
Ferrous ion (oxidation)
Thiobacillus ferrooxidans NCIMB 9490
Formaldehyde (fixation)
Hyphomicrobium sp. NCIMB 12162
Gluconate (oxidation)
Pseudomonas fluorescens NCIMB 9493
Glycerol (oxidation)
Enterococcus faecium NCIMB 8442
Glycoside hydrolase inhibitors L-deoxynojirmycin
Bacillus coagulans NCIMB 9365
Heavy metal ions (accumulation)
Zooglea ramigera NCIMB 11941
Rhizobiaceae sp. NCIMB 10340
Hydrocarbon (oxidation)
Micococcus sp. NCIMB 10405
Pseudomonas aeruginosa NCIMB 9571
Rhodococcus rhodochrous NCIMB 9703
Arthrobacterc sp. NCIMB 10407
Corynebacterium sp. NCIMB 10406
5-anilino 1,2,3,4-thiatriazole (Bioconv/Hydroxylates)
Rhizopus stolonifer CABI-IMI 061269
Steroid (hydroxylation)
Rhizopus arrhizus CABI-IMI 090340
Aspergillus niger CABI-IMI 041874
Ion exchange / complexation of the uranyl ion
Rhizopus oryzae CABI-IMI 309924
Rhizopus oryzae CABI-IMI 309922
Iron uptake phage
Listonella anguillarum NCIMB 2286
Lactose in dairy products (hydrolysis of)
Bacillus stearothermus NCIMB 11408
L-Histidinol (bioconversion)
Arthrobacter histidinolovorans NCIMB 9541
Lincomycin (bioconversion)
Streptomyces spectabilis NCIMB 9750
Streptomyces spectabilis NCIMB 9733
L-Tryptophan (oxidation via aromatic pathway)
Pseudomonas fluorescens NCIMB 10098
Olefins (epoxidation of)
Mycobacterium sp. NCIMB 11626
Orotic acids (accumulation)
Neurospora crassa CABI-IMI 075730ii
Orotidine (accumulation)
Neurospora crassa CABI-IMI 075730ii
p- Hydroxybenzoate (ester oxidation of)

Acinetobacter sp. NCIMB 8250
Parathion (hydrolysis of)
Flavobacterium sp. NCIMB 11635
Penicillic acid (production deficient)
Penicillium viridicatum CABI-IMI 039758ii
Phenol (oxidation)
Pseudomonas putida NCIMB 8249
Pseudomonas putida NCIMB 8248
Phenolic acid (study of synthesis)
Bacillus subtilis NCIMB 11824
Phenols 0-methylation of (halogenated)
Rhodococcus sp. NCIMB 12689
Rhodococcus sp. NCIMB 12690
Acinetobacter sp. NCIMB 12962
Phenoxymethylpenicillin tetrazole (deacylation)
Escherichia coli NCIMB 8666
Progesterone (oxidation) to 16-alpa-hydroxyprogesterone
Streptomyces argenteolus NCIMB 9625
Quinic acid (reduction to dihydroshikmic acid)
Lactobacillus collinoides NCIMB 8847
Lactobacillus collinoides NCIMB 8848
Riboflavin (oxidation) to lumichrome
Pseudomonas riboflavina NCIMB 8177
Riboflavin to beta hydroxyethylflavin
Eubacterium fissicatena NCIMB 10445
Eubacterium fissicatena NCIMB 10446
RNA (hydrolysis of)
Kleibsiella planticola NCIMB 8153
Salicylic acid (hydroxylation) to gentisic acid
Unnamed NCIMB 10467
Sesquiterpene lacton costunolide (Patent)
Penicillium spinulosum CABI-IMI 167427
Sesquiterpene lactone costunolide (transformation)
Rhizopus stolonifer CABI-IMI 057762
Geotrichum suaveolens CABI-IMI 096837
Cochliobolus lunatus CABI-IMI 061535
Rhizopus arrhizus CABI-IMI 090340
Penicillium chrysogenum CABI-IMI 037767
Penicillium glabrum CABI-IMI 040234
Mucor circinelloides f. griseocyanus CABI-IMI 116946
*Fennellia flavipes*CABI-IMI 135424
Rhizopus stolonifer CABI-IMI 057761
Steroid (dehydrogenation)
Rhizobiaceae sp. NCIMB 12157
Rhodococcus equi NCIMB 10701
Steroid (hydroxylation
Nocardia italica NCIMB 9386
Steroid (modification)
Coryneform sp. NCIMB 9324
Steroid (oxidation)
Corynebacterium mediolanum NCIMB 7206
Micromonospora sp. NCIMB 12734
Steroid (oxidation)
Micromonospora sp.NCIMB 12885
Steroid (oxygenation)
Rhizoctonia muneratii CABI-IMI 236679
Steroid (research)
Cunninghamella blakesleena CABI-IMI 063877
Steroid (transformation)
Cunninghamella elegans CABI-IMI 314507
Steroids (bioconversion)
Mycobacterium sp. NCIMB 11677
Mycobacterium sp. NCIMB 11678
Mycobacterium sp. NCIMB 11679
Streptomycin (dependent on)
Escherichia coli NCIMB 8667
Amino acid sequence of cytochrome c` (study of)
Alcaligenes xylosoxidans NCIMB 11015
Sulphide (oxidation)
Thiobacillus ferroxidans NCIMB 9490
Sulphite (reduction)
Shewanella putrefaciens NCIMB 12577
Shewanella putrefaciens NCIMB 12583
Shewanella putrefaciens NCIMB 12578
Shewanella putrefaciens NCIMB 12582
Sulphur isotope (fractionation)
Shewanella putrefaciens NCIMB 12578
Sulphur isotope (fractionation)
Shewanella putrefaciens NCIMB 12577
Sulphur (oxidation)
Sulfolobus acidocaldarius NCIMB 11770
Thiobacillus ferroxidans NCIMB 9490
Sulfolobus acidocaldarius NCIMB 11770
Superoxide dismutase (regulation of)
Escherichia coli NCIMB 12052
Tannin-gallic acid (fermentation)
Aspergillus niger CABI-IMI 050566ii
Taxifolin (hydroxylation)
Pseudomonas sp. NCIMB 9940
Terpene (modification)
Bacillus pumulis NCIMB 11617
Thiosulphate (reduction) to sulphide
Shewanella putrefaciens NCIMB 12583
Shewanella putrefaciens NCIMB 12577
Shewanella putrefaciens NCIMB 12582
Shewanella putrefaciens NCIMB 12581
Shewanella putrefaciens NCIMB 12580
Shewanella putrefaciens NCIMB 12578
Shewanella putrefaciens NCIMB 12579
Tropin (conversion) to pseudotropin
Enterococcus faecalis NCIMB 9633
Tryptophan (oxidation via quinoline)
Comamonas acidovorans NCIMB 9289
Tryptophan (synthesis study)
Escherichia coli NCIMB 10199
Klebsiella pneumoniae subsp. *pneumoniae* NCIMB 10198
Klebsiella pneumoniae subsp. *pneumoniae* NCIMB 10197
Zinc oxide(dissociation)
Mortierella elongate CABI-IMI 375268*
Zinc phophate (dissociation)
Mortierella elongate CABI-IMI 375268*

Vectors, phages, transposons, genetically modified organisms (auxotrophs, resitant, sensitive, producers), mutants

The Property and map (where available) is given for each organism with its name, collection acronym and number.

Acid phosphatase h- pho1-44
Schizosaccharomyces pombe NCYC 2087
Acid phosphatase h+ pho1-44
Schizosaccharomyces pombe NCYC 2088
Adenine auxotroph h+ ade 9-8H
Schizosaccharomyces pombe NCYC 1856
Adenine auxotroph h+ ade103H
Schizosaccharomyces pombe NCYC 1858
Adenine auxotroph h-ade ade 6-M210
Schizosaccharomyces pombe NCYC 1845
Adenine auxotroph h+ ade1-40
Schizosaccharomyces pombe NCYC 1836
Adenine auxotroph h+ ade7-84
Schizosaccharomyces pombe NCYC 1862
Adenine auxotroph h- ade1-3
Schizosaccharomyces pombe NCYC 1829
Adenine auxotroph h- ade1-25
Schizosaccharomyces pombe NCYC 1833
Adenine auxotroph h+ ade1-3
Schizosaccharomyces pombe NCYC 1830
Adenine auxotroph h+ ade8-106
Schizosaccharomyces pombe NCYC 1854
Adenine auxotroph h- ade1-40
Schizosaccharomyces pombe NCYC 1835
Adenine auxotroph h- ade9-8H
Schizosaccharomyces pombe NCYC 1855
Adenine auxotroph h- ade3-58
Schizosaccharomyces pombe NCYC 1839
Adenine auxotroph h- ade6-706
Schizosaccharomyces pombe NCYC 1868
Adenine auxotroph h+ ade6-704
Schizosaccharomyces pombe NCYC 1860
Adenine auxotroph h- ade6-704
Schizosaccharomyces pombe NCYC 1859
Adenine auxotroph h+ ade6-250
Schizosaccharomyces pombe NCYC 1848
Adenine auxotroph h- ade6-250
Schizosaccharomyces pombe NCYC 1847
Adenine auxotroph h+ ade5-36
Schizosaccharomyces pombe NCYC 1844
Adenine auxotroph h- ade5-36
Schizosaccharomyces pombe NCYC 1843
Adenine auxotroph h+ ade1-25
Schizosaccharomyces pombe NCYC 1834
Adenine auxotroph h- ade6-M216
Schizosaccharomyces pombe NCYC 1849
Adenine auxotroph h+ ade4-31
Schizosaccharomyces pombe NCYC 1842
Adenine auxotroph h+ ade3-58
Schizosaccharomyces pombe NCYC 1840
Adenine auxotroph h+ ade6-M26
Schizosaccharomyces pombe NCYC 2039
Adenine auxotroph h+ ade2-17
Schizosaccharomyces pombe NCYC 1838
Adenine auxotroph h- ade2-17
Schizosaccharomyces pombe NCYC 1837
Adenine auxotroph h+ ade1-51
Schizosaccharomyces pombe NCYC 1832
Adenine auxotroph h+ ade6-M210
Schizosaccharomyces pombe NCYC 1846
Adenine auxotroph h- ade1-51
Schizosaccharomyces pombe NCYC 1831
Adenine auxotroph h- ade103H
Schizosaccharomyces pombe NCYC 1857
Adenine auxotroph h+ ade7-413
Schizosaccharomyces pombe NCYC 1867
Adenine auxotroph h- ade8-106
Schizosaccharomyces pombe NCYC 1853
Adenine auxotroph h+ ade6-M216
Schizosaccharomyces pombe NCYC 1850
Adenine auxotroph h- ade6-M375
Schizosaccharomyces pombe NCYC 2040
Adenine auxotroph h+ ade6-M375
Schizosaccharomyces pombe NCYC 2041
Adenine auxotroph h- ade7-50
Schizosaccharomyces pombe NCYC 1851
Adenine auxotroph h+ ade7-50
Schizosaccharomyces pombe NCYC 1852
Adenine auxotroph h- ade7-84
Schizosaccharomyces pombe NCYC 1861
Adenine auxotroph h+ ade6-706
Schizosaccharomyces pombe NCYC 1869
Adenine auxotroph h- ade7-413
Schizosaccharomyces pombe NCYC 1866
Adenine auxotroph h- ade6-M26
Schizosaccharomyces pombe NCYC 2038
Adenine auxotroph h- ade4-31
Schizosaccharomyces pombe NCYC 1841
Adenine requiring mutants a ade 2
Saccharomyces cerevisiae NCYC 830
Adenine requiring mutants a ade 6
Saccharomyces cerevisiae NCYC 838
Adenine requiring mutants Alpha ade 4 (ura)
Saccharomyces cerevisiae NCYC 835
Adenine requiring mutants a ade1 leu1
Saccharomyces cerevisiae NCYC 1577
Adenine requiring mutants a ade 1 leu2
Saccharomyces cerevisiae NCYC 1652
Adenine requiring mutants a ade4
Saccharomyces cerevisiae NCYC 834
Adenine requiring mutants Alpha ade3 (ura)
Saccharomyces cerevisiae NCYC 833
Adenine requiring mutants Alpha ade5 (ura)
Saccharomyces cerevisiae NCYC 837
Adenine requiring mutants Alpha ade 6 (trp)
Saccharomyces cerevisiae NCYC 839
Adenine requiring mutants a ade7
Saccharomyces cerevisiae NCYC 840
Adenine requiring mutants a ade 5
Saccharomyces cerevisiae NCYC 836
Adenine requiring mutants Alpa ade 2(lys)
Saccharomyces cerevisiae NCYC 802
Adenine requiring mutants a ade 8
Saccharomyces cerevisiae NCYC 842
Adenine requiring mutants a ade1
Saccharomyces cerevisiae NCYC 828
Adenine requiring mutants alpa ade1
Saccharomyces cerevisiae NCYC 829

Adenine requiring mutants alpha ade7 *Saccharomyces cerevisiae* NCYC 841
Adenine requiring mutants a ade3 *Saccharomyces cerevisiae* NCYC 832
Adenine requiring mutants alpha ade8 (lys trp) *Saccharomyces cerevisiae* NCYC 843
Alkaline phosphatase h+pho2-1 *Schizosaccharomyces pombe* NCYC 2055
Alkaline phosphatase h- pho2-1 *Schizosaccharomyces pombe* NCYC 2054
Alkaline phosphatase h- pho3-1 *Schizosaccharomyces pombe* NCYC 2056
Alkaline phosphatase h+ pho3-1 *Schizosaccharomyces pombe* NCYC 2057
Alteration to nuclear structure h-leu1 nuc2 *Schizosaccharomyces pombe* NCYC 2245
Alteration to nuclear structure h- leunuc1-632 *Schizosaccharomyces pombe* NCYC 2244
Aminoglycoside resistance antibact mechanism AAD(2") p FCT3103 NCTC 50478
Aminoglycoside resistance antibact mechanism apHA p UZ8 NCTC 50228
Aminoglycoside resistance antibact mechanism APH(3')-II p IP1433 NCTC 50453
Aminoglycoside resistance antibact mechanism APH(3')-II Tn5 NCTC 50189
Aminoglycoside resistance antibact mechanism APH(3')-II JR66a NCTC 50122
Aminoglycoside resistance antibact mechanism APH(3')-II p SAY16 NCTC 50477
Aminoglycoside resistance antibact mechanism APH(3')-1 p GH54 NCTC 50476
Aminoglycoside resistance antibact mechanism ACC(3)-IV TP305 NCTC 50335
Aminoglycoside resistance antibact mechanism ACC(3)-IV TP307 NCTC 50338
Aminoglycoside resistance antibact mechanism ACC(3)-1 p TH1 NCTC 50113
Aminoglycoside resistance antibact mechanism AAD(4')(4") p UB 110 NCTC 50595
Aminoglycoside resistance antibact mechanism ACC(3)-IV R1535 NCTC 50320
Anisomycin resistant h- Ani1 F1 *Schizosaccharomyces pombe* NCYC 1349
Anisomycin resistant h- Ano2 F5 *Schizosaccharomyces pombe* NCYC 1350
Antibiotic resistance gene probes antibact mechanism TET-B p RT11 NCTC 50365
Antibiotic resistance gene probes antibact mechanism TET-E p SL 1504 NCTC 50272
Antibiotic resistance gene probes antibact mechanism TET-D p SL106 NCTC 50271
Antibiotic resistance gene probes antibact mechanism TET-B p KT007 NCTC 50269
Antibiotic resistance gene probes antibact mechanism TET-O p AT121 NCTC 50500
Antibiotic resistance gene probes antibact mechanism TET-A p SL18 NCTC 50268
Antibiotic resistance gene probes antibact mechanism DHFR-V p LK09 NCTC 50514
Antibiotic resistance gene probes antibact mechanism DHFR-IV p UK1148 NCTC 50515
Antibiotic resistance gene probes antibact mechanism DHFR-II p WZ820 NCTC 50536
Antibiotic resistance gene probes antibact mechanism DHFR-I p FE872 NCTC 50535
Antibiotic resistance gene probes antibact mechanismAPH(3')-II p SAY16 NCTC 50477
Antibiotic resistance gene probes antibact mechanism APH(3')-I p GH54 NCTC 50476
Antibiotic resistance gene probes antibact mechanism AAD(2") p FCT3103 NCTC 50478
Antibiotic resistance gene probes antibact mechanism DHFR-III p FE1242 NCTC 50537
Antibiotic resistance gene probes antibact mechanism TET-C p BR322 NCTC 50270
Antisurpressor Trna MODIFIED h- ade7-413SUP3-18 SIN3-193 *Schizosaccharomyces pombe* NCYC 2227
Antisurpressor Trna MODIFIED h+ ade7-413SUP3-18 sin1-26 *Schizosaccharomyces pombe* NCYC 2224
Antisurpressor Trna MODIFIED h+ ade7-413sup3-18 sin4-15 *Schizosaccharomyces pombe* NCYC 2226
Antisurpressor Trna MODIFIED h+ ade7-413SUP3-18 SIN3-193 Schizosaccharomyces pombe NCYC 2228
Antisurpressor Trna MODIFIED h- ade7-413SUP3-18 SIN1-26 *Schizosaccharomyces pombe* NCYC 2223
Antisurpressor Trna MODIFIED h- ade7-413sup3-18 sin4-15 *Schizosaccharomyces pombe* NCYC 2225
Apiculate cells Teaching strain *Saccharomyces ludwigii* NCYC 731
Arginine auxotroph h- arg3-20 *Schizosaccharomyces pombe* NCYC 2046
Arginine auxotroph h- arg2-3 *Schizosaccharomyces pombe* NCYC 2008
Arginine auxotroph h+ arg7-1 *Schizosaccharomyces pombe* NCYC 1885
Arginine auxotroph h+ arg1-2 *Schizosaccharomyces pombe* NCYC 2007
Arginine auxotroph h+ arg4-55 *Schizosaccharomyces pombe* NCYC 2011
Arginine auxotroph h- arg1-230 *Schizosaccharomyces pombe* NCYC 1872
Arginine auxotroph h+ arg 1-14 *Schizosaccharomyces pombe* NCYC 1871
Arginine auxotroph h+ arg1-230 *Schizosaccharomyces pombe* NCYC 1873
Arginine auxotroph h+ arg2-3 *Schizosaccharomyces pombe* NCYC 2009
Arginine auxotroph h+ arg3-16 *Schizosaccharomyces pombe* NCYC 2043
Arginine auxotroph h- arg2-89 *Schizosaccharomyces pombe* NCYC 1874
Arginine auxotroph h+ arg11-124 *Schizosaccharomyces pombe* NCYC 1877
Arginine auxotroph h- arg3-15 *Schizosaccharomyces pombe* NCYC 2044

Arginine auxotroph h- arg6-328
Schizosaccharomyces pombe NCYC 1882
Arginine auxotroph h+ arg3-15
Schizosaccharomyces pombe NCYC 2045
Arginine auxotroph h- arg3-16
Schizosaccharomyces pombe NCYC 2042
Arginine auxotroph h- arg1-2
Schizosaccharomyces pombe NCYC 2006
Arginine auxotroph h+ arg2-89
Schizosaccharomyces pombe NCYC 1875
Arginine auxotroph h+ arg8-1
Schizosaccharomyces pombe NCYC 1887
Arginine auxotroph h+ arg10-1
Schizosaccharomyces pombe NCYC 1891
Arginine auxotroph h- arg10-1
Schizosaccharomyces pombe NCYC 1890
Arginine auxotroph h+ arg9-1
Schizosaccharomyces pombe NCYC 1889
Arginine auxotroph h- arg9-1
Schizosaccharomyces pombe NCYC 1888
Arginine auxotroph h+ arg3-20
Schizosaccharomyces pombe NCYC 2047
Arginine auxotroph h- arg8-1
Schizosaccharomyces pombe NCYC 1886
Arginine auxotroph h- arg 1-14
Schizosaccharomyces pombe NCYC 1870
Arginine auxotroph h+ arg6-328
Schizosaccharomyces pombe NCYC 1883
Arginine auxotroph h- arg4-55
Schizosaccharomyces pombe NCYC 2010
Arginine auxotroph h+ arg5-189
Schizosaccharomyces pombe NCYC 1881
Arginine auxotroph h- arg5-189
Schizosaccharomyces pombe NCYC 1880
Arginine auxotroph h- arg11-124
Schizosaccharomyces pombe NCYC 1876
Arginine auxotroph h- arg7-1
Schizosaccharomyces pombe NCYC 1884
Aromatic amino acidauxotroph h+ aro5-110
Schizosaccharomyces pombe NCYC 2053
Aromatic amino acidauxotroph h- aro4-98
Schizosaccharomyces pombe NCYC 2069
Aromatic amino acidauxotroph h+ aro3-63
Schizosaccharomyces pombe NCYC 2051
Aromatic amino acidauxotroph h+ aro4-98
Schizosaccharomyces pombe NCYC 2070
Aromatic amino acidauxotroph h- aro5-110
Schizosaccharomyces pombe NCYC 2052
Aromatic amino acidauxotroph h- aro3-63
Schizosaccharomyces pombe NCYC 2050
Arsenate resistant Resistant R773
NCTC 50058
Arsenite resistant Resistant p I258
NCTC 50581
Arsenite resistant Resistant p I524
NCTC 50599
Arsenite resistant Resistant R828
NCTC 50346
Azaguanidine resistant h- aza 2-1
Schizosaccharomyces pombe NCYC 2115
Azaguanidine resistant h+ aza4-1
Schizosaccharomyces pombe NCYC 2120
Azaguanidine resistant h- aza3-1
Schizosaccharomyces pombe NCYC 2117
Azaguanidine resistant h+ aza3-1
Schizosaccharomyces pombe NCYC 2118
Azaguanidine resistant h+ aza 2-1
Schizosaccharomyces pombe NCYC 2116
Azaguanidine resistant h- aza4-1
Schizosaccharomyces pombe NCYC 2119
Benomyl resistant h- ben4.D19
Schizosaccharomyces pombe NCYC 1713
Benomyl resistant h- ben4.C10
Schizosaccharomyces pombe NCYC 1712
Benomyl resistant h- ben4.D23
Schizosaccharomyces pombe NCYC 1714
b-Lactam resistance antibact mechanism tem1 R6K
NCTC 50005
b-Lactam resistance antibact mechanism TEM-2
Tn802 NCTC 50077
b-Lactam resistance antibact mechanism TEM-2
Tn401 NCTC 50076
b-Lactam resistance antibact mechanism TEM-2
Tn1 NCTC 50188
b-Lactam resistance antibact mechanism TEM-2
RP4 NCTC 50078
b-Lactam resistance antibact mechanism TEM-2
RP1-1 NCTC 50077
b-Lactam resistance antibact mechanism TEM-2
RP1 NCTC 50076
b-Lactam resistance antibact mechanism TEM-2
R667 NCTC 50463
b-Lactam resistance antibact mechanism TEM-2
R91 NCTC 50015
b-Lactam resistance antibact mechanism tem1
Tn2660 NCTC 50005
b-Lactam resistance antibact mechanism tem1
Tn3 NCTC 50001
b-Lactam resistance antibact mechanism tem1
R1033 NCTC 50070
b-Lactam resistance antibact mechanism tem1
R934 NCTC 50372
b-Lactam resistance antibact mechanism tem1
R692 NCTC 50469
b-Lactam resistance antibact mechanism OXA-3
RIP64 NCTC 50075
b-Lactam resistance antibact mechanism OXA-1
R753 NCTC 50521
b-Lactam resistance antibact mechanism OXA-1
RGN238 NCTC 50529
b-Lactam resistance antibact mechanism OXA-1
Tn2604 NCTC 50034
b-Lactam resistance antibact mechanism OXA-2
R46 NCTC 50011
b-Lactam resistance antibact mechanism OXA-3
R62 NCTC 50450
b-Lactam resistance antibact mechanism tem1
R839 NCTC 50371
b-Lactam resistance antibact mechanism OXA-3
R57b NCTC 50458
b-Lactam resistance antibact mechanism PSE 3
Rms 149 NCTC 50081
b-Lactam resistance antibact mechanism SHV1
R1010 NCTC 50069
b-Lactam resistance antibact mechanism SHV1
Tn721 NCTC 50069
b-Lactam resistance antibact mechanism tem1
R1 NCTC 50001
b-Lactam resistance antibact mechanism tem1
R2 NCTC 50004
b-Lactam resistance antibact mechanism OXA-3
R205 NCTC 50369

Bronze mutant
Aspergillus parasiticus CABI-IMI 091019bii
Brown mutant
Aspergillus fumigatus CABI-IMI 131705
Buff mutant
*Aspergillus aurantiobrunneus*CABI-IMI 201980
Canavanine resistant h+ can1-1
Schizosaccharomyces pombe NCYC 2200
Canavanine resistant h- can1-1
Schizosaccharomyces pombe NCYC 1711
Canavanine resistant h+ can2-1
Schizosaccharomyces pombe NCYC 2197
Canavanine resistant h- can2-1
Schizosaccharomyces pombe NCYC 2197
Canavanine resistant h- can2-1
Schizosaccharomyces pombe NCYC 1710
Canavanine resistant h- can1-1
Schizosaccharomyces pombe NCYC 2199
CDC Diploids cdc6 (MH18)
Saccharomyces cerevisiae NCYC 1753
CDC Diploids JC2 (L31-9a [A]/l31-2c{alpha])
Saccharomyces cerevisiae NCYC 1718
CDC Diploids cdc13 (MH20)
Saccharomyces cerevisiae NCYC 1754
CDC Diploids cdc15-1 (MH15)
Saccharomyces cerevisiae NCYC 1752
CDC Diploids cdc36 (MH30)
Saccharomyces cerevisiae NCYC 1797
CDC Diploids cdc17 (MH21)
Saccharomyces cerevisiae NCYC 1755
CDC Diploids cdc21 (MH21)
Saccharomyces cerevisiae NCYC 1756
Cell Division cycle h- cdc10-`129
Schizosaccharomyces pombe NCYC 1686
Cell Division cycle h+cdc20-m10
Schizosaccharomyces pombe NCYC 1701
Cell Division cycle h- cdc26-1
Schizosaccharomyces pombe NCYC 1706
Cell Division cycle h- cdc12-112
Schizosaccharomyces pombe NCYC 1693
Cell Division cycle h- cdc7-24
Schizosaccharomyces pombe NCYC 1691
Cell Division cycle h- cdc3-6
Schizosaccharomyces pombe NCYC 1687
Cell Division cycle h- cdc8-134 Chr1
Schizosaccharomyces pombe NCYC 1692
Cell Division cycle h- cdc28-p8
Schizosaccharomyces pombe NCYC 1708
Cell Division cycle h- cdc11-136
Schizosaccharomyces pombe NCYC 1685
Cell Division cycle h- cdc2-33 Chr 2
Schizosaccharomyces pombe NCYC 1688
Cell Division cycle h- cdc13-117
Schizosaccharomyces pombe NCYC 1694
Cell Division cycle h- cdc1-7
Schizosaccharomyces pombe NCYC 1683
Cell Division cycle h+ cdc21-m68
Schizosaccharomyces pombe NCYC 1702
Cell Division cycle h- cdc14-118
Schizosaccharomyces pombe NCYC 1695
Cell Division cycle h- cdc5-120
Schizosaccharomyces pombe NCYC 1689
Cell Division cycle h+ cdc23-m36
Schizosaccharomyces pombe NCYC 1704
Cell Division cycle h- cdc15-140
Schizosaccharomyces pombe NCYC 1696
Cell Division cycle h+ cdc24-m38
Schizosaccharomyces pombe NCYC 1705
Cell Division cycle h+ cdc22-m45
Schizosaccharomyces pombe NCYC 1703
Cell Division cycle h+ cdc19-p-1
Schizosaccharomyces pombe NCYC 1700
Cell Division cycle h- cdc25-22
Schizosaccharomyces pombe NCYC 1684
Cell Division cycle h- cdc4-8 Chr1
Schizosaccharomyces pombe NCYC 1690
Cell Division cycle h+ cdc17-K42
Schizosaccharomyces pombe NCYC 1698
Cell Division cycle h- cdc16-116
Schizosaccharomyces pombe NCYC 1697
Cell Division cycle h+ cdc18-K46
Schizosaccharomyces pombe NCYC 1699
Cell division cycle mutants cdc31-1
Saccharomyces cerevisiae NCYC 1676
Cell division cycle mutants cdc36-16 SR661-2
Saccharomyces cerevisiae NCYC 1722
Cell division cycle mutants cdc28-4 L31-7a
Saccharomyces cerevisiae NCYC 1665
Cell division cycle mutants cdc9-8
Saccharomyces cerevisiae NCYC 1671
Cell division cycle mutants cdc9-7 (L82-2B)
Saccharomyces cerevisiae NCYC 1807
Cell division cycle mutants cdc10-1
Saccharomyces cerevisiae NCYC 1674
Cell division cycle mutants cdc41
Saccharomyces cerevisiae NCYC 1677
Cell division cycle mutants cdc9-7 (L94-4d)
Saccharomyces cerevisiae NCYC 1808
Cell division cycle mutants cdc30-1
Saccharomyces cerevisiae NCYC 1675
Cell division cycle mutants cdc39-1 SR665-1
Saccharomyces cerevisiae NCYC 1641
Cell division cycle mutants cdc9-7
Saccharomyces cerevisiae NCYC 1670
Cell division cycle mutants cdc37-1 SR672-1
Saccharomyces cerevisiae NCYC 1666
Cell division cycle mutants cdc6-1
Saccharomyces cerevisiae NCYC 1723
Cell division cycle mutants cdc14-1
Saccharomyces cerevisiae NCYC 1735
Cell division cycle mutants cdc13-1
Saccharomyces cerevisiae NCYC 1734
Cell division cycle mutants cdc12-1
Saccharomyces cerevisiae NCYC 1733
Cell division cycle mutants cdc9-7 p-
Saccharomyces cerevisiae NCYC 1633
Cell division cycle mutants cdc18-1
Saccharomyces cerevisiae NCYC 1736
Cell division cycle mutants cdc9-1 (L89-6C)
Saccharomyces cerevisiae NCYC 1771
Cell division cycle mutants cdc7-1
Saccharomyces cerevisiae NCYC 1729
Cell division cycle mutants cdc9-12
Saccharomyces cerevisiae NCYC 1672
Cell division cycle mutants cdc9-4
Saccharomyces cerevisiae NCYC 1731
Cell division cycle mutants cdc9-3
Saccharomyces cerevisiae NCYC 1791
Cell division cycle mutants cdc9-1 rev1
Saccharomyces cerevisiae NCYC 1778
Cell division cycle mutants cdc19-1
Saccharomyces cerevisiae NCYC 1737

Cell division cycle mutants cdc8-198
Saccharomyces cerevisiae NCYC 1667
Cell division cycle mutants cdc8-141
Saccharomyces cerevisiae NCYC 1730
Cell division cycle mutants cdc 11-1
Saccharomyces cerevisiae NCYC 1655
Cell division cycle mutants cdc5-1
Saccharomyces cerevisiae NCYC 1643
Cell division cycle mutants cdc4-1
Saccharomyces cerevisiae NCYC 1642
Cell division cycle mutants cdc3-1
Saccharomyces cerevisiae NCYC 1654
Cell division cycle mutants cdc9-6
Saccharomyces cerevisiae NCYC 1732
Cell division cycle mutants cdc26-1
Saccharomyces cerevisiae NCYC 1738
Cell division cycle mutants cdc9-13
Saccharomyces cerevisiae NCYC 1673
Cell division without nuclear division h- leu1 tws1
Schizosaccharomyces pombe NCYC 2243
Cell division without nuclear division h- leu1 endf1-458
Schizosaccharomyces pombe NCYC 2246
Cell division without nuclear division h- ley1 top2-191
Schizosaccharomyces pombe NCYC 2250
Cell division without nuclear division h- leu1 cut2-364
Schizosaccharomyces pombe NCYC 2248
Cell division without nuclear division h- leu1 cut1-645
Schizosaccharomyces pombe NCYC 2247
Cell division without nuclear division h- leu1 top1-710
Schizosaccharomyces pombe NCYC 2249
Cell wall defective
Chlamydomonas reinhardtii CCAP 11/32 Cw15+
Changed division response h- cdr1-76
Schizosaccharomyces pombe NCYC 2368
Changed division response h- cdr2-96
Schizosaccharomyces pombe NCYC 2369
Chemically induced mutant (patent)
Penicillium chrysogenum CABI-IMI 142387
Chemically induced mutant (patent)
Penicillium chrysogenum CABI-IMI 142384
Chemically induced mutant (patent)
Penicillium chrysogenum CABI-IMI 142385
Chemically induced mutant (patent)
Penicillium chrysogenum CABI-IMI 142386
Chemically induced mutant (patent)
Penicillium chrysogenum CABI-IMI 142383
Chloramphenicol Resistance NON-ENZYMIC R1033 NCTC 50070
Chloramphenicol Resistance antibact mechanism CAT-III R387 NCTC 20022
Chloramphenicol Resistance antibact mechanism CAT-IISa NCTC 50128
Chloramphenicol Resistance antibact mechanism CAT-1 R726 NCTC 50055
Citrate plasmid ECO encoded*R27*
NCTC 50010
Citrate plasmid ECO encoded*R726*
NCTC 50055
Cloning vector ECO for gram -ve bacteria *p ACYC184* NCTC 50570
Cloning vector ECO for gram -ve bacteria *p BR322*
NCTC 5008
Cloning vector ECO for gram -ve bacteria *p AT153*
NCTC 50242
Cloning vector ECO for gram -ve bacteria *p NO1517* NCTC 50588
Clonig vector ECO for gram -ve bacteria *p KT279*
NCTC 50559
Cloning vector ECO for gram -ve bacteria *p NO1523* NCTC 50525
Cloning vector ECO for gram -ve bacteria *p BR325*
NCTC 50293
Cloning vector ECO for gram -ve bacteria *p HA10*
NCTC 50526
Cloning vector STA for gram +ve bacteria *p UB110*
NCTC 5055
Cloning vector STA for gram +ve bacteria *p E194*
NCTC 50591
Cloning vector STA for gram +ve bacteria *p C194*
NCTC 50586
Colonuial mutants
Escherichia coli NCIMB 11595
Cyclohexamide
Schizosaccharomyces pombe NCYC 1347
Schizosaccharomyces pombe NCYC 1361
Schizosaccharomyces pombe NCYC 1360
Schizosaccharomyces pombe NCYC 1351
Schizosaccharomyces pombe NCYC 1352
Schizosaccharomyces pombe NCYC 1345
Schizosaccharomyces pombe NCYC 1348
Schizosaccharomyces pombe NCYC 1346
Dark mutant
Glomerella cingulata CABI-IMI 085091ii
Deformed plastids
Chlamydomonas moewusii CCAP 11/16E
Disomic strains
Saccharomyces cerevisiae NCYC 960
Saccharomyces cerevisiae NCYC 961
Drug resistant mutants
Saccharomyces cerevisiae NCYC 1628
Saccharomyces cerevisiae NCYC 1716
Saccharomyces cerevisiae NCYC 1717
Saccharomyces cerevisiae NCYC 1621
Saccharomyces cerevisiae NCYC 1622
Dumbell formers
Saccharomyces cerevisiae NCYC 1815
Saccharomyces cerevisiae NCYC 1794
Saccharomyces cerevisiae NCYC 1748
Saccharomyces cerevisiae NCYC 1795
Saccharomyces cerevisiae NCYC 1820
Saccharomyces cerevisiae NCYC 1819
Saccharomyces cerevisiae NCYC 1818
Saccharomyces cerevisiae NCYC 1813
Saccharomyces cerevisiae NCYC 1816
Saccharomyces cerevisiae NCYC 1814
Saccharomyces cerevisiae NCYC 1747
Saccharomyces cerevisiae NCYC 1825
Saccharomyces cerevisiae NCYC 1750
Saccharomyces cerevisiae NCYC 1824
Saccharomyces cerevisiae NCYC 1823
Saccharomyces cerevisiae NCYC 1796
Saccharomyces cerevisiae NCYC 1751
Saccharomyces cerevisiae NCYC 1817

Ethionine resistance
Schizosaccharomyces pombe NCYC 2192
Schizosaccharomyces pombe NCYC 2196
Schizosaccharomyces pombe NCYC 2195
Schizosaccharomyces pombe NCYC 2194
Schizosaccharomyces pombe NCYC 2193
Schizosaccharomyces pombe NCYC 2191
Fermentation markers
Saccharomyces cerevisiae NCYC 1787
Saccharomyces cerevisiae NCYC 1636
Saccharomyces cerevisiae NCYC 866
Saccharomyces cerevisiae NCYC 1757
Saccharomyces cerevisiae NCYC 1625
Saccharomyces cerevisiae NCYC 1638
Saccharomyces cerevisiae NCYC 1789
Saccharomyces cerevisiae NCYC 864
Saccharomyces cerevisiae NCYC 1635
Saccharomyces cerevisiae NCYC 861
Saccharomyces cerevisiae NCYC 1624
Saccharomyces cerevisiae NCYC 1634
Saccharomyces cerevisiae NCYC 867
Saccharomyces cerevisiae NCYC 2252
Saccharomyces cerevisiae NCYC 863
Saccharomyces cerevisiae NCYC 862
Saccharomyces cerevisiae NCYC 859
Saccharomyces cerevisiae NCYC 807
Saccharomyces cerevisiae NCYC 865
Flocculation marker
Saccharomyces cerevisiae NCYC 860
Saccharomyces cerevisiae NCYC 868
Saccharomyces cerevisiae NCYC 869
Saccharomyces cerevisiae NCYC 870
Fluorouracil resistance
Schizosaccharomyces pombe NCYC 2122
Schizosaccharomyces pombe NCYC 2123
Schizosaccharomyces pombe NCYC 2124
Schizosaccharomyces pombe NCYC 2121
General amino acid permease mutant
Saccharomyces cerevisiae NCYC 1390
Saccharomyces cerevisiae NCYC 1391
Glutamte auxotroph
Schizosaccharomyces pombe NCYC 2027
Schizosaccharomyces pombe NCYC 2026
Schizosaccharomyces pombe NCYC 2021
Schizosaccharomyces pombe NCYC 2020
Schizosaccharomyces pombe NCYC 1893
Schizosaccharomyces pombe NCYC 2013
Schizosaccharomyces pombe NCYC 2028
Schizosaccharomyces pombe NCYC 2029
Schizosaccharomyces pombe NCYC 2012
Schizosaccharomyces pombe NCYC 1892
Histidine auxotroph
Schizosaccharomyces pombe NCYC 1898
Schizosaccharomyces pombe NCYC 1909
Schizosaccharomyces pombe NCYC 2016
Histidine auxotrophh+ his8-24
Schizosaccharomyces pombe NCYC 2017
Histidine auxotrophh- his9-62
Schizosaccharomyces pombe NCYC 1911
Histidine auxotrophh+ his9-62
Schizosaccharomyces pombe NCYC 1912
Histidine auxotrophh- his6-365
Schizosaccharomyces pombe NCYC 1906
Histidine auxotrophh+ his1-102
Schizosaccharomyces pombe NCYC 1897
Histidine auxotrophh- his5-303
Schizosaccharomyces pombe NCYC 1904
Histidine auxotrophh- his7-366
Schizosaccharomyces pombe NCYC 1908
Histidine auxotrophh+ his3-21
Schizosaccharomyces pombe NCYC 2015
Histidine auxotrophh+ his3-237
Schizosaccharomyces pombe NCYC 1901
Histidine auxotrophh+ his6-365
Schizosaccharomyces pombe NCYC 1907
Histidine auxotrophh- his1-102
Schizosaccharomyces pombe NCYC 1896
Histidine auxotrophh- his3-21
Schizosaccharomyces pombe NCYC 2014
Histidine auxotrophh+ his2-245
Schizosaccharomyces pombe NCYC 1899
Histidine auxotrophh- his3-237
Schizosaccharomyces pombe NCYC 1900
Histidine auxotrophh- his4-239
Schizosaccharomyces pombe NCYC 1902
Histidine auxotrophh+ his5-303
Schizosaccharomyces pombe NCYC 1905
Histidine auxotrophh+ his4-239
Schizosaccharomyces pombe NCYC 1903
Hyaline mutant
*Cochliobolus ativus*CABI-IMI 166173
Fulvia fulva CABI-IMI 296520
Hyaline mutant SML1Streptomycin bleached
Euglena gracilis var. saccharophilia
CCAP 1224/7B
Incompatibility/replicon probes Replicon M
pULB2423 NCTC 50538
Incompatibility/replicon probes Replicon W
pULB2426 NCTC 50539
Incompatibility/replicon probes Replicon U
pULB2429 NCTC 50558
Incompatibility/replicon probes Replicon T
pULB2425 NCTC 50542
Incompatibility/replicon probes Replicon Q
pULB2424 NCTC 50557
Incompatibility/replicon probes Replicon X
pULB2405 NCTC 50545
Incompatibility/replicon probes Replicon N
pULB2432 NCTC 50555
Incompatibility/replicon probes Replicon com9
pULB2422 NCTC 50552
Incompatibility/replicon probes Replicon HI
pULB2433 NCTC 50546
Incompatibility/replicon probes Replicon HIT
pULB2443 NCTC 50541
Incompatibility/replicon probes Replicon K
pULB2442 NCTC 50554
Incompatibility/replicon probes Replicon K
pULB2439 NCTC 50540
Incompatibility/replicon probes Replicon FIA
pULB2154 NCTC 50547
Incompatibility/replicon probes Replicon P
pULB2420 NCTC 50556
Incompatibility/replicon probes Replicon FIA
pULB2401 NCTC 50551
Incompatibility/replicon probes Replicon IT
pULB2428 NCTC 50553
Incompatibility/replicon probes Replicon FIC
pULB2440 NCTC 50550
Incompatibility/replicon probes Replicon B
pULB2406 NCTC 50548
Incompatibility/replicon probes Replicon FIB
pULB2404 NCTC 50549

Incompatibility/replicon probes Replicon Y pULB2410 NCTC 50543
Lactose plasmid ECO encoded Plasmid NCTC 50089
Lactose plasmid ECO encoded Plasmid NCTC 50068
Lactose plasmid ECO encoded Plasmid NCTC 50115
Lactose plasmid ECO encoded Plasmid NCTC 50097
Lactose plasmid ECO encoded Plasmid NCTC 50127
Lactose plasmid ECO encoded Plasmid NCTC 50120
Lactose plasmid ECO encoded Plasmid NCTC 50090
Leucine auxotroph h- leu1-32 *Schizosaccharomyces pombe* NCYC 1913
Leucine auxotroph h+leu3-241 *Schizosaccharomyces pombe* NCYC 1920
Leucine auxotroph h- leu3-241 *Schizosaccharomyces pombe* NCYC 1919
Leucine auxotroph h+ leu3-155 *Schizosaccharomyces pombe* NCYC 1918
Leucine auxotroph h+ leu2-120 *Schizosaccharomyces pombe* NCYC 1916
Leucine auxotroph h- leu2-120 *Schizosaccharomyces pombe* NCYC 1915
Leucine auxotroph h+ leu1-32 *Schizosaccharomyces pombe* NCYC 1914
Leucine auxotroph h- leu3-155 *Schizosaccharomyces pombe* NCYC 1917
Light coloured mutant *Aspergillus fumigatus* var. *helvolus* CABI-IMI 016153ii
Lysine auxotroph h+ lys7-2 *Schizosaccharomyces pombe* NCYC 2023
Lysine auxotroph h+ lys8-1 *Schizosaccharomyces pombe* NCYC 1937
Lysine auxotroph h- lys7-2 *Schizosaccharomyces pombe* NCYC 2022
Lysine auxotroph h- lys8-1 *Schizosaccharomyces pombe* NCYC 1936
Lysine auxotroph h- lys9-1 *Schizosaccharomyces pombe* NCYC 1938
Lysine auxotroph h+ lys7-1 *Schizosaccharomyces pombe* NCYC 1935
Lysine auxotroph h- lys6-1 *Schizosaccharomyces pombe* NCYC 1932
Lysine auxotroph h- lys6-3 *Schizosaccharomyces pombe* NCYC 2018
Lysine auxotroph h- lys2-97 *Schizosaccharomyces pombe* NCYC 1923
Lysine auxotroph h+ lys2-97 *Schizosaccharomyces pombe* NCYC 1924
Lysine auxotroph h- lys3-97 *Schizosaccharomyces pombe* NCYC 1925
Lysine auxotroph h+ lys3-97 *Schizosaccharomyces pombe* NCYC 1926
Lysine auxotroph h- lys4-95 *Schizosaccharomyces pombe* NCYC 1927
Lysine auxotroph h- lys5-931 *Schizosaccharomyces pombe* NCYC 1929
Lysine auxotroph h- lys1-131 *Schizosaccharomyces pombe* NCYC 1921
Lysine auxotroph h+ lys6-1 *Schizosaccharomyces pombe* NCYC 1933
Lysine auxotroph h+ lys4-95 *Schizosaccharomyces pombe* NCYC 1928
Lysine auxotroph h+ lys6-3 *Schizosaccharomyces pombe* NCYC 2019
Lysine auxotroph h+ lys1-131 *Schizosaccharomyces pombe* NCYC 1922
Lysine auxotroph h+ lys9-1 *Schizosaccharomyces pombe* NCYC 1939
Lysine auxotroph h+ lys5-931 *Schizosaccharomyces pombe* NCYC 1930
Lysine auxotroph h- lys7-1 *Schizosaccharomyces pombe* NCYC 1934
Lysine ECO decarboxylaseplasmid encoded Plasmid NCTC 50197
Mapping strain K396-22B *Saccharomyces cerevisiae* NCYC 1617
Mapping strain K382-19D *Saccharomyces cerevisiae* NCYC 1612
Mapping strain K393-35C *Saccharomyces cerevisiae* NCYC 1616
Mapping strain K399-7D *Saccharomyces cerevisiae* NCYC 1615
Mapping strain X4120-19D *Saccharomyces cerevisiae* NCYC 1661
Mapping strain K398-4D *Saccharomyces cerevisiae* NCYC 1613
Mapping strain X4119-19C *Saccharomyces cerevisiae* NCYC 1618
Mapping strain K381-9D *Saccharomyces cerevisiae* NCYC 1614
Mapping strain STX66-4A *Saccharomyces cerevisiae* NCYC 1619
Mapping strain STX 147-4C *Saccharomyces cerevisiae* NCYC 1786
Mapping strain X2928-3D-1C *Saccharomyces cerevisiae* NCYC 959
Mapping strain X2928-3D-1A *Saccharomyces cerevisiae* NCYC 958
Mapping strain STX77-6C *Saccharomyces cerevisiae* NCYC 1620
Mapping strain K382-23A *Saccharomyces cerevisiae* NCYC 1611
Further Mapping strains of *Schizosaccharomyces pombe consult* NCYC
Membrane ATPase esistant to diguanidines h+ pma1 leu1-32 *Schizosaccharomyces pombe* NCYC 1726
Membrane ATPase esistant to diguanidines h- pma1 ade7-413 *Schizosaccharomyces pombe* NCYC 1725
Membrane ATPase esistant to diguanidines h- pma prototroph *Schizosaccharomyces pombe* NCYC 1728
Membrane mitochondrial ATPAse h- atp2-1 leu1-32 *Schizosaccharomyces pombe* NCYC 1727
Membrane mitochondrial ATPAse h- atp1-1 leu1-32 *Schizosaccharomyces pombe* NCYC 1724
Methionine auxotroph h- met5-1 *Schizosaccharomyces pombe* NCYC 1878
Methionine auxotroph h- met3-15 *Schizosaccharomyces pombe* NCYC 1946
Methionine auxotroph h+ met3-1 *Schizosaccharomyces pombe* NCYC 1945

Methionine auxotroph h+ met3-15
Schizosaccharomyces pombe NCYC 1947
Methionine auxotroph h+ met4-3
Schizosaccharomyces pombe NCYC 2190
Methionine auxotroph h- met1-1
Schizosaccharomyces pombe NCYC 1940
Methionine auxotroph h+ met5-1
Schizosaccharomyces pombe NCYC 1879
Methionine auxotroph h+ met2-1
Schizosaccharomyces pombe NCYC 1943
Methionine auxotroph h- met4-3
Schizosaccharomyces pombe NCYC 2189
Methionine auxotroph h+ met1-1
Schizosaccharomyces pombe NCYC 1941
Methionine auxotroph h- met3-1
Schizosaccharomyces pombe NCYC 1944
Methionine auxotroph h- met2-1
Schizosaccharomyces pombe NCYC 1942
MINI INC -GAL plasmids Inc group F11 pMU 610
NCTC 50257
MINI INC -GAL plasmids Inc group FV pMU 1462
NCTC 50448
MINI INC -GAL plasmids Inc group FV pMU 605
NCTC 50252
MINI INC -GAL plasmids Inc group I2pMU 615
NCTC 50260
MINI INC -GAL plasmids Inc group B pMU 602
NCTC 50249
MINI INC -GAL plasmids Inc group 0 pMU 601
NCTC 50248
MINI INC -GAL plasmids Inc group F1 pMU 2205
NCTC 50444
MINI INC -GAL plasmids Inc group P pMU 606
NCTC 50253
MINI INC -GAL plasmids Inc group MpMU 604
NCTC 50251
MINI INC -GAL plasmids Inc group K pMU 2209
NCTC 50447
MINI INC -GAL plasmids Inc group 1GAMMA
pMU 1530 NCTC 50442
MINI INC -GAL plasmids Inc group N pMU 603
NCTC 50250
MINI INC -GAL plasmids Inc group Q pMU 608
NCTC 50255
MINI INC -GAL plasmids Inc group FIpMU 614
NCTC 50528
MINI INC -GAL plasmids Inc group T pMU 607
NCTC 50254
MINI INC -GAL plasmids Inc group U pMU 2208
NCTC 20446
MINI INC -GAL plasmids Inc group WpMU 613
NCTC 50259
MINI INC -GAL plasmids Inc group X pMU 609
NCTC 50256
MINI INC -GAL plasmids Inc group Y pMU 2206
NCTC 50445
MINI INC -GAL plasmids Inc group Z pMU 2200
NCTC 50443
Missense surpressor h+ ade7-465 sup7-465
Schizosaccharomyces pombe NCYC 2230
Missense surpressor h- ade7-465 sup7-465
Schizosaccharomyces pombe NCYC 2229
Missense surpressor h- ade7-465 sup6-465
Schizosaccharomyces pombe NCYC 2207
Missense surpressor h+ ade7-541 sup5-541
Schizosaccharomyces pombe NCYC 2206
Missense surpressor h- ade7-541 sup5-541
Schizosaccharomyces pombe NCYC 2205
Missense surpressor h+ ade7-465 sup6-465
Schizosaccharomyces pombe NCYC 2208
Monster mutant
Chlamydomonas moewusii CCAP 11/6B
Mutant that does not form gas-vacuoles
Anabaena flo aquae CCAP 1403/13d
Mutation affecting Mating type switching h90 swi3-1
Schizosaccharomyces pombe NCYC 1990
Mutation affecting Mating type switching h90 swi 10-154
Schizosaccharomyces pombe NCYC 2004
Mutation affecting Mating type switching h90 swi 9-136
Schizosaccharomyces pombe NCYC 2005
Mutation affecting Mating type switching h90 swi8-1
Schizosaccharomyces pombe NCYC 1991
Mutation affecting Mating type switching h90 swi5-39
Schizosaccharomyces pombe NCYC 1987
Mutation affecting Mating type switching h90 swi6-115 ade 6-M210
Schizosaccharomyces pombe NCYC 1986
Mutation affecting Mating type switching h90 swi2-3
Schizosaccharomyces pombe NCYC 1989
Mutation affecting Mating type switching h90 swi1-1
Schizosaccharomyces pombe NCYC 1993
Mutation affecting Mating type switching h90 swi4-1
Schizosaccharomyces pombe NCYC 1992
Mutation affecting Mating type switching h90 swi7-155
Schizosaccharomyces pombe NCYC 1988
Mutation affecting meiosis h90
Schizosaccharomyces pombe NCYC 1783
Mutation affecting sporulation h90 spo15-B225 ade6-M210
Schizosaccharomyces pombe NCYC 1780
Mutation affecting sporulation h90 spo6-B79 ade6-M210
Schizosaccharomyces pombe NCYC 1777
Mutation affecting sporulation h90 spo5 B37 ade6-M210
Schizosaccharomyces pombe NCYC 1776
Mutation affecting sporulation h90 spo4-B4 ade6-M210
Schizosaccharomyces pombe NCYC 1775
Mutation affecting sporulation h90 spo3-B3 ade6-M210
Schizosaccharomyces pombe NCYC 1774
Mutation affecting sporulation h90 spo20 -KC104 ade6-M216 ura1 leu1
Schizosaccharomyces pombe NCYC 1782
Mutation affecting sporulation h90 h90 spo18-B317 ade6-M210
Schizosaccharomyces pombe NCYC 1781
Mutation affecting sporulation h90 spo14-B221 ade6-M210
Schizosaccharomyces pombe NCYC 1779
Mutation affecting sporulation h90 spo13-B90 ade6-M210
Schizosaccharomyces pombe NCYC 1778

Mutation affecting sporulation h90 spo2-B1 ade6-M210
Schizosaccharomyces pombe NCYC 1773
Mutation affecting sterility h90 ste1101 leu1
Schizosaccharomyces pombe NCYC 1784
Mutation affecting sterility h90 leu1 ste7
Schizosaccharomyces pombe NCYC 2001
Mutation affecting sterility h90 leu1 ste3
Schizosaccharomyces pombe NCYC 1998
Mutation affecting sterility h90 leu1 ste8
Schizosaccharomyces pombe NCYC 2002
Mutation affecting sterility h90 leu1 ste6
Schizosaccharomyces pombe NCYC 2000
Mutation affecting sterility h90 leu1 ste5
Schizosaccharomyces pombe NCYC 1999
Mutation affecting sterility h- ste11-1 his2 ade5-M216
Schizosaccharomyces pombe NCYC 1785
Mutation affecting sterility h+N ura5-294 ste1-1
Schizosaccharomyces pombe NCYC 2003
Mutation affecting sterility h90 leu1 ste2
Schizosaccharomyces pombe NCYC 1997
Mutation affecting sterility h90 ade6-M216 ste4
Schizosaccharomyces pombe NCYC 1996
Mutation affecting vegataive iodine reaction h-S vir1 leu1
Schizosaccharomyces pombe NCYC 1995
Mutation affecting vegataive iodine reaction mat2-102
Schizosaccharomyces pombe NCYC 2172
Mutation affecting vegataive iodine reaction h+N vir1 leu1
Schizosaccharomyces pombe NCYC 1994
Non-gas vacuolate
Microcystis sp. CCAP 1450/14
Nuclear divisin arrest h+ his2 nda7-KM465
Schizosaccharomyces pombe NCYC 2237
Nuclear divisin arrest h- leu1 nda4 KM108
Schizosaccharomyces pombe NCYC 2234
Nuclear divisin arrest h-leu1 nda1-KM376
Schizosaccharomyces pombe NCYC 2231
Nuclear divisin arrest h- arg1 nda8-KM476
Schizosaccharomyces pombe NCYC 2238
Nuclear divisin arrest h- arg1 nda6-KM48
Schizosaccharomyces pombe NCYC 2236
Nuclear divisin arrest h- leu1 nda3-KM311
Schizosaccharomyces pombe NCYC 2233
Nuclear divisin arrest h- arg1 nda5-KM4
Schizosaccharomyces pombe NCYC 2235
Nuclear divisin arrest h- arg1 nda9-KM52J
Schizosaccharomyces pombe NCYC 2239
Nuclear divisin arrest h- arg1 nda11-KM138
Schizosaccharomyces pombe NCYC 2241
Nuclear divisin arrest h- arg1 nda12-KM170
Schizosaccharomyces pombe NCYC 2242
Nuclear divisin arrest h- arg1 nda10-KM3
Schizosaccharomyces pombe NCYC 2240
Nuclear fission defective ABq21
Saccharomyces cerevisiae NCYC 917
Nuclear fission defective JCK5-5A
Saccharomyces cerevisiae NCYC 916
Phage strain Virulent
Escherichia coli NCIMB 11289
Phage strain Virulent- T series mutant
Escherichia coli NCIMB 11320
Phage strain Virulent
Escherichia coli NCIMB 10093
Phage strain Virulent
Escherichia coli NCIMB 10116
Phage strain Virulent
Escherichia coli NCIMB 10108
Phage strain Virulent
Escherichia coli NCIMB 10382
Phage strain Temperate
Pseudomonas aeruginosa NCIMB 10882
Phage strain Virulent- T series wildtype
Escherichia coli NCIMB 11297
Phage strain Virulent- T series mutant
Escherichia coli NCIMB 10359
Escherichia coli NCIMB 11233
Escherichia coli NCIMB 11234
Escherichia coli NCIMB 11235
Escherichia coli NCIMB 11236
Escherichia coli NCIMB 11319
Escherichia coli NCIMB 11321
Phage strain Virulent- T series mutant
Escherichia coli NCIMB 11325
Escherichia coli NCIMB 11332
Escherichia coli NCIMB 11323
Escherichia coli NCIMB 11291
Phage strain RNA-single stranded
Escherichia coli NCIMB 11289
Phage strain Virulent
Bacillus subtilis NCIMB 10705
Phage strain Temperate
Pseudomonas aeruginosa NCIMB 10883
Salmonella typhimarium NCIMB 10441
Phage strain Rapid lysing mutants
Escherichia coli NCIMB 10359
Phage strain Temperate sensitive
Escherichia coli NCIMB 11235
Escherichia coli NCIMB 11236
Phage strain Transducer
Pseudomonas aeruginosa NCIMB 10880
Pseudomonas aeruginosa NCIMB 10882
Phage strain T4 mutants -deletion mutation
Escherichia coli NCIMB 11323
Phage strain T4 mutants - point mutation
Escherichia coli NCIMB 11319
Escherichia coli NCIMB 11320
Escherichia coli NCIMB 11321
Escherichia coli NCIMB 11322
Phage strain Virulent- T series mutant
Escherichia coli NCIMB 11732
Phage strain Amber / surpreesive mutant
Escherichia coli NCIMB 11234
Phage strain Virulent
Micrococcus luteus NCIMB 10458
Phage strain Virulent
Pseudomonas aeruginosa NCIMB 10884
Pseudomonas syringae NCIMB 11267
Phage strain Indicator strain lysed by phage lamda
Escherichia coli NCIMB 9481
Escherichia coli NCIMB 10084
Escherichia coli NCIMB 11459
Phage strain Indicator strain
Pseudomonas aerunginosa NCIMB 10522
Phage strain Prevents production of T4amN120 and T4amB17 (infe)
Escherichia coli NCIMB 11838
Phage strain Generalised transduces
Salmonella typhimarium NCIMB 10411
Phage strain Restricted transducer
Escherichia coli NCIMB 10451

Phage strain Virulent
Escherichia coli NCIMB 11325
Phage strain DNA single stranded
Escherichia coli NCIMB 11332
Phage strain Indicator strain
Pseudomonas aerunginosa NCIMB 10523
Phage strain DNA single stranded
Escherichia coli NCIMB 10382
Phage strain Clear plaque mutant
Escherichia coli NCIMB 11324
Phage strain Amber / surpreesive mutant
Escherichia coli NCIMB 11233
Phage strain Virulent- T series wildtype
Escherichia coli NCIMB 10380
Escherichia coli NCIMB 10092
Escherichia coli NCIMB 10358
Escherichia coli NCIMB 10360
Escherichia coli NCIMB 10377
Escherichia coli NCIMB 10379
Phage strain Virulent
Serratia marcscens NCIMB 10644
Phage strain Extended host range
Escherichia coli NCIMB 10359
Phage strain Rapid lysing
Escherichia coli NCIMB 11321
Phage strain Generalised transduces
Escherichia coli NCIMB 11291
Phage strain Rapid lysing mutants
Escherichia coli NCIMB 11320
Escherichia coli NCIMB 11319
Phage strain Inducible non-transducer
Pseudomonas aerunginosa NCIMB 10881
Pseudomonas aerunginosa NCIMB 10882
Phage strain Lamda lysogen used to prepare packing extracts
Escherichia coli NCIMB 12191
Phage strain Lamda lysogen used to prepare packing extracts
Escherichia coli NCIMB 12192
Phage strain Lipid containing
Pseudomonas phaswolicola NCIMB 11267
Phage strain Male specific
Escherichia coli NCIMB 10108
Phage strain Rapid lysing mutants
Escherichia coli NCIMB 11323
Phage strain RNA-double stranded
Pseudomonas syringae NCIMB 11267
Phage strain Temperate
Pseudomonas aeruginosa NCIMB 10881
Phage strain Non-inducible transducer
Pseudomonas aeruginosa NCIMB 10883
Pseudomonas aerunginosa NCIMB 10880
Phage strain Lysogenic for phage lamda
Escherichia coli NCIMB 9483
Phage strain Male specific
Escherichia coli NCIMB 11289
Phage strain RNA-single stranded
Escherichia coli NCIMB 11108
Phage strain Temperate
Pseudomonas aeruginosa NCIMB 10880
Escherichia coli NCIMB 11733
Escherichia coli NCIMB 11324
Escherichia coli NCIMB 11291
Escherichia coli NCIMB 10451

Phage strain Male specific
Escherichia coli NCIMB 11332
Plasmids used for molecular weight determinations
MW 36 RP4 NCTC 50078
Plasmids used for molecular weight determinations
MW 110 R716 NCTC 50052
Plasmids used for molecular weight determinations
MW 2.6 Pbr322 NCTC 50088
Plasmids used for molecular weight determinations
MW 5.7 R3OOB NCTC 50020
Plasmids used for molecular weight determinations
MW 13.2 ColE1::Tn7 NCTC 50117
Plasmids used for molecular weight determinations
MW 26 R6K NCTC 50005
Plasmids used for molecular weight determinations
MW 36 RP1 NCTC 50076
Plasmids used for molecular weight determinations
MW 166 R478 NCTC 80038
Plasmids used for molecular weight determinations
MW 18.6, 3.1 Strain SK18 NCTC 50580
Plasmids used for molecular weight determinations
MW 32,5.2,3.5,3.0,2.2,1.7,1.5,1.2 train V517 NCTC 50193
Plasmids used for molecular weight determinations
MW 98,42,23.9,4.6 Strain 39R861 NCTC 50192
Plasmids used for molecular weight determinations
MW 95 RIP64 NCTC 50075
Plasmids used for molecular weight determinations
MW 300 R931 NCTC 50066
Plasmids used for molecular weight determinations
MW 44 R2 NCTC 50004
Plasmids used for molecular weight determinations
MW 145 Rms163 NCTC 80082
Plasmids used for molecular weight determinations
MW 120 Rms148 NCTC 50080
Plasmids used for molecular weight determinations
MW 112 R27 NCTC 50010
Plasmids used for molecular weight determinations
MW 96 pIP40a NCTC 50102
Plasmids used for molecular weight determinations
MW 72 R64 NCTC 50012
Plasmids used for molecular weight determinations
MW 62 R1 NCTC 50001
Plasmids used for molecular weight determinations
MW 46 R702 NCTC 50047
Plasmids used for molecular weight determinations
MW 312 Pmg1 NCTC 50108
Polarised sex linked mutant
Chlamydomonas moewusii CCAP 11/16H
Proline auxotroph h- pro2-1
Schizosaccharomyces pombe NCYC 1950
Proline auxotroph h- pro1-1
Schizosaccharomyces pombe NCYC 1948
Proline auxotroph h+ pro1-1
Schizosaccharomyces pombe NCYC 1949
Proline auxotroph h+ pro2-1
Schizosaccharomyces pombe NCYC 1951
Pseudomycelium Teaching strain
Candida krusei NCYC 1398
Purine auxotroph h- hyp4-24
Schizosaccharomyces pombe NCYC 2101
Purine auxotroph h- hyp5-23
Schizosaccharomyces pombe NCYC 2103
Purine auxotroph h- hyp2-26
Schizosaccharomyces pombe NCYC 2097

Purine auxotroph h+ all1-2
Schizosaccharomyces pombe NCYC 2092
Purine auxotroph h+ ure4-1
Schizosaccharomyces pombe NCYC 2112
Purine auxotroph h- ure4-1
Schizosaccharomyces pombe NCYC 2111
Purine auxotroph h- ure1-1
Schizosaccharomyces pombe NCYC 2105
Purine auxotroph h- hyp3-20
Schizosaccharomyces pombe NCYC 2099
Purine auxotroph h+ hyp3-20
Schizosaccharomyces pombe NCYC 2100
Purine auxotroph h- all1-2
Schizosaccharomyces pombe NCYC 2091
Purine auxotroph h+ hyp2-26
Schizosaccharomyces pombe NCYC 2098
Purine auxotroph h- all2-1
Schizosaccharomyces pombe NCYC 2093
Purine auxotroph h+ hyp4-24
Schizosaccharomyces pombe NCYC 2102
Purine auxotroph h+ hyp5-23
Schizosaccharomyces pombe NCYC 2104
Purine auxotroph h- uro1-1
Schizosaccharomyces pombe NCYC 2113
Purine auxotroph h+ ure1-1
Schizosaccharomyces pombe NCYC 2106
Purine auxotroph h- ure2-1
Schizosaccharomyces pombe NCYC 2107
Purine auxotroph h+ ure2-1
Schizosaccharomyces pombe NCYC 2108
Purine auxotroph h- ure3-1
Schizosaccharomyces pombe NCYC 2109
Purine auxotroph h+ ure3-1
Schizosaccharomyces pombe NCYC 2110
Purine auxotroph h- hyp1-50
Schizosaccharomyces pombe NCYC 2095
Purine auxotroph h+ all2-1
Schizosaccharomyces pombe NCYC 2094
Purine auxotroph h+ hyp1-50
Schizosaccharomyces pombe NCYC 2096
Purine auxotroph h+ ala1-1
Schizosaccharomyces pombe NCYC 2090
Purine auxotroph h+ uro1-1
Schizosaccharomyces pombe NCYC 2114
Purine auxotroph h- ala1-1
Schizosaccharomyces pombe NCYC 2089
Rad diploid strains
Saccharomyces cerevisiae NCYC 1637
Saccharomyces cerevisiae NCYC 1630
Saccharomyces cerevisiae NCYC 1792
Radiation Sensitice strains CM8/1a
Saccharomyces cerevisiae NCYC 1803
Radiation Sensitice strains CM30/2c
Saccharomyces cerevisiae NCYC 1806
Radiation Sensitice strains CM5/1B
Saccharomyces cerevisiae NCYC 1764
Radiation Sensitice strains CM31/1d
Saccharomyces cerevisiae NCYC 1799
Radiation Sensitice strains CM4/1d
Saccharomyces cerevisiae NCYC 1763
Radiation Sensitice strains CM1/8a
Saccharomyces cerevisiae NCYC 1801
Radiation Sensitice strains CM21/9a
Saccharomyces cerevisiae NCYC 1805
Radiation Sensitice strains CM26/4c
Saccharomyces cerevisiae NCYC 1800
Radiation Sensitice strains
Saccharomyces cerevisiae NCYC 1639
Radiation Sensitice strains g739-2a
Saccharomyces cerevisiae NCYC 1640
Radiation Sensitice strains CM1/1c
Saccharomyces cerevisiae NCYC 1802
Radiation Sensitice strains CM9/1a
Saccharomyces cerevisiae NCYC 1804
Radiation Sensitice strains g725-12a
Saccharomyces cerevisiae NCYC 1749
Radiation Sensitice strains g739-2d
Saccharomyces cerevisiae NCYC 1721
Radiation sensitive h- rad15-P
Schizosaccharomyces pombe NCYC 1976
Radiation sensitive h- rad4-116
Schizosaccharomyces pombe NCYC 1965
Radiation sensitive h- rad5-158
Schizosaccharomyces pombe NCYC 1966
Radiation sensitive h+ rad1-rad 10-98
Schizosaccharomyces pombe NCYC 1984
Radiation sensitive h- rad13A
Schizosaccharomyces pombe NCYC 1974
Radiation sensitive h- rad11-404
Schizosaccharomyces pombe NCYC 1972
Radiation sensitive h- rad10-198
Schizosaccharomyces pombe NCYC 1971
Radiation sensitive h- rad9-192
Schizosaccharomyces pombe NCYC 1970
Radiation sensitive h- rad8-190
Schizosaccharomyces pombe NCYC 1969
Radiation sensitive h- rad17W
Schizosaccharomyces pombe NCYC 1978
Radiation sensitive h- rad14G
Schizosaccharomyces pombe NCYC 1975
Radiation sensitive h- rad16U
Schizosaccharomyces pombe NCYC 1977
Radiation sensitive h- rad3-136
Schizosaccharomyces pombe NCYC 1964
Radiation sensitive h- rad2-44
Schizosaccharomyces pombe NCYC 1963
Radiation sensitive h- rad1
Schizosaccharomyces pombe NCYC 1962
Radiation sensitive h- rad7-185
Schizosaccharomyces pombe NCYC 1968
Radiation sensitive h+ rad21 rad22
Schizosaccharomyces pombe NCYC 1985
Radiation sensitive h- rad22-67
Schizosaccharomyces pombe NCYC 1983
Radiation sensitive h- rad12-502
Schizosaccharomyces pombe NCYC 1973
Radiation sensitive h- rad21-45
Schizosaccharomyces pombe NCYC 1982
Radiation sensitive h- rad20M25
Schizosaccharomyces pombe NCYC 1981
Radiation sensitive h- rad18X
Schizosaccharomyces pombe NCYC 1979
Radiation sensitive h- rad19M9
Schizosaccharomyces pombe NCYC 1980
Radiation sensitive h- rad6-165
Schizosaccharomyces pombe NCYC 1967
RNA mutants ts96
Saccharomyces cerevisiae NCYC 1758
RNA mutants rna3-3 (D43)
Saccharomyces cerevisiae NCYC 1745
RNA mutants rna3-4 (D167)
Saccharomyces cerevisiae NCYC 1746

Sporulation deficient mutants K396-22B
Saccharomyces cerevisiae NCYC 1617
Sporulation deficient mutants K398-4D
Saccharomyces cerevisiae NCYC 1613
Sporulation deficient mutants K381-9D
Saccharomyces cerevisiae NCYC 1614
Sporulation deficient mutants K399-7D
Saccharomyces cerevisiae NCYC 1615
Sporulation deficient mutants K382-23A
Saccharomyces cerevisiae NCYC 1611
Sporulation deficient mutants K393-35C
Saccharomyces cerevisiae NCYC 1616
Sporulation deficient mutants K382-19D
Saccharomyces cerevisiae NCYC 1612
Strontium chromateFungicide resistant
Acremonium strictum CABI-IMI 178506
Sterile mutant
Chlamydomonas sphagnophila v. *dysomos*
CCAP 11/36B
Sucinate (accumulation) Fungicide resistant
Brettanomyces bruxellensis NCYC 370
Sulphonamide resistance antibact mechanism DHPS-II R300B NCTC 50020
Sulphonamide resistance antibact mechanism DHPS-I R1 NCTC 50001
Sulphonamide resistance antibact mechanism DHPS-I R388 NCTC 50023
Surpressor (nonsense) h- ade7-413 sup1-35
Schizosaccharomyces pombe NCYC 2201
Surpressor (nonsense) h+ ade7-413 sup1-35
Schizosaccharomyces pombe NCYC 2202
Surpressor (nonsense) h+ ade7-413 sup2-413
Schizosaccharomyces pombe NCYC 2204
Surpressor (nonsense) h- ade7-413 sup2-413
Schizosaccharomyces pombe NCYC 2203
Surpressor (UAA urprsessor) h+ ade7-413 sup3-18
Schizosaccharomyces pombe NCYC 2212
Surpressor (UAA urprsessor) h- ade7-413 sup 3-18
Schizosaccharomyces pombe NCYC 2209
Surpressor (UAA urprsessor) h- ade7-413 sup3-18
Schizosaccharomyces pombe NCYC 2211
Surpressor (UAA urprsessor) h+ ade7-413 sup 3-18
Schizosaccharomyces pombe NCYC 2210
Surpressor (UGA) h- ade6-704 sup3-5
Schizosaccharomyces pombe NCYC 2221
Surpressor (UGA) h+ ade6-704sup8-4
Schizosaccharomyces pombe NCYC 2214
Surpressor (UGA) h- ade6-704sup9-169
Schizosaccharomyces pombe NCYC 2215
Surpressor (UGA) h+ ade6-704sup10-152
Schizosaccharomyces pombe NCYC 2218
Surpressor (UGA) h+ ade6-704sup9-169
Schizosaccharomyces pombe NCYC 2216
Surpressor (UGA) h+ ade6-704 sup3-5
Schizosaccharomyces pombe NCYC 2222
Surpressor (UGA) h- ade6-704sup10-152
Schizosaccharomyces pombe NCYC 2217
Surpressor (UGA) h- ade6-704 sup3-UCGsup 12-1
Schizosaccharomyces pombe NCYC 2219
Surpressor (UGA) h+ ade6-704 sup3-UCGsup 12-1
Schizosaccharomyces pombe NCYC 2220
Surpressor (UGA) h- ade6-704sup8-4
Schizosaccharomyces pombe NCYC 2213
Surpressor 9cdc25) h- stf1-1
Schizosaccharomyces pombe NCYC 2367
Temperature sensitice lethals h- tps19-17
Schizosaccharomyces pombe NCYC 2034
Temperature sensitice lethals h- tps16-26
Schizosaccharomyces pombe NCYC 2067
Temperature sensitice lethals h+ tps15-119
Schizosaccharomyces pombe NCYC 2082
Temperature sensitice lethals h- tps14-5
Schizosaccharomyces pombe NCYC 2032
Temperature sensitice lethals h+ tps16-26
Schizosaccharomyces pombe NCYC 2068
Temperature sensitice lethals h+ tps14-5
Schizosaccharomyces pombe NCYC 2033
Temperature sensitice lethals h- tps17-4
Schizosaccharomyces pombe NCYC 2061
Temperature sensitice lethals h+ tps13-25
Schizosaccharomyces pombe NCYC 2031
Temperature sensitice lethals h- tps13-24
Schizosaccharomyces pombe NCYC 2030
Temperature sensitice lethals h+ tps25-97
Schizosaccharomyces pombe NCYC 2064
Temperature sensitice lethals h- tps28-9
Schizosaccharomyces pombe NCYC 2059
Temperature sensitice lethals h+ tps19-17
Schizosaccharomyces pombe NCYC 2035
Temperature sensitice lethals h+ tps25-97
Schizosaccharomyces pombe NCYC 2060
Temperature sensitice lethals h+ tps17-4
Schizosaccharomyces pombe NCYC 2062
Temperature sensitice lethals h- tps25-97
Schizosaccharomyces pombe NCYC 2063
Temperature sensitice lethals h+ tps24-113
Schizosaccharomyces pombe NCYC 2074
Temperature sensitice lethals h- tps24-113
Schizosaccharomyces pombe NCYC 2073
Temperature sensitice lethals h+ tps20-104
Schizosaccharomyces pombe NCYC 2066
Temperature sensitice lethals h- tps20-104
Schizosaccharomyces pombe NCYC 2065
Temperature sensitice lethals h- tps15-119
Schizosaccharomyces pombe NCYC 2081
Tetracycline resistance antibact mechanism TET-C p BR322 NCTC 50270
Tetracycline resistance antibact mechanism TET-E p SL 1504 NCTC 50272
Tetracycline resistance antibact mechanism TET-M TP-TC NCTC 50354
Tetracycline resistance antibact mechanism TET-O p UA466 NCTC 50452
Tetracycline resistance antibact mechanism TET-D p SL106 NCTC 50271
Tetracycline resistance antibact mechanism TET-A RP1 NCTC 50076
Tetracycline resistance antibact mechanism TET-C p SC 101 NCTC 50112
Tetracycline resistance antibact mechanism TET-B Tn10 NCTC 50243
Tetracycline resistance antibact mechanism TET-A RP4 NCTC 50078
Tetracycline resistance antibact mechanism TET-E p SL1456NCTC 50273
Tetracycline resistance antibact mechanism TET-A p SL18 NCTC 50268
Tetracycline resistance antibact mechanism TET-B p RT1 1 NCTC 50365

Tetracycline resistance antibact mechanism TET-B p KT007 NCTC 50269
Tetracycline resistance antibact mechanism TET-B R222Jap NCTC 50019
Tetracycline resistance antibact mechanism TET-D ra1 NCTC 50073
THIABenoDAZOLE resistant h- ben4.D3 leu1.320 *Schizosaccharomyces pombe* NCYC 1715
Thrichodermin resistant h- Tri3 N1 *Schizosaccharomyces pombe* NCYC 1353
Transformable strains LL20 *Saccharomyces cerevisiae* NCYC 1445
Transformable strains MD40/C *Saccharomyces cerevisiae* NCYC 1527
Transformable strains AH22 *Saccharomyces cerevisiae* NCYC 1528
Transformable strains D13-1A (YNN6) *Saccharomyces cerevisiae* NCYC 1627
Transformable strains cdc9-1 (L89-6C) *Saccharomyces cerevisiae* NCYC 1771
Transformable strains DBY 747 *Saccharomyces cerevisiae* NCYC 1383
Transformable strains MC16 *Saccharomyces cerevisiae* NCYC 1392
Transposon donor vectors Tn 1733 p RU738 NCTC 50233
Transposon donor vectors Tn 1721 p JOE105 NCTC 50230
Transposon donor vectors Tn 1732 p RU666 NCTC 50232
Transposon donor vectors Tn 1725 p RU667 NCTC 50234
Trimethroprin resistance antibact mechanism DHFR-I Tn7 NCTC 50190
Trimethroprin resistance antibact mechanism DHFR-V p LK09 NCTC 50515
Trimethroprin resistance antibact mechanism DHFR-I R483 NCTC 50039
Trimethroprin resistance antibact mechanism DHFR-I p FE872 NCTC 50535
Trimethroprin resistance antibact mechanism DHFR-IV p UK1148 NCTC 50536
Trimethroprin resistance antibact mechanism DHFR-III p FE1242 NCTC 50514
Trimethroprin resistance antibact mechanism DHFR-II p WZ280 NCTC 50537
Trimethroprin resistance antibact mechanism DHFR-II R388 NCTC 50023
Trimethroprin resistance antibact mechanism DHFR-II R751 NCTC 50057
Trimethroprin resistance antibact mechanism DHFR-I p HH1508a NCTC 50516
Tryptophan auxotroph h- trp3-25 *Schizosaccharomyces pombe* NCYC 2048
Tryptophan auxotroph h+ trp1-S24 *Schizosaccharomyces pombe* NCYC 2086
Tryptophan auxotroph h+ trp3-25 *Schizosaccharomyces pombe* NCYC 2049
Tryptophan auxotroph h+ trp 2-21 *Schizosaccharomyces pombe* NCYC 2130
Tryptophan auxotroph h- trp4-47 *Schizosaccharomyces pombe* NCYC 2077
Tryptophan auxotroph h- trp2-21 *Schizosaccharomyces pombe* NCYC 2129
Tryptophan auxotroph h+ tryp1-S11 *Schizosaccharomyces pombe* NCYC 2084
Tryptophan auxotroph h- tryp1-S11 *Schizosaccharomyces pombe* NCYC 2083
Tryptophan auxotroph h+ tryp1-556 *Schizosaccharomyces pombe* NCYC 2188
Tryptophan auxotroph h- tryp1-556 *Schizosaccharomyces pombe* NCYC 2187
Tryptophan auxotroph h+ tryp1-3 *Schizosaccharomyces pombe* NCYC 2126
Tryptophan auxotroph h- tryp1-3 *Schizosaccharomyces pombe* NCYC 2125
Tryptophan auxotroph h+ trp4-47 *Schizosaccharomyces pombe* NCYC 2078
Tryptophan auxotroph h- trp1-S24 *Schizosaccharomyces pombe* NCYC 2085
Tyrosine-phenyalanine auxotroph h- phe2-87 *Schizosaccharomyces pombe* NCYC 2079
Tyrosine-phenyalanine auxotroph h- tyr1-62 *Schizosaccharomyces pombe* NCYC 2127
Tyrosine-phenyalanine auxotroph h+ phe2-87 *Schizosaccharomyces pombe* NCYC 2080
Tyrosine-phenyalanine auxotroph h+ phe1-115 *Schizosaccharomyces pombe* NCYC 2076
Tyrosine-phenyalanine auxotroph h- tyr2-78 *Schizosaccharomyces pombe* NCYC 2072
Tyrosine-phenyalanine auxotroph h+ tyr1-62 *Schizosaccharomyces pombe* NCYC 2128
Tyrosine-phenyalanine auxotroph h- tyr2-78 *Schizosaccharomyces pombe* NCYC 2071
Tyrosine-phenyalanine auxotroph h- phe1-115 *Schizosaccharomyces pombe* NCYC 2075
Uracil auxotroph h- ura2-10 *Schizosaccharomyces pombe* NCYC 1956
Uracil auxotroph h- ura5-294 *Schizosaccharomyces pombe* NCYC 1894
Uracil auxotroph h+ ura4D18 *Schizosaccharomyces pombe* NCYC 2037
Uracil auxotroph h- ura4D18 *Schizosaccharomyces pombe* NCYC 2036
Uracil auxotroph h- ura4-294 *Schizosaccharomyces pombe* NCYC 1959
Uracil auxotroph h- ura3-34 *Schizosaccharomyces pombe* NCYC 2024
Uracil auxotroph h+ ura1-171 *Schizosaccharomyces pombe* NCYC 1953
Uracil auxotroph h+ ura3-34 *Schizosaccharomyces pombe* NCYC 2025
Uracil auxotroph h+ ura2-10 *Schizosaccharomyces pombe* NCYC 1957
Uracil auxotroph h+ ura1-61 *Schizosaccharomyces pombe* NCYC 1955
Uracil auxotroph h- ura1-61 *Schizosaccharomyces pombe* NCYC 1954
Uracil auxotroph h- ura1-171 *Schizosaccharomyces pombe* NCYC 1952
Uracil auxotroph h+ ura5-294 *Schizosaccharomyces pombe* NCYC 1895
Uracil auxotroph h+ ura4-294 *Schizosaccharomyces pombe* NCYC 1960

Vaccine producers

Cholera vaccine production
Vibrio cholerae NCTC 10733
Vibrio cholerae NCTC 10732
Farmers lung (Antigen production)
Saccharopolyspora rectivirgula NCIMB 9984
Fish vibriosis
Vibrio ordalii NCIMB 2168
Human virus vaccines
WI 38 ECCAC 90020107
Rubbella vaccine production
Duck embryo ECCAC 89051503
Vaccine experiments
Aeromonas salmonicida subsp. *salmonicida* NCIMB 13076
Vaccine experiments
Aeromonas salmonicida subsp. *salmonicida* NCIMB 13077
Vaccine Production
Pseudomonas aeruginosa NCIMB 10707
Pseudomonas aeruginosa NCIMB 10708
Pseudomonas aeruginosa NCIMB 10709
Pseudomonas aeruginosa NCIMB 10710
Pseudomonas aeruginosa NCIMB 10711
Pseudomonas aeruginosa NCIMB 10712
Pseudomonas aeruginosa NCIMB 10713
Verro ECCAC 88020401
Virus vaccine research
4647 ECCAC 90091902
Yellow fever virus
2D12 ECCAC 91051509

Appendix B Media recipes

The formula for media commonly used by the UKNCC member collections are detailed under five organism groups; algae and protozoa; bacteria; fungi; yeasts and animal cell lines. Further advice and information can be obtained from the appropriate collection.

Media for algae and protozoa

ANT (Antia's Medium) for marine algae

Stock

Trace metals stock solution	
$EDTA.Na_2\ 2H_20$	3.24g
$FeCl_3.4H_20$	1.08g
$MnSO_4.4H_2O$	0.450g
$ZnSO_4.7H_20$	0.230g
$Na_2MoO4.2H_20$	0.097g
$CuSO_4$ 5H20	0.01g
$CoSO_4$ $7H_20$	0.0056g
Distilled water	to 1 litre

Make up to 1 litre with distilled water and adjust to pH 7.6 - 7.8 with dilute HCl or NaOH. Store frozen

Medium

KNO_3	0.05g
$NaH_2PO_4.2H_2O$	0.0078g
Tris (tris(hydroxymethyl) aminomethane)	1.0g
Glycine	0.3g
Trace metals solution stock (chelated)	2.5ml
Thiamine HCl	500.0µg
Cyanocobalamin	2.0µg
Biotin	1.0µg
Filtered natural seawater	800.0ml
Distilled water	to 1 litre

Autoclave at 121°C. Final pH should be between 7.6-7.8

ASW (Artificial Seawater) for marine algae

Stocks

1. Extra salts	
$NaNO_3$	30.0g
Na_2HPO_4	1.2g
K_2HPO_4	1.0g
Distilled water	to 1 litre
2. Vitamin solution	
Biotin	0.0002g
Calcium pantothenate	0.02g
Cynanocobalamin	0.004g
Folic acid	0.0004g
Inositol	1.0g
Nicotinic acid	0.02g
Thiamine HCl	0.01g
Thymine	0.6g
Distilled water	to 1 litre

Stored frozen at -20^{o}C

Medium

Ultramarine Synthetica Sea salts*	33.6g
Extra salts stock solution	3.75ml
Vitamin stock solution	2.5ml
Soil extract (SE 1 see recipe)	25.0ml
Tricine	0.50g
Distilled water	to 1 litre

Adjust to pH 7.6-7.8 with 1M NaOH or HCl
Autoclave at 121°C
*Waterlife Research Industries Ltd, 476 Bath Road, Longford, West Drayton, Middlesex UB7 OED, U.K.

ASW + barley (Artificial Seawater + barley grains) for marine dinoflagellate *Oxyrrhis*

Medium

As for ASW except one grain of barley is added for each 25ml of prepared medium before autoclaving

2ASW (double strength Artificial Seawater) for marine algae

Medium

As for ASW except 35g per litre of NaCl is added before autoclaving

ASW:BG for marine algae

Medium

A 1:1 mixture of ASW and BG : See separate recipes, mix aseptically.

ASWP (Artificial Seawater for Protozoa) for marine protozoa

Stock

Solution (1)	
$NaNO_3$	5.625g
Na_2HPO_4	0.225g
K_2HPO_4	0.188g
Distilled water	to 1 litre

Medium

Ultramarine Synthetica Sea salts*	33.6g
Solution 1	10ml
Soil extract (SE 2 see recipe)	50ml
Tricine	0.50g
Distilled water	to 1 litre

Adjust to pH 7.6-7.8 with 1M NaOH or HCl
Autoclave at 121°C
*Waterlife Research Industries Ltd, 476 Bath Road, Longford, West Drayton, Middlesex UB7 OED, U.K.

BB (Bolds Basal Medium) for freshwater algae

Stocks

Each in 200ml distilled water

1. $NaNO_3$	5.0g
2. $MgSO_4\ 7H_2O$	1.5g
3. NaCl	0.5g
4. K_2HPO_4	1.5g
5. KH_2PO_4	3.5g
6. $CaCl_2 2H_20$	0.5g
7. Trace elements solution	
$ZnSO_4.7H_2O$	8.82g
$MnCl_2.4H_2O$	1.44g
MoO_3	0.71g
$CuSO_4.5H_2O$	1.57g
$Co(NO_3)_2.6H_2O$	0.49
Distilled water	1 litre

May need autoclaving to dissolve

Each in 100ml distilled water

8. H_3BO_3	1.14g
9. EDTA-KOH Solution	
$EDTANa_2$	5.0g
KOH	3.1g
10. $FeS0_4.7H_2O$	4.98g
conc. H_2SO_4	1.0ml
Distilled water	1 litre

Medium

Stock solutions 1-6 each	10.0ml
Stock solutions 7-10 each	1.0ml
Distilled water	to 1 litre

Autoclave at 121C for 15min

For Solid Medium

Add 15.0g per litre of bacteriological agar (Oxoid L11)

BB:MErds for freshwater/brackish water algae

Medium

8:2 mixture or 1:1 mixture
See separate recipes, mix and autoclave at 121°C for 15min

BG (Blue-Green Medium) for marine cyanobacteria

Stocks

1. Extra nutrient salts	
$NaNO_3$	3.0g
K_2HPO_4	10.12g
Na_2HPO_4	0.10g
Distilled water	100ml
2. Trace metal solution	
$ZnSO_4.7H_2O$	0.022g
$MnCl_2.4H_2O$	0.181g
H_3BO_3	0.286g
$CuSO_4.5H_2O$	0.008g
$Co(NO_3)_2.6H_2O$	0.005g
$Na_2MoO_4.2H_2O$	0.039g
Distilled water	100ml

Medium *(The medium is made up in 2 parts)*

Part 1

Tricine	0.50g
Soil extract (SE1 - see recipe)	25ml
Extra nutrient salts (1)	3.75ml
Filtered natural seawater	to 1 litre

Adjust to pH 7.6-7.8 with 1m NaOH or HCl

Part 2

$NaNO_3$	1.5g
$K_2HPO_4.3\ H_2O$	0.040g
$MnSO_4.7H_2O$	0.075g
$CaCl_2.2H_2O$	0.036g
Citric acid	0.006g
Ammonium ferric citrate green	0.006g
EDTA Na_2	0.001g
Na_2CO_3	0.020g
Trace metal; solution (2)	1.0ml
Distilled water	to 1 litre

Adjust to pH7.4

Final

Autoclave parts 1 and 2 separately at 121°C, cool and mix aseptically

For Solid Medium

Add 15g non-nutrient agar per litre of medium

BG11 (Blue-green Medium) for freshwater algae and protozoa

Stocks

In 1 litre distilled water

1. $NaNO_3$

Each in 50ml distilled water

2. K_2HPO_4
3. $MgSO_4\ 7H_2O$
4. $CaCl_2.2H_2O$
5. Citric acid
6. Ammonium ferric citrate green
7. EDTA Na_2
8. Na_2CO_3
9. Trace metal solution

$ZnSO_4.7H_2O$	0.22g
$MnCl_2.4H_2O$	1.81g
H_3BO_3	2.86g
$CuSO_4.5H_2O$	0.08g
$Co(NO_3)_2.6H_2O$	0.05g
$Na_2MoO_4.2H_2O$	0.39g
Distilled water	1 litre

Medium

Stock solution 1	100ml
Stock solutions 2-8	10.0ml each
Stock solution 9	1.0ml
Deionized water	to 1 litre

Adjusted to pH 7.1 with 1M NaOH or HCl
Autoclaved at 121°C for 15min

For Solid Medium

Add 15.0g bacteriological agar (Oxoid L11) per litre of medium

CH (Chalkleys Medium) for freshwater protozoa

Stocks

Each in 100ml deionised water

1. NaCl	2.0g
2. KCl	0.08g
3. $CaCl_2$	0.12g

Medium

Stock solution 1	5ml
Stock solution 2	5ml
Stock solution 3	5ml
Deionised water	to 1 litre

Autoclaved at 121°C for 15min

CHM (Chilomonas Medium) for freshwater protozoa

Sodium acetate trihydrate	1.0g
"Lab Lemco" powder (Oxoid L29)	1.0g
Deionised water	to 1 litre

Autoclaved at 121°C for 15min

CMA (Corn Meal Glucose Agar) for freshwater protozoa

Corn Meal Agar (Oxoid CM103)	17.0g
D-glucose	2.0g
Yeast Extract (Oxoid L21)	1.0g
Deionised water	to 1 litre

Autoclave at 110°C for 15min

DM (Diatom Medium) for freshwater algae

Stocks

Each in 200ml deionised water

1. $Ca(NO_3)$	4.00g
2. KH_2PO_4	2.48g
3. $MgSO_4$.7H2O	5.00g
4. $NaHCO_3$	3.18
5. EDTA FeNa	0.45g
EDTA Na_2	0.45g
6. H_3BO_3	0.496g
$MnCl_2.4H_2O$	0.278g
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.20g
7. Cyanocobalamin	0.008g
Thiamine HCl	0.008g
Biotin	0.008g
8. $NaSiO_3.9H_2O$	11.4g

Medium

Stock solutions 1-8	1.0ml
Deionised water	to 1 litre

Adjusted to pH 6.9 with 1M HCl and autoclaved at 121°C for 15min

EG (Euglena Gracilis Medium) for freshwater algae and protozoa

Stock

1. $CaCl_2$	1.0g
Deionised water	1 litre

Medium

Sodium acetate trihydrate	1.0g
"Lab-Lemco" powder (Oxoid)	1.0g
Tryptone (Oxoid)	2.0g
Yeast Extract (Oxoid)	2.0g
$CaCl_2$ Stock 1	10.0ml
Deionised water	to 1 litre

Autoclave at 121°C for 15min

For Solid Medium

Add 15g Bacteriological agar (Oxoid) per litre of medium

EG:JM

Medium

1:1 mixture
See separate recipes, mix and autoclave at 121°C for 15min

E27 (E27 Medium) for marine algae and sterility testing

Medium

Part1

Soil extract (SE1- see recipe)	25.0ml
KNO_3	0.050g
K_2HPO_4	0.005g
$MgSO_4$ $7H_2O$	0.005g
Glucose	0.250g
Tryptone (Oxoid L42)	0.025g
Liver digest (Oxoid L27)	0.025g
Cyanonobalamin	100.00ng
Thiamine HCl	50.00ug
Distilled water	to 500ml

Part 2

Filtered natural seawater	500ml

Final

Autoclave parts 1 and 2 separately at 121°C, cool and mix aseptically

E31 (E31 Medium) for marine algae

Medium

Part 1

Soil extract (SE1- see recipe)	50.0ml
KNO_3	0.10g
K_2HPO_4.	0.01g
$MgSO_4.7H_2O$	0.01g
Cyanonobalamin	100.00ng
Thiamine HCl	50.00ug
Biotin	100.0ng
Distilled water	to 500ml

Part 2

Filtered natural seawater	500ml

Final

Autoclave parts 1 and 2 separately at 121°C, cool and mix aseptically

E31:ANT

Medium

1:1 mixture
See separate recipes, mix and autoclave at 121°C for 15min

F/2 (f/2 Medium) for marine algae

Stocks

1. Trace metals stock solution

$MnCl_2.4H_2O$	0.180g
$EDTANa_2$	4.160g
$FeCl_3.6H_2O$	3.150g
$CuSO_4.5H_2O$	0.010g
$ZnSO_4.7H_2O$	0.022g
$CoCl_2 .6H_2O$	0.010g
$Na_2MoO_4.2H_2O$	0.006g
Distilled water	1 litre

2. Vitamin stock solution

Cyanocobalamin	0.0005g
Thiamine HCl	0.1g
Biotin	0.0005g
Deionised water	1 litre

Medium

$NaNO_3$	0.075g
$NaH_2PO_4.2H_2O$	0.00565g
Trace metals stock solution	1.0ml
Vitamin stock solution	1.0ml
Filtered natural seawater	to 1 litre

pH adjusted to 8.0 with 1ml NaOH or HCl

F/2 + Si (f/2 Medium + sodium metassilicate) for marine diatoms

As for F/2 except: an additional sodium metasilicate stock solution (100 g/l-1 Na2SiO3.5H2O) is required. The media is made as F/2 except that the pH is balanced to 3.0-4.0 and then 0.3ml of the sodium metasilicate stock solution is added before the final pH is adjusted to pH 8.

HSM (Jones's Horse Serum Medium) for freshwater protozoa

Stocks

1. Sterile buffered saline

$Na_2HPO_4.12H_2O$	2.65g
KH_2PO_4	0.41g
NaCl	7.36g
Deionised water	1 litre
2. Sterilised Horse serum (Oxoid)	0.5ml

per test tube
Filter sterilised (0.22um filter)
3. Sterile "Marmite" solution

Marmite	1.0g
Deionised water	100ml

Filter sterilised (0.22um filter)
4. Rice starch suspension

Rice starch	5.0g
Deionised water	20ml

Place dry rice starch into a dry 50ml bottle, and cap. Dispense 20ml deionised water into a separate bottle. Autoclave separately at 121°C for 15min. When cool, aseptically combine and mix thoroughly by vigorous agitation.

Medium

Per tube

Sterile buffered saline (1)	8.5ml
Sterile horse serum (2)	0.5ml
Sterile 1% "Marmite" solution (3)	1.0ml
Rice starch suspension (4)	One drop

Aseptically add stock solutions 2,3 and 4, to each tube. Incubate at room temperature for 3 days to check sterility prior to use.

JM (Jaworski's Medium) for freshwater algae

Stocks

Each in 200ml deionised water

1. $Ca(NO_3)_2.4H_2O$	4.00g
2. KH_2PO_4	2.48g
3. $MgSO_4.7H_2O$	10.00g
4. $NaHCO_3$	3.18
5. EDTA FeNa	0.45g
EDTA Na_2	0.45g
6. H_3BO_3	0.496g
$MnCl_2.4H_2O$	0.278g
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.20g
7. Cyanocobalamin	0.008g
Thiamine HCl	0.008g
Biotin	0.008g
8. $Na_2HPO_4.12H_2O$	7.2g
9. $NaNO_3$	16.0g

Medium

Stock solutions 1-9	1.0ml
Deionised water	to 1 litre

Autoclaved at 121°C for 15min

For Solid Medium

Add 15g Bacteriological agar (Oxoid) per litre of medium

JM:SE
Medium

7:3 mixture (JM:SE)
See separate recipes, mix and autoclave at 121°C for 15min

MC (Modified Changs Serum-Casein-Glucose-Yeast Extract Medium) for freshwater protozoa
Medium

Casein digest (Gibco GRL Peptone No140)	10.0g
$Na_2HPO_4.7H_2O$	2.5g
KH_2PO_4	0.8g
Yeast extract (Oxoid L21)	5.0g
D-glucose	2.5g
Liver digest (Oxoid L27)	2.5g
Deionised water	to 900ml

Adjust to pH 6.9, dispense into 5X 180ml aliquots and sterilise at 110°C for 15min
Add aseptically to each aliquot

Sterile foetal calf serum	5ml

Store at 4°C

MCH (Modified Chalkey's Medium) for freshwater protozoa
Stocks

Each in 100ml deionised water

1. NaCl	8.0g
KCl	0.2g
2. $NaHCO_3$	0.4g
3. $Na_2HPO_4.12H_2O$	0.1g
$CaHPO_4$	trace

Medium

Stock solution 1	1ml
Sock solution 2	1ml
Stock solution 3	1ml
Deionised water	to 1 litre

Autoclaved at 121°C for 15min

MErds (Modified Foyns Erdschreiber Medium) for marine protozoa
Stocks

Each in 100ml deionised water

1.$NaNO_3$	20.0g
2. Na_2HPO_4	1.2g

Medium

Soil extract with salts (SES-see recipe)	100ml
Stock solutions 1 and 2	1.0ml each
Filtered seawater	898.0ml

Autoclaved at 121°C for 15min
Precipitates can be removed by filtration

MErds/MY75S
Medium Biphasic (1:1) see separate recipes

MP (Chapman-Andersen's Modified Pringsheim's Solution) for protozoa
Stocks

Each in 100ml deionised water

1. $Ca(NO_3)_2.H_2O$	20.0g
2. $MgSO_4.7H_2O$	2.0g
3. $Na_2HPO_4.2H_2O$	2.0g
4. KCl	2.6g
5. Fe $SO_4.7H_2O$	0.2g
conc. H_2SO_4	0.1ml

Medium

Stock solutions 1-5	1ml each
Deionised water	to 1 litre

Autoclaved at 121°C for 15min

MW (Mineral Water) for freshwater protozoa
Medium

Many commercial brands available. CCAP uses "Volvic" (Perrier Group, 6 Lygon Place, London, UK)

MY75S (Malt and Yeast Exteract-75% Seawater Agar) for marine protozoa
Medium

Malt extract (Oxoid)	0.1g
Yeast extract (Oxoid)	0.1g
Bacteriological agar (Oxoid)	15.0g
Deionised water	250ml
Natural filtered seawater (GF/C)	750ml

Disperse agar in cold liquid. Bring to the boil and stir continuously. Add other ingredients. Transfer molten agar to suitable vessel and autoclave at 121°C for 15min

NN (Non-Nutrient (Amoeba Saline) Agar) for freshwater protozoa
Medium

Bacteriological agar (Oxoid)	15g
Page's Amoeba Saline Solution (PAS)	1litre

See recipe
Autoclave at 121°C for 15min

NSW (Natural Seawater) for marine organisms
Medium

Filter natural seawater through GF/C filters
Autoclaved at 121°C for 15min

PAS (Page's Amoeba Saline constituent of protozoa media
Stocks

Each in 500ml deionised water

1. NaCl	12.0g
$MgSO_4.7H_2O$	0.40g
$CaCl_2.6H_2O$	0.60g
2. Na_2HPO_4	14.20g
KH_2PO_4	13.60g

Medium

Stock solutions 1 and 2	5ml each
Deionised water	to 1 litre

PC (Prerscott's and Carriers Solution) for freshwater protozoa

Stocks

Each in 1000ml deionised water

1. NaCl	1.0g
$MgSO_4.7H_2O$	0.20g
KCl	0.5g
$CaCl_2$	1.0g
2. $CaPO_4$	0.36g

Autoclaved at 121°C for 15min

Medium

Stock solutions 1 and 2	10ml each
Deionised water (sterile)	to 1 litre

Aseptically mix. Decant supernatant from precipitate if necessary

PE (Plymouth Erdschreiber Medium) for marine algae

Stocks

1. Filtered 95% natural seawater
2. Nutrient salts solution

$NaNO_3$	200.0g
$Na_2HPO_4.12H_2O$	20.0g
Deionised water	1 litre

Medium

Filtered Natural seawater (1)	950.0ml
Soil extract (SE1-see recipe)	50.0ml
Nutrient salts solution (2)	1.0ml

Autoclave ingredients separately at 121°C and mix aseptically when cold

PER (Peranema Medium) for freshwater protozoa

Medium

Soil extract with salts (SES- see recipe)	1.0 litre
Complan*	1.0g

Autoclaved at 121°C for 15min

* HJ Heinz Co Ltd, Hayesa, Middlesex, UK, UB4 8AL

PJ (Prescott & James Solution) for freshwater protozoa

Stocks

Each in 100ml deionised water

1. $CaCl_2.2H_2O$	0.43g
KCl	0.16g
2. K_2HPO_4	0.51g
3. $MgSO_4.7H_2O$	0.28g

Medium

Stock solutions 1 -3	1.0ml each
Deionised water	to 1 litre

Autoclaved at 121°C for 15min

PJ/NN

Medium Biphasic (1:1) See separate recipes

PM (Polytoma Medium) for protozoa

Medium

Sodium acetate trihydrate	2.0g
Yeast extract (Oxoid L21)	1.0g
Tryptone (Oxoid L42)	1.0g
Deionised water	to 1 litre

Autoclaved at 121°C for 15min

PP (Proteose Peptone Medium) for freshwater algae

Stocks

Each in 1 litre of deionised water

1. KNO_3	10.0g
2. K_2HPO_4.	1.00g
3. $MgSO_4.7H_2O$	01.00g

Medium

Proteose peptone (Oxoid L85)	1.0g
Stock solutions 1-3	20ml each
Deionised water	to 1 litre

Autoclaved at 121°C for 15min

For Solid Medium

Add 15g Bacteriological agar (Oxoid)/litre medium

PPG (Proteose Peptone Glucose Medium) for freshwater protozoa

Medium

Proteose Peptone (Oxoid L85)	15g
D-glucose	18g
Page Amoeba Saline solution (see PAS recipe)	1 litre

Autoclaved at 110°C for 20min

PPY (Proteose Peptone Yeast extract Medium) for freshwater protozoa

Medium

Proteose peptone (Oxoid L85)	20.0g
Yeast Extract (Oxoid L21)	2.5g
Deionised water	to 1 litre

Autoclaved at 121°C for 15min

SE (Soil Extract)

- constituent of several CCAP media

Preparing the soil

Site selection for a good soil is very important and for most purposes a soil from undisturbed deciduous woodland is best. Sites to avoid are those

showing obvious signs of man's activity and particular care should be taken to avoid areas where fertilisers, crop sprays or other toxic chemicals may have been used.

A rich loam with a good crumb structure should be sought. Stones, roots and larger invertebrates should be removed during an initial sieving though a 1cm mesh. The sieved soil should be spread to air dry and hand picked for smaller invertebrates and roots. It should be turned periodically and picked over again, When dry it may be sieved through a finer mesh (2-4mm) or stored as it is prior to use. Two slightly different preparations are used at CCAP, as follows:

SE1 (Soil Extract 1) used in media for marine algae

Method

Soil is prepared as above. Air-dried soil and twice its volume of supernatant distilled water are autoclaved together at 121°C for 2h and left to cool. The supernatant is then decanted and filtered through Whatman No1 filter paper, then distributed to containers in volumes suitable for making up batches of media. The aliquots and their containers are autoclaved for an appropriate length of time (e.g. 1 litre or less for 15min) and are then kept in a cool place until required

SE2 (Soil Extract 2) used for freshwater and terrestrial protozoa

Method

Soil is prepared as above. 105g of air-dried soil and 660ml of deionised water are placed in a 1 litre bottle and autoclaved once at 121°C for 15min, then again after 24h. The contents of the bottle are left to settle (usually for at least a week). The final pH should be between 7.0 and 8.0.

SES (Soil Extract with Added Salts) for freshwater and terrestrial protozoa and marine algae

Stocks

Each in 1 litre of deionised water

1. KNO_3	10.0g
2. K_2HPO_4.	1.00g
3. $MgSO_4.7H_2O$	1.00g

Medium

Stock solutions 1-3	20ml each
Soil extract (*SE-see recipe)	100ml
Deionised water	to 1 litre

Autoclaved at 121°C for 15min
*SE1 for marine algae
*SE2 for freshwater and terrestrial protozoa

SES: MP for freshwater protozoa

Medium

3:1 mixture (see separate recipes)
Autoclave separately and mix aseptically

SNA (Seawater Nutrient Agar) for marine algae

Medium

Nutrient agar (Oxoid CM3)	28g
a) Filtered natural sea water	500ml **OR**
b) "Ultramarine synthetica" sea salts	17.5g
Distilled water	to 1 litre

Steam for 30min to ensure homogeneity, dispense and autoclave at 121°C.

2SNA (Saline Seawater Nutrient Agar) for marine algae

Medium

Nutrient agar (Oxoid CM3)	28g
a) Filtered natural sea water	1000ml **OR**
b) "Ultramarine synthetica" sea salts	35g
Distilled water	to 1 litre

Steam for 30min to ensure homogeneity, dispense and autoclave at 121°C.

SNA/5 (Brackish Seawater Nutrient Agar) for brackish water algae

Medium

As above (a) but 200ml seawater and 800ml distilled water used per litre

SP (Spirulina Medium) for marine cyanobacteria

Stocks

1. Micronutrient solution	
$ZnSO_4.7H_2O$	0.001g
$MgSO_4.7H_2O$	0.002g
H_3BO_3	0.010g
$Na_2MoO_4.2H_2O$	0.001g
$Co(NO_3)_2.6H_2O$	0.001g
$CuSO_4.5H_2O$	0.00005g
$FeSO_4.7H_2O$	0.70g
EDTA Na_2	0.80g
Deionised water	1 litre
2. Vitamin solution	
Biotin	0.0002g
Calcium panthothenate	0.02g
Cyanocobalamin	0.004g
Folic acid	0.0004g
Inositol	1.0g
Nicotinic acid	0.02g
Thiamine HCl	0.10g
Thymine	0.60g
Deionised water	1 litre

Medium

Part 1

$NaHCO_3$	27.22g
Na_2CO_3	8.06g
K_2HPO_4	1.00g
Deionised water	1 litre

Part 2

$NaNO_3$	5.0g
K_2SO_4	2.0g
NaCl	2.0g
$MgSO_4.7H_2O$	0.4g
$CaCl_2.2H_2O$	0.02g
$FeSO_4.7H_2O$	0.02g
$EDTANa_2$	0.16g
Micronutrient solution (1)	10.0ml
Vitamin solution (2)	5.0ml

Final

Part 1	500.0ml
Part 2	500.0ml

Autoclave parts 1 and 2 separately at 121°C, cool and mix aseptically

SPA (Sigma Leaf-Prescott Agar) for fresh water protozoa)
0.1% SPA
Medium

Mix 1 litre of Sigma Cereal Leaf-Prescott liquid (SPL: see recipe) with 15g of bacteriological agar. Autoclave at 121°C for 15min

0.01% SPA
Medium

Mix 1 litre of Sigma Cereal Leaf-Prescott liquid (SPL: see recipe) with 0.1g of bacteriological agar. Autoclave at 121°C for 15min

SPL (Sigma Cereal Leaf-Prescott Liquid) for protozoa
Medium

Sigma cereal leaves (Sigma C7141)	1.0g
Prescott & James Solution (PJ: see recipe)	1 litre

Bring PJ to the boil and then add cereal leaves. Continue to boil for 5min. Allow to cool and restore to 1 litre with deionised water. Filter through GF/C paper and autoclave at 121°C for 15min

SPL:MP for freshwater protozoa
Medium

2:1 mixture
See separate recipes, Autoclave separately and mix aseptically when cool

SPL: PJ for freshwater protozoa
Medium

3:1 mixture
See separate recipes, Autoclave separately and mix aseptically when cool

SPL:0.01%SPA for protozoa
Medium

Biphasic (1:1) mixture
See separate recipes, Autoclave separately and mix aseptically when cool

SPL:PJ/0.001%SPA for freshwater protozoa
Stocks SPL:PJ (1:1)
Medium

Biphasic (1:1) mixture (SPL:PJ:0.001%SPA)
See separate recipes, Autoclave separately and mix aseptically when cool

S/W (Soil/Water Biphasic Medium) for protozoa
Medium

Put a layer about 1cm deep of air-dried, sieved good calcareous garden loam into a test tube or jar. (The use of mud from rivers or ponds is seldom satisfactory). Carefully add deionised water to a depth of 7 to 10cm, plug or cover, and steam for one hour or autoclave for 15min at 121°C (longer for larger vessels) on each of 2 consecutive days; further sterilisation is not needed. Allow to stand for a further day before inoculating, when the pH should be between 7.00 and 8.00.

S/W + AMP (Soil/Water Biphasic Medium + ammonium magnesium phosphate) for freshwater algae
Medium

As for S/W, but 0.01g ammonium magnesium phosphate is placed into the base of the test tube before the soil and water is added.

S/W + Ca (Soil/Water Biphasic Medium + calcium carbonate) for freshwater algae
Medium

As for S/W, but 0.01g calcium carbonate is placed into the base of the test tube before the soil and water is added.

S75S (Sigma Cereal Leaf-75% Seawater) for marine protozoa
Medium

Natural filtered seawater	750ml

Deionised water	250ml
Sigma cereal leaves (C7141)	1.0g

Bring 75% seawater to the boil, add the powdered cereal leaves and boil for 5min. Cool and restore volume to 1 litre with deionised water. Filter through Whattman GF/C paper. Autoclave at 121°C for 15min

S75S:NSW for marine protozoa

Medium

2:1 mixture
See separate recipes, Autoclave separately and mix aseptically when cool

S77 + Vitamins (S77 Medium + vitamins) for marine diatoms

Medium

Major salts	
NaCl	16.00g
$MgSO_4.7H_20$	2.50g
$CaSO_4.2H_2O$	0.50g
KCl	0.40g
Buffers	
Tris (tris(hydroxymethyl)aminomethane)	0.05g
Glycine	0.25g
Nutrients	
KNO_3	0.10g
K_2HPO_4	0.01g
Minor salts	
KBr	32.5mg
$SrCl_2.6H_2O$	6.50mg
$AlCl_3.6H_2O$	250.00µg
RbCl	100.00µg
$LiCl.H_2O$	50.00µg
KI	25.00µg
Chelated trace metals	
$EDTANa_2$	50.00mg
$FeSO_4.7H_2O$	2.50mg
$MnSO_4.4H_2O$	203.00µg
$ZnSO_4.7H_2O$	22.00µg
$CuSO_4.5H_2O$	19.60µg
$CoSO_47H_2O$	2.38µg
$NaMoO_4.2H_2O$	1.26µg
Vitamins	
Cyanocobalamin	100.0ηg
Distilled water	to 1 litre

Adjust to between pH 4.0 to 5.0

Add $NaSiO_3.5H_2O$	0.10g

While stirring continuously.
Adjust to pH 8

S88+ vitamins (S88 + Vitamins) for marine algae

Medium As for S77 EXCEPT:

1. Omit tris and replace with 0.50g glycylglycine
2. Add 50.0µg per litre thiamine HCl in addition to cyanocobalamin
3. Omit $NaSiO_3.5H_2O$ and associated step of adjusting pH to 4.0-5.0

UM (Uronema Medium) for protozoa

Medium

*Complan	10.0g
ASWP (see recipe)	1 litre

Autoclaved at 121°C for 15min
(* H.J. Heinz Co Ltd, Hayes, Middlesex)

YEL (Yeast Extract-Liver Digest Medium) for protozoa

Medium

Yeast extract (Oxoid L21)	4.0g
Licer digest (Oxoid L27)	4.0g
Deionised water	to 1 litre

Mix thoroughly and autoclave at 121°C for 15min

Media for bacteria

Detailed formulae are given for all media except those readily available commercially, in which case only the name of the medium is given. It can be assumed that dehydrated media from any reputable manufacturer are suitable, if prepared according to the manufacturer's instructions, unless a specific brand is recommended.

AC Broth (Difco 0317)

Commercial preparation

Acetate agar

Yeast extract	2.0g
Tryptone	1.0g
Sodium acetate	1.0g
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 7.4-7.6. Autoclave at 121°C for 15min

Acetobacter diazotrophicus medium

Glucose	50.0g
Yeast extract	10.0g
$CaCO_3$	30.0g
Agar	25.0g
Distilled water	1.0 litre

Mix $CaCO_3$ thoroughly and cool rapidly. Adjust pH to 5.5

Acetobacterium medium

NH_4Cl	1.00g
KH_2PO_4	0.33g
K_2HPO_4	0.45g
$MgSO_4.7H_2O$	0.10g
*Trace element solution	20.00ml
**Vitamin solution	20.00ml
Yeast extract	2.00g
Fructose	1.00g
Resazurin	1.00mg
$NaHCO_3$	10.0g
Cysteine hydrochloride	0.50g
$Na_2S.9H_2O$	0.50g
Distilled water	1.00 litre

**Trace element solution:*

Nitrilotriacetic acid	1.500g
$MgSO_4.7H_2O$	3.000g
$MnSO_4.2H_2O$	0.500g
NaCl	1.000g
$FeSO_4.7H_2O$	0.100g
$CaSO_4.7H_2O$	0.180g
$CaCl_2.2H_2O$	0.100g
$ZnSO_4.7H_2O$	0.180g
$CuSO_4.5H_2O$	0.010g
$KAl(SO_4)_2.12H_2O$	0.020g
H_3BO_3	0.010g
$Na_2MoO_4.2H_2O$	0.010g
$NiCl_2.6H_2O$	0.025g
$Na_2SeO_3.5H_2O$	0.300mg
Distilled water	1.0 litre

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

***Vitamin solution:*

Biotin	2.0mg
Folic acid	20mg
Pyridoxine-HCl	10.0mg
Thiamine-HCl	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
DL-Calcium pantothenate	5.0mg
Vitamin B_{12}	0.1mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Distilled water	1.0 litre

Dissolve ingredients except $NaHCO_3$, fructose, cysteine and sodium sulphide, bring to the boil for a few min and cool to room temperature under N_2 + CO_2 (80 + 20) gas mixture. Add $NaHCO_3$ (solid) and equilibrate the medium with the gas until a pH of approximately 7.4 is reached. Then distribute and autoclave under the same gas. Before use adjust the pH to 8.2 by adding sterile anaerobic Na_2CO_3 solution (approximately 0.25ml of 5% Na_2CO_3 per 10ml medium) and add fructose, cysteine and sodium sulphide from anaerobic sterile stock solutions.

Acid glucose salts medium

$(NH_4)_2SO_4$	0.15g
KCl	50.00mg
$MgSO_4.7H_2O$	0.50g
KH_2PO_4	0.10g
$Ca(NO_3)_2$	10.00mg
Glucose	5.00g
Distilled water	to 1.00 litre

Adjust pH to 3.0 and autoclave at 121°C for 15min

Acidic rhodospirillaceae medium

Yeast extract	0.2g
Disodium succinate	1.0g
Ferric citrate (0.1%)	5.0ml
KH_2PO_4	0.5g
$MgSO_4.7H_2O$	0.4g
NaCl	0.4g

NH_4Cl	0.4g
$CaCl_2.H_2O$	50.0mg
*Trace element solution	1.0ml
Distilled water	1.0 litre

Adjust pH to 5.7 and autoclave at 121°C for 15min.
*Trace element solution: See yeast malate medium

Acidic tomato juice agar
Tomato Juice agar at pH 4.8.

Acid nutrient agar
Nutrient agar adjusted to pH 5.0 with HCl.

Acidiphilium medium

$MgSO.7H_2O$	0.5g
$(NH_4)_2SO_4$	0.1g
KH_2PO_4	50.0mg
KCl	50.0mg
$Ca(NO_3)_2$	10.0mg
Mannitol	1.0g
Tryptone soya broth	0.1g
Agar	12.0g
Distilled water	to 1.0 litre

Prepare medium at double strength without agar. Adjust pH to 3.5 and autoclave at 121°C for 15min. Add to an equal volume of hot sterile, double strength agar solution.

Acidobacterium medium

$(NH_4)_2SO_4$	2.0g
KCl	0.5g
$MgSO_4.7H_2O$	0.5g
Glucose	1.0g
Yeast extract (Difco)	0.1g
Distilled water	1.0 litre

Adjust pH to 3.5 with H_2SO_4 before sterilisation.

Actinobolin medium
Lactobacilli AOAC medium plus 1mg/ml actinobolin.

Actinomadura madurae medium

Bacto yeast extract (Difco)	1.0g
Bacto beef extract (Difco)	1.0g
N-Z amine, type A (Sheffield Chem. Co.)	2.0g
Sucrose	10.0g
Agar	15.0g
Distilled water	1.0 litre

Adjust pH to 7.3.

Actinomyces broth (Difco 0840)
Commercial preparation

Actinomyces humiferus medium
Tryptone soya broth plus 5% horse blood.

"*Alcaligenes tolerans*" agar
Nutrient agar plus 0.3% ammonium lactate (60% syrup). Autoclave at 115°C for 20 min. Add 0.02% ferric citrate (sterile solution). Final pH 7.0.

Alicyclobacillus acidoterrestris medium
Solution A:

$CaCl_2.7H_2O$	0.25g
$MgSO_4.7H_2O$	0.50g
$(NH_4)_2SO_4$	0.20g
Yeast extract	2.00g
Glucose	5.00g
KH_2PO_4	3.00g
Distilled water (for liquid medium)	1.00 litre
Distilled water (for solid medium)	500.00ml

Adjust to pH 4.0.
Solution B:

*Trace element solution SL-6	1.00ml

Solution C:

Agar	15.00g
Distilled water	500.00ml

**Trace element solution SL-6:*

$ZnSO_4.7H_2O$	0.10g
$MnCl_2.4H_2O$	0.03g
H_3BO_3	0.30g
$CoCl_2.6H_2O$	0.20g
$CuCl_2.2H_2O$	0.01g
$NiCl_2.6H_2O$	0.02g
$Na_2MoO_4.2H_2O$	0.03g
Distilled water	to 1.00 litre

Sterilise solutions separately. For liquid medium combine solutions A and B. For solid medium combine solutions A, B and C.

Alkaline plate count agar (modified Oxoid CM325)
Adjust pH to 8.0 and autoclave at 121°C for 15min.

Alkaline nutrient agar 1
Nutrient agar + 0.5% NaCl.
After sterilisation add sterile 1M Na-sesquicarbonate solution (1ml in 10ml) to achieve a pH of 9.7.
Na-sesquicarbonate solution:

$NaHCO_3$	4.2g
Na_2CO_3 anhydrous	5.3g
Distilled water	100.0ml

Alkaline nutrient agar 2
Nutrient agar + 10% NaCl.
After sterilisation add sterile 1M Na-sesquicarbonate solution (1ml in 10ml) to achieve a pH of 9.7.
Na-sesquicarbonate solution:

$NaHCO_3$	4.2g

Na_2CO_3 anhydrous	5.3g
Distilled water	100.0ml

Alkaline polypectate medium

Sodium polypectate	5.0 g
Peptone	6.0g
Yeast extract	3.0g
K_2HPO_4	1.0g
$MgSO_4.7H_2O$	0.2g
$MnSO_4$	40.0mg
Na_2CO_3	10.0g
Agar	16.0g
Distilled water	to 1.0 litre

Adjust pH to 10.0 and autoclave at 121°C for 15min

Alkaline PPYG medium

Peptone	5.00g
Yeast extract	1.50g
Glucose	5.00g
$Na_2HPO_4.12H_2O$	1.50g
NaCl	1.50g
$MgCl_2.6H_2O$	0.10g
Na_2CO_3	5.03g
Agar	15.00g
Distilled water	1.00 litre

Adjust pH to 10.5-11.0. Sterilise solutions of glucose and Na_2CO_3 separately, add to the rest of the medium after autoclaving at 121°C for 15min

Alkaline starch medium

Peptone	6.0g
Yeast extract	3.0g
K_2HPO_4	1.0g
$MgSO_4.7H_2O$	0.2g
$MnSO_4$	40.0mg
Na_2CO_3	10.0g
Starch	20.0g
Agar	16.0g
Distilled water	to 1.0 litre

Adjust pH to 9.7 and autoclave at 121°C for 15min

Alkalophile medium

Nutrient agar adjusted to about pH 9.5 with 9% Na sesquicarbonate. If the agar is sterilised in 12ml amounts, 0.2ml sterile sesquicarbonate solution added aseptically should produce a suitable pH.

Alkalophilic halophile medium

Yeast extract	10.00g
Casamino acids	7.50g
Trisodium citrate	3.00g
KCl	2.00g
$MgSO_4.7H_2O$	1.00g
$MnCl_2.4H_2O$	0.36mg
$FeSO_4.7H_2O$	50.00mg
NaCl	200.00g
$Na_2CO_3.10H_2O$	50.00g

Make up NaCl plus $Na_2CO_3.10H_2O$ in 500ml distilled water and the rest of the components in 500ml distilled water and autoclave separately. Adjust pH to 9.5-10.5. For solid medium add 20.0g agar at 65-70°C and pour immediately.

Alteromonas denitrificans medium

Peptone (Difco)	0.5g
Tryptone (Difco)	0.5g
Yeast extract (Difco)	0.5g
Aged sea water	800.0ml
Tap water	200.0ml

To avoid precipitation, the nutrients should be autoclaved separately in 100ml of the tap water and added to the medium after autoclaving. Prior to revival of ampoule and subsequent subculturing chill medium. Use broth to rehydrate culture and only subculture onto solid medium when good growth is apparent. For solid medium add 15.0g Bacto agar (Difco).

Ampicillin l broth medium

LB (LURIA) plus 50mcg/ml ampicillin.

Ampicillin kanamycin nutrient agar

Nutrient agar plus 50µg/ml ampicillin and 25mcg/ml kanamycin.

Ampicillin TY salt medium

TY salt medium plus 50mcg/ml ampicillin.

Ancalomicrobium adetum medium

Prosthecomicrobium and Ancalomicrobium medium plus 5.0mg nicotinamide added to the vitamin solution.

Anderson's marine medium

Peptone	2.5g
Yeast extract	2.5g
$FePO_4$	0.1g
Filtered, aged sea water	750.0ml
Distilled water	250ml

Adjust pH to 7.4-7.6. For solid medium add 15.0g agar.

Antibiotic medium no.1 (Difco 0263)

Commercial preparation

Apple juice medium

Apple juice	500.0ml
Difco yeast extract	5.0g
Agar	15.0g

Adjust pH to 4.8 with acetic acid. Steam at 110°C for 10min to sterilise.

Artificial organic lake peptone medium

NaCl	30.0g
$MgSO_4.7H_2O$	9.5g
KCl	5.0g
$CaCl_2.2H_2O$	0.2g
$(NH_4)_2SO_4$	0.1g
KNO_3	0.1g
Peptone	5.0g
Yeast extract	1.0g
Distilled water	960.0ml

Adjust pH to 7.3 with 0.1M KOH and autoclave at 121°C for 15min. Cool medium to 60°C and add aseptically 20ml of sterile HMSS, 20ml of sterile PS and 1ml of AOLV. For solid medium add 15g of agar prior to sterilisation.

Hutner's modified salts solution (HMSS):

Nitrilotriacetic acid	10.0g
$MgSO_4.7H_2O$	23.0g
$CaCl_2.2H_2O$	2.9g
$FeSO_4.7H_2O$	1.1g
$ZnSO_4.7H_2O$	0.1g
$MnSO_4.7H_2O$	0.1g
$CuSO_4.5H_2O$	0.1g
$Na_2MoO_4.2H_2O$	0.1g
$CoCl_2.6H_2O$	0.1g
$NaVO_3$	0.1g
$NaWO_4.2H_2O$	0.1g
$NiCl_2.6H_2O$	0.1g
Na_2SeO_3	0.1g
H_3BO_3	0.1g
Distilled water to	1.0 litre

Adjust pH to 6.5 with KOH and autoclave at 121°C for 15min.

Hutner's modified salts solution (HMSS):

See brackish prosthecomicrobium medium

Phosphate supplement (PS):

K_2HPO_4	2.5g
KH_2PO_4	2.5g
Distilled water	1.0 litre

Artificial Organic Lake vitamin solution (AOLV):

Cyanocobalamine	0.1mg
Biotin	2.0mg
Calcium pantothenate	5.0mg
Folic acid	2.0mg
Nicotinamide	5.0mg
Pyridoxine HCl	10.0mg
Riboflavin	5.0mg
Thiamine HCl	5.0mg
Distilled water to	1.0 litre

Sterilise by filtration (0.2 mm). Store at 4°C.

Artificial organic lake medium

NaCl	80.0g
$MgSO_4.7H_2O$	9.5g
KCl	0.5g
$CaCl_2.2H_2O$	0.2g
$(NH_4)2SO_4$	0.1g
KNO_3	0.1g
Yeast extract	1.0g
Distilled water	60.0ml

Adjust pH to 8.0 and autoclave at 121°C for 15min. On cooling add aseptically 20ml of sterile HMSS, 20ml of sterile phosphate supplement and 1ml of vitamin solution.

Hutner's modified salts solution (HMSS): See brackish prosthecomicrobium medium

Phosphate supplement:

K_2HPO_4	50.0mg
KH_2PO_4	50.0mg
Distilled water	20.0ml

Vitamin solution:

Cyanocobalamine	10.0mg
Biotin	2.0mg
Calcium pantothenate	5.0mg
Folic acid	2.0mg
Nicotinamide	5.0mg
Pyridoxine HCl	10.0mg
Thiamine HCl	10.0mg
Distilled water to	1.0 litre

Sterilise by filtration (0.2 mm), store at 4°C.

ASM medium

NH_4Cl	535.00g
KH_2PO_4	531.00g
Na_2HPO_4	866.00g
K_2SO_4	174.00g
$MgSO_4.7H_2O$	37.00mg
$CaCl_2.2H_2O$	7.35mg
*Trace elements	1.00ml
Distilled water	1.00 litre

**Trace elements solution:*

$ZnSO_4.7H_2O$	288.0mg
$MnSO_4.7H_2O$	224.0mg
H_3BO_3	61.8mg
$CuSO_4.5H_2O$	125.0mg
$Na_2MoO_4.2H_2O$	48.4mg
$CoCl_2.6H_2O$	47.6mg
KI	83.0mg
1M H_2SO_4	1.0ml

Autoclave at 121°C for 15min and add 0.2ml filter sterilised 0.1M $FeSO_4$ to 1.0 L of ASM.

For solid medium add 15g agar and 15mg vitamin B_{12} to 1.0 L ASM before autoclaving and 0.2ml filter sterilised 0.1M $FeSO_4$ after.

ATB acid tomato broth

Glucose	1.0%
Peptone (Oxoid)	1.0%
Yeast Extract Oxoid	0.5%
$MgSO_4.7H_2O$	0.02%
$MgSO_4.4H_2O$	0.005%
Tomato juice	25.0%

Can be supplemented with 0.05% cysteine; 0.1% Tween 80 or 0.1% Tween 80 + 10% ethanol if required

ATCC bacteriostasis medium (Difco 0931)

Bacto peptone	10.0g
Bacto beef extract	5.0g
NaCl	5.0g
Agar	15.0g

Suspend 35g in 1.0 litre distilled water and boil to dissolve. Autoclave at 121°C for 15min. Final pH 7.2 at 25°C.

Azorhizobium medium

Peptone	5g
Yeast extract	1g
Beef extract	5g
Sucrose	5g
$MgSO_4$	0.24g
Agar	15g
Distilled water	1.0 litre

Azospirillum amazonense medium

Nutrient agar adjusted to pH 6.0.

B_{12} nutrient agar

Nutrient agar plus 400 mcg/L vitamin B_{12}.

Bacillus acidocaldarius medium

Solution (a)

$(NH_4)2SO_4$	1.30g
KH_2PO_4	0.37g
$MgSO_4.7H_2O$	0.25g
$CaCl_2.6H_2O$	0.10g
$FeCl_3.6H_2O$	30.00mg
Yeast extract	1.00g
Distilled water	500.00ml

Adjust pH to 3.5 with 0.5M H_2SO_4.

Solution (b)

Glucose	1.0g
Agar	20.0g
Distilled water	500.0ml

Autoclave solutions (a) and (b) separately at 121°C for 15min, cool to 50°C, then combine.

Bacillus benzoevorans medium

50:50 mixture of modified Palleroni and Doudoroff mineral base medium and enriched cytophaga agar with only 0.5g tryptone/litre. A filter-sterilised solution of 0.05% sodium benzoate is added to the medium after sterilisation.

Bacillus lentimorbus mediun

Mueller-Hinton broth	10.0g
Yeast extract	10.0g
K_2HPO_4	3.0g
Glucose (autoclaved separately)	0.5g
Sodium pyruvate	1.0g
Distilled water	to 1.000ml

Adjust pH to 7.1. For solid medium add 20g agar.

Bacillus racemilacticus agar

Glucose	5.0g
Peptone	5.0g
Yeast extract	5.0g
$CaCO_3$	5.0g
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 6.8. Autoclaved at 121°C for 15min

Bacillus schlegelii medium

$Na_2HPO_4.2H_2O$	4.50g
KH_2PO_4	1.50g
NH_4Cl	1.00g
$MnSO_4.2H_2O$	0.01g
$MgSO_4.7H_2O$	0.20g
$CaCl_2.2H_2O$	0.01g
Ferric ammonium citrate	5.00mg
Trace element solution SL-6	3.00ml
Pyruvate (Na salt)	1.50g
Distilled water	1.00 litre

See *Alicyclobacillus Acidoterrestris* Medium for trace element solution SL-6.

Adjust to pH 7.1. Autoclave at 121°C for 15min For solid medium add 15g agar.

Bacillus thermoglucosidasius medium

Soluble starch	10.0g
Peptone	5.0g
Meat extract	3.0g
Yeast extract	3.0g
KH_2PO_4	3.0g
Agar	30.0g
Distilled water	1.0 litre

Adjust pH to 7.0 and autoclave at 121°C for 15min

Bacillus thermoleovorans medium

$(NH_4)_2HPO_4$	1.0g
KCl	0.2g
$MgSO_4.7H_2O$	0.2g
Yeast extract	1.0g
Distilled water	1.0 litre

Add 0.1% (v/v) n-heptadecane. Autoclave at 121°C for 15min

Bacto marine medium 2216 (Difco)

Commercial preparation

Basic cultivation medium

K_2HPO_4	1.000g
$(NH_4)_2PO_4$	1.500g
$MgSO_4.7H_2O$	0.200g
$Fe_2(SO_4)_3.5H_2O$	0.010g
$ZnSO_4.7H_2O$	0.002g
Yeast extract	10.000g
Glucose	5.00g
Distilled water	1.00 litre

Adjust pH to 7.0.

Basic mineral medium

$MgSO_4.7H_2O$	0.500g
$Na_2HPO_4.2H_2O$	1.000g
KH_2PO_4	0.500g
NH_4NO_3	2.500g

$CaCl_2.2H_2O$	1.000mg
$Fe(SO_4)_3.5H_2O$	0.010g
$MnSO_4.2H_2O$	0.100mg
$Co(NO_3)_2.6H_2O$	0.005mg
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.100mg
Distilled water	1.000litre.

Bdellovibrio medium

Host medium:

Yeast extract	3.0g
Peptone	0.6g
Distilled water	to 1.0 litre

Adjust pH to 7.2

Base layer agar: As host medium, but with the addition of 1.9% agar.

Semi-solid agar: As host medium, but with the addition of 0.6% agar.

Distribute in 10ml amounts and autoclave at 115°C for 20min. Grow up the appropriate host (see individual catalogue entries) in the host medium for 24-48h at 30°C. Melt the base layer agar and semi-solid agar. Pour the base layer into a Petri dish and allow to set. Cool the semi-solid agar to 40-45°C, add 1ml host culture, mix and pour over the base layer agar. Incubate at 30°C for 18-24h, agar surface uppermost. Spot the *Bdellovibrio* culture, resuspended in a small quantity of host medium on to the surface of the plate and incubate at 30°C until zones of clearing appear in the host organism layer (3-5 days).

Bennett's agar

Yeast extract	1.0g
Beef extract	1.0g
N-Z Amine A (or casitone)	2.0g
Glucose	10.0g
Agar	15.0g
Distilled water	1.0 litre

Adjust pH to 7.3 with NaOH and autoclave at 121°C for 15min

Bifidobacterium medium

Casein peptone, tryptic digest	10.0g
Meat extract	5.0g
Yeast extract	5.0g
Glucose	10.0g
K_2HPO_4	3.0g
Tween 80	1.0ml
Distilled water	1.0 litre

Adjust pH to 6.8. After sterilisation, aseptically add solutions of sodium ascorbate and L-cysteine HCl to final concentration of 1.0% and 0.05% respectively. Heat medium not freshly prepared in a steamer for 10min before addition of the reducing substances.

Blastobacter denitrificans medium

Enriched cytophaga medium plus 0.025% separately sterilised glucose.

Blood agar

Blood agar base No 2 (Oxoid) plus 10% cow or sheep blood

Blood agar base

Commercial preparation

Blood agar base with 2.5% NaCl

Blood agar base plus 2.5% NaCl.

Blood agar base with 3.5% NaCl

Blood agar base plus 3.5% NaCl.

Bogoriella medium

Glucose	10.0g
Peptone (Difco)	5.0g
Yeast extract (Difco)	5.0g
KH_2PO_4	1.0g
$MgSO_4.7H_2O$	0.2g
NaCl	40.0g
Na_2CO_3	10.0g
Agar	20.0g
Aqua dest.	1000.0ml

Adjust pH to 9.6. NaCl and Na_2CO_3 were autoclaved separately and added to the organic compounds at 60°C before pouring the agar medium.

Brackish prosthecomicrobium medium

Peptone	0.25g
Yeast extract	0.25g
Glucose	0.25g
**Modified Hutner's basal salts	20.00ml
*Vitamin solution	10.00ml
Agar	15.00g
Distilled water	500.00ml
Sea water	500.00ml

**Vitamin solution:*

Biotin	2.0mg
Folic acid	2.0mg
Thiamine HCl	5.0mg
D Calcium pantothenate	5.0mg
Vitamin B_{12}	0.1mg
Riboflavin	5.0mg
Distilled water	1.0 litre

***Modified Hutner's Basal Salts:*

Nitrilotriacetic acid	10.00g
$MgSO_4.7H_2O$	29.70g
$CaCl_2.2H_2O$	3.34g
Ammonium molybdate	9.25mg
$FeSO_4.7H_2O$	99.00mg
***Metals "44	50.00ml
Distilled water to	1.00 litre

***Metals "44"*

EDTA	0.250g
$ZnSO_4.7H_2O$	1.100g
$FeSO_4.7H_2O$	0.500g
$MnSO_4.7H_2O$	0.154g
$CuSO_4.5H_2O$	0.025g
$Na_2B_4O_7.10H_2O$	0.018g
Distilled water	1.00 litre

Initially add a few drops of H_2SO_4 to the distilled water to retard precipitation.
Dissolve the nitrilotriacetic acid first and neutralise the solution with KOH. Add other ingredients and readjust the pH with KOH and/or H_2SO_4 to 7.2. There may be a slight precipitate. Store at 5°C.

Brigg's liver tomato broth

Tomato juice	400.0ml
Neopeptone	15.0g
Yeast extract	6.0g
Liver extract	75.0ml
Glucose	20.0g
Soluble starch	0.5g
NaCl	5.0g
Cysteine HCl	0.2g
Tween 80	1.0g
Distilled water to	1.0 litre

Adjust pH to 5.0.
3.0g of proteolysed liver (Oxoid L25) may be used instead of the liver extract.

Brain heart infusion agar

Commercial preparation
Can be supplemented with 0.05% cysteine if required

Brain heart infusion broth (Oxoid)

Commercial preparation

Caffeine medium

Solution (a):

NaCl	0.58g
KH_2PO_4	3.00g
Na_2HPO_4	7.80g
Caffeine	1.00g

Solution (b):

$MgSO_4.7H_2O$	0.12g
$CaCl_2.2H_2O$	11.00mg

Solution (c):

$FeCl_3$	0.16mg

Dissolve each constituent of solution (a) separately in distilled water, adjust pH to 5.0, followed by the addition of solution (b). Volume is made up to almost 1.0 L. Solution (c) is added, pH adjusted to 5.0 and volume made up to 1.0 litre. Add 1.5% agar and autoclave at 121°C for 15min

Carboxymethyl cellulose medium

$(NH_4)2SO_4$	0.1%
$MgSO_4.7H_2O$	0.1%
$CaCl_2.2H_2O$	0.1%
$FeCl_3$	0.02%
K_2HPO_4 (autoclaved separately)	0.1%
Casitone (Difco)	0.2%
Carboxymethyl cellulose	1.5%
Agar	0.6%

Carboxymethyl cellulose Simga no. C-5013, sodium salt, high viscosity, works well.

Carnobacterium medium

Oxoid nutrient broth No.2	25.0g
Yeast extract	3.0g
Glucose	5.0g
Oxoid agar No.3	15.0g
Distilled water	1.0 litre

Adjust pH to 6.8.

Casamino acids and yeast extract agar

Casamino acids	7.5g
Yeast extract	10.0g
$MgSO_4.7H_2O$	20.0g
Na_3 citrate	3.0g
KCl	2.0g
NaCl	200.0g
$FeSO_4.7H_2O$ (4.98% in 0.001M HCl)	1.0ml
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 7.4. Autoclaved at 121°C for 10 min

Casamino acids medium

Casamino acids (Difco 0230)	1.00g
Glucose	1.00g
Modified Hutner's basal salts (See recipe)	20.00ml
Biotin	0.02mg
Distilled water	1.00 litre

Casein agar

Skim milk powder	10.0g
Agar	4.5g
Distilled water	to 1.0 litre

Add agar to 200ml water and heat to dissolve. Dissolve milk powder in 100ml distilled water, add to agar and autoclave at 121°C for 15min

Casitone agar

Casitone	3.0g
$CaCl_2$	1.0g
Agar	15.0g

Adjust pH to 7.2, autoclave at 121°C for 15min

Casitone phosphate agar

Casitone (Difco)	20.00g
$MgSO_4.7H_2O$	2.00g

K_2HPO_4	1.25g
KH_2PO_4	0.48g
Agar	20.00g
Distilled water	1.00 litre

Adjust pH to 7.2. Autoclave at 121°C for 15min

Casitone yeast extract agar

Casitone	5.0g
Yeast extract	1.0g
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 7.2. Autoclaved at 121°C for 15min

Castenholz tye medium

Castenholz Salts, 2X:

Nitrilotriacetic acid	0.2g
*Nitsch's Trace elements (see below)	2.0ml
$FeCl_3$ solution (0.03%)	2.0ml
$CaSO_4.2H_2O$	0.12g
$MgSO_4.7H_2O$	0.2g
NaCl	0.016g
KNO_3	0.21g
$NaNO_3$	1.4g
Na_2HPO_4	0.22g
Agar (if needed)	30.0g
Distilled water	1.0litre

Adjust pH to 8.2.

Nitsch's Trace elements:

H_2SO_4	0.5ml
$MnSO_4$	2.2g
$ZnSO_4$	0.5g
H_3BO_3	0.5g
$CuSO_4$	0.016g
Na_2MoO_4	0.025g
$CoCl_2.6H_2O$	0.046g
Distilled water	1.0 litre

1% TYE:

Tryptone (Difco 0123)	10.0g
Yeast extract	10.0g
Distilled water	1.0 litre

Mix aseptically 5 parts double strength Castenholz Salts with one part 1% TYE and 4 parts distilled water. Final pH of complete medium should be 7.6.

Caulobacter medium

Peptone	2.0g
Yeast extract	1.0g
$MgSO_4.7H_2O$	0.2g
Riboflavin	1.0mg
Agar powder	10.0g
Distilled water	1.0 litre

Dissolve all ingredients except the agar in the water and adjust the pH to 7.0. Add the agar and dissolve by steaming. Distribute as required and sterilise by autoclaving at 121°C for 15min

Chloramphenicol ampicillin lb medium

Chloramphenicol LB medium no.2 plus 40 µg/ml ampicillin.

Chloramphenicol brain heart infusion agar

Brain-heart infusion agar plus 50 µg/ml chloramphenicol.

Chloramphenicol l broth medium no.3

Luria broth plus 50µg/ml chloramphenicol.

Chloramphenicol l broth medium no.1

L (Luria) broth plus 5µg/ml chloramphenicol.

Chloramphenicol l broth medium no.2

L (Luria) broth plus 12.5µg/ml chloramphenicol.

Chloramphenicol erythromycin lb medium

Chloramphenicol LB medium no.2 plus 10µg/ml erythromycin.

Chlorobium thiosulfatophilum medium

(a)Trace element solution:

$FeCl_3.6H_2O$	2.7 g
H_3BO_3	0.1 g
$ZnSO_4.7H_2O$	0.1 g
$Co(NO_3)_2.6H_20$	50.0mg
$CuSO_4.5H_2O$	5.0 mg
$MnCl_2.6H_2O$	5.0 mg
Distilled water	to 1.0 litre

(b) Basal medium:

KH_2PO_4	1.0 g
NH_4Cl	1.0 g
$MgCl_2.6H_2O$	0.5 g
NaCl	10.0g
Trace elements solution	1.0ml
Distilled water	999.0ml
(c)$NaHCO_3$	10%
(d)$Na_2S.9H_2O$	10%
(e)$Na_2S_2O_3.5H_2O$	10%

All solutions are autoclaved separately at 121°C for 15min. For every 10ml of freshly boiled medium (b), are added, aseptically, 0.2ml solution (c), 0.02ml solution (d) and 0.1ml solution (e). Adjust pH to 7.0-7.2 with sterile phosphoric acid. The complete medium should be used immediately.

Chloroflexus medium

Nitrilotriacetic acid	0.100g
**Micronutrient solution	1.000ml
*$FeCl_3$ solution	1.000ml
$CaSO_4.2H_2O$	0.060g
$MgSO_4.7H_2O$	0.100g
NaCl	0.008g
KNO_3	0.103g
$NaNO_3$	0.689g

Na_2HPO_4	0.111g
NH_4Cl	0.200g
Yeast extract	0.500g
Glycl-glycine	0.500g
Distilled water	1.000 litre

Prepare above medium and adjust pH to 8.2-8.4. Add 0.5g Na sulphide. Readjust pH to 8.2-8.4. For broth, filter-sterilise and dispense in tubes. For solid medium add 15g agar.

**FeCl$_3$ Solution:*

$FeCl_3$	0.2905g
Distilled water	1.0 litre

***Micronutrient Solution:*

H_2SO_4 (concentrated)	0.500ml
$MnSO_4.7H_2O$	2.280g
$ZnSO_4.7H_2O$	0.500g
H_3BO_3	0.500g
$CuSO_4.2H_2O$	0.025g
$Na_2MoO_4.2H_2O$	0.025g
$CoCl_2.6H_2O$	0.045g
Water	1.000litre

Chocolate agar

Blood agar base plus 10% horse blood. Add 10ml horse blood to 100ml sterile molten agar at 55°C, mix well and steam for about 10min. Dispense as slopes or plates and allow to set.

Choline medium

K_2HPO_4	1.00g
$MgSO_4$	0.50g
$FeSO_4$	0.01g
NaCl	30.00g
Choline chloride	5.00g
Distilled water	1.00 litre

Adjust pH to 7.4. For solid medium add 15.0g agar.

Chondromyces VYZ medium

Fresh baker's yeast cake	5.0g
$CaCl_2$	1.0g
Agar	15.0g
Distilled water	1.0 litre

Adjust pH to 7.2 with KOH. Autoclave at 121°C for 15min

Chromatium/thiocapsa medium

(a)Trace element solution:

$FeCl_3.6H_2O$	2.7 g
H_3BO_3	0.1 g
$ZnSO_4.7H_2O$	0.1 g
$Co(NO_3)_2.6H_20$	50.0 mg
$CuSO_4.5H_2O$	5.0 mg
$MnCl_2.6H_2O$	5.0 mg
Distilled water	to 1.0 litre

(b) Basal medium:

KH_2PO_4	1.0 g
NH_4Cl	1.0 g
$MgCl_2.6H_2O$	0.5 g
Trace elements solution	1.0ml
Distilled water	999.0ml

(c)$NaHCO_3$	10%
(d)$Na_2S.9H_2O$	10%
(e)$Na_2S_2O_3.5H_2O$	10%
(f)Sodium malate	10%

All solutions are autoclaved separately at 121°C for 15min. For every 10ml of freshly boiled medium (b), are added, aseptically, 0.2ml solution (c), 0.02ml solution (d),0.1ml solution (e) and 0.1ml solution (f). The complete medium should be used immediately.

Clostridium acetobutylicum medium

Fresh milk	100ml
Resazurin	0.1mg

Adjust pH to 7.1 Tube and autoclave under 100% N_2 for 12min at 121°C.

Clostridium acidi-urici medium

KOH	0.67g
K_2HPO_4	0.91g
Uric acid	2.00g
$MgSO_4.7H_2O$	0.25g
$CaCl_2.2H_2O$	0.02g
$FeSO_4.7H_2O$	6.00mg
Yeast extract	1.00g
Sodium thioglycollate	0.50g
$NaHCO_3$	5.00g
Distilled water	1.00 litre
Agar	15.00g

Add KOH and K_2HPO_4 to 900ml H_2O and heat. Add uric acid and boil until acid is dissolved. Add all the other chemicals except the sodium thioglycollate and $NaHCO_3$ at this stage. Adjust pH to 7.4 - 7.8 with NaOH. Add agar if plates/slopes are required. Autoclave at 121°C for 15min.

Dissolve sodium thioglycollate in 50ml H2O and autoclave separately at 121°C for 15min. Dissolve $NaHCO_3$ in 50ml H_2O and filter sterilise. Add thioglycollate and $NaHCO_3$ solutions to uric acid base aseptically when the base has cooled to approximately 50°C. Dispense in 20ml amounts in McCartney bottles or as plates. Do not allow the temperature of the medium to fall below 35°C as this will cause the precipitation of uric acid. Store the medium at 35°C to 45°C, preferably anaerobically and use within 7 to 10 days of preparation.

Clostridium cellobioparum medium

Ground beef (fat free)	500.0g
Distilled water	1.0 litre
1N NaOH	25.0ml

Use lean beef or horsemeat. Remove fat and connective tissue before grinding. Mix meat, water and NaOH, then boil for 15min stirring. Cool to room temperature, skim fat off surface and filter retaining both meat particles and filtrate. To the filtrate add water to a final volume of 1 litre and then add:

Casitone	30.0g
Yeast extract	5.0g
K_2HPO_4	5.0g
Resazurin	1.0mg
Glucose	4.0g
Cellobiose	1.0g
Maltose	1.0g
Soluble starch	1.0g

Boil, cool, add 0.5g cysteine and adjust pH to 7.0. Dispense 7ml into tubes containing meat particles (use 1 part meat particles to 4 or 5 parts fluid). Autoclave at 121°C for 30min. For agar slants use 15g agar per litre of medium.

Clostridium kluyveri medium

Potassium acetate	1.0 g
K_2HPO_4	31.0mg
KH_2PO_4	23.0mg
NH_4Cl	25.0mg
$CaCl_2.H_2O$	1.0mg
$MgSO_4.7H_2O$	20.0mg
$MnSO_4.H_2O$	0.2mg
$FeSO_4.7H_2O$	2.0mg
$Na_2MoO_4.2H_2O$	0.2mg
Biotin	10.0µg
p-Aminobenzoic acid	20.0µg
Resazurin	50.0µg
Agar	50.0mg
Distilled water	100.0ml

Adjust pH to 7.0. Boil 5 min, cool quickly to 50°C, then add 50mg sodium thioglycollate and 2ml ethanol. Dispense into tubes containing little $CaCO_3$, autoclave at 121°C for 15min and store anaerobically.

Clostridium lentocellum medium

NH^4SO^4	1.60g
Yeast extract	1.00g
Agar	30.0g
K_2HPO_4	1.65g
Cysteine hydrochloride	0.50g
NaCl	0.96g
$MgSO_4$	96.00mg
$CaCl_2$	96.00mg
Resazurin solution (0.1% w/v)	1.00ml
*Cellulose suspension	200.00ml
Distilled water	1.00 litre

Adjust pH to 7.2 with 5M NaOH. For broth medium exclude agar.

**Cellulose suspension*

4% (w/v) Whatman CF cellulose powder. (If cellulose suspension is not available cellulose can be provided by a strip (4.5 x 1cm) of Whatman No. 1 filter paper in each tube). Autoclave 121°C

Clostridium oroticum medium

Tryptone	5.00g
Yeast extract	0.50g
KH_2PO_4	1.36g
K_2HPO_4	6.95g
Sodium orotate	2.50g
Riboflavin	15.00mg
Sodium thioglycollate	0.50 g
Distilled water	to 1.0 litre

Adjust pH to 7.5. Autoclave at 121°C for 15min. Add aseptically 100ml, filter-sterilised, 5% L-arabinose solution. Dispense the complete medium separately.

Clostridium sticklandii medium

K_2HPO_4	1.75g
$CaCl_2.2H_2O$	10.00mg
$MgSO_4.7H_2O$	0.20g
$FeSO_4.7H_2O$	10.00mg
L-arginine HCl	2.00g
L-lysine HCl	2.00g
Yeast extract	5.00g
Sodium formate	2.00g
NH_4Cl	2.00g
Distilled water	1.00 litre

Adjust pH to 7.0. Autoclave at 121°C for 15min. Add 3ml/100ml sterile $Na_2S.9H_2O$ solution.

Clostridium thermocellum medium

Cellobiose	2.00g
Cellulose	10.00g
Yeast extract	2.00g
$(NH_4)SO_4$	1.30g
KH_2PO_4	1.50g
$K_2HPO_4.3H_2O$	2.90g
$MgCl.6H_2O$	1.00g
$CaCl_2$	0.15g
Resazurin (0.2%)	1.00ml
$FeSO_4.7H_2O$ (5%)	25.00ml
Distilled water	1.00 litre

Adjust pH to 7.8 with NaOH and autoclave at 121°C for 15min. Add 5ml/100ml of the following reductants solution, freshly prepared and filter-sterilised:-

L-cysteine HCl	0.5g
$NaHCO_3$	5.0g
Distilled water	50.0ml

Clostridium thermohydrosulfuricum medium

Tryptone	10.0g
Sucrose	10.0g
Yeast extract	2.0g
$FeSO_4.7H_2O$	0.2g
Na_2SO_3	0.2g
$Na_2S_2O_3.5H_2O$	80.0mg
Distilled water	1.0 litre

Adjust pH to 6.8-7.8, autoclave, 121°C for 15min

Colby and Zatman medium

K_2HPO_4	1.20g
KH_2PO_4	0.62g
$CaCl_2.6H_2O$	0.05g
$MgSO_4.7H_2O$	0.20g
NaCl	0.10g
$FeCl_3.6H_2O$	0.001g
$(NH_4)_2SO_4$	0.50g
Solution A (see below)	1ml
Distilled water	1 litre
Agar (Oxoid Purified)	(2.0%)

Adjust pH to 7.0. Autoclave at 121°C for 15min. Cool to 50°C. Add a filter-sterilised solution of trimethylamine to give a final concentration of 0.1%. Make 10% sol., add 1ml per 100ml base.

Solution A (per litre):

$CuSO_4.5H_2O$	5mg
$MnSO_4.5H_2O$	10mg
$NaMoO_4.2H_2O$	10mg
H_3BO_3	10mg
$ZnSO_4.7H_2O$	70mg
$CoCl_2.6H_2O$	5mg

Colby and Zatman thiamine medium

Colby and Zatman medium plus 0.5mg of thiamine per litre.

Columbia agar

Adjust pH to 7.0 - 7.2.
Autoclave at 121°C for 15min

Columbia blood agar + 5% blood

Commercial preparation

Columbia blood agar + 5% citrated sheep blood

Commercial preparation

Colwellia psychroerythrus medium

NaCl	29.0g
$MgCl_2.6H_2O$	8.0g
KH_2PO_4	5.4g
$FeCl_2.4H_2O$	2.0mg
$CaCl_2.6H_2O$	33.0mg
Tryptone	8.0g
Distilled water	1.0 litre

Adjust pH to 7.0. For solid medium add 15.0g agar.

Cooked meat medium (Difco)

Commercial preparation

Cooked meat carbohydrate medium

Cooked meat glucose medium with the supernatant replaced with the following:-

Peptone	30.0g
Yeast extract	5.0g
K_2HPO_4	5.0g
Glucose	4.0g
Cellobiose	1.0g
Maltose	1.0g
Starch.	1.0g
Resazurin (0.1%)	1.0ml
Distilled water	1.0 litre

Boil, cool, add 0.5g cysteine, adjust pH to 7.0 and dispense. Autoclave at 121°C for 15min. Use immediately or store anaerobically.

Cooked meat glucose medium

Cooked meat medium (Southern Group Laboratories:0503) with the supernatant removed and replaced with an equal volume of medium 3. To prepare this medium from basic ingredients, heat 500g fat-free minced beef in 1 litre distilled water containing 25ml 1M NaOH, stirring until the mixture boils. Cool to room temperature, skim fat from the surface and filter. Dispense 1 volume meat particles to 4 volumes medium 3 in screw-capped bottles and sterilise at 121°C for 15min

Cook's cytophaga agar

Tryptone	2.0g
Agar	10.0g
Distilled water	1.0 litre

Adjust pH to 7.3. Autoclave at 121°C for 15min

Corn steep starch nutrient agar

Half strength nutrient agar plus 0.1% corn steep liquor and 1% soluble starch.

Corynebacterium agar

Trypticase peptone	10.0g
Yeast extract	5.0g
Glucose	5.0g
NaCl	5.0g
Agar	15.0g
Distilled water	1000.0ml

Adjust pH to 7.2 - 7.4

Coryneform agar
Coryneform Broth + 1.5% Agar

Coryneform broth
Tryptone	1.0%
Yeast Extract	0.5%
NaCl	0.5%
Glucose	0.5%

pH 7.2-7.4

Creatinine medium
K_2HPO_4	2.0g
Creatinine	5.0g
Fumaric acid	2.0g
Yeast extract	1.0g
Salt solution (see below)	10.0ml

Adjust pH to 6.8 with NaOH. Autoclave at 121°C for 15min

Salt solution

$MgSO_4$	12.20g
$CaCl_2.2H2O$	0.76g
$MnSO_4.H_2O$	1.70g
$FeSO_4.7H_2O$	2.80g
$ZnSO_4.7H_2O$	0.06g
NaCl	0.60g
$Na_2MoO_4.2H_2O$	0.10g
0.1M HCl	to 1.00 litre

CSY-3 medium
Casitone (Difco)	1.0g
Bacto soytone (Difco)	1.0g
Yeast extract (Difco)	1.0g
Ferric ammonium citrate	0.4g
Agar	15.0g
Seawater	750ml
Distilled water	250ml

Curtobacterium and *Psychrobacter* medium
Peptone	1.0%
Yeast extract	0.5%
Glucose	0.1%
Agar	1.5%

Adjust pH to 7.0. Incubation temperature 10°C

CYC medium
Czapek Dox liquid medium powder	33.4g
Yeast extract	2.0g
Casamino acids (vitamin free)	6.0g
Agar	16.0g
Distilled water	to 1.0 litre

Adjust pH to 7.2 and autoclave at 121°C for 15min

Cytophaga medium
Casitone	0.3%
$CaCl_2.H_2O$	0.136%
Yeast Extract	0.1%
Agar	1.5%
Cellobiose	0.5%

pH 7.2

Cytosine nutrient agar
Nutrient agar plus 20mcg/ml cytosine.

Czapek peptone agar
Sucrose	30.00g
K_2HPO_4	1.00g
$MgSO_4.7H_2O$	0.50g
KCl	0.50g
$FeSO_4.7H_20$	0.01g
Peptone	5.00g
Agar	15.00g
Distilled water	to 1.0 litre

Dissolve all except the agar, adjust the pH to 7.0-7.3, add the agar and steam to dissolve. Autoclave at 121°C for 15min

Czapek peptone yeast agar
Czapek peptone agar plus 0.2% yeast extract. Autoclave at 121°C for 15min

Czapek (sucrose nitrate) agar
Sucrose	30.00g
$NaNO_3$	2.00g
K_2HPO_4	1.00g
$MgSO_4.7H_2O$	0.50g
KCl	0.50g
$FeSO_4.7H_2O$	0.01g
Agar	15.00g
Distilled water	to 1.00 litre

Adjust pH to 7.3. Autoclaved at 121°C for 15min

DAP nutrient agar
Nutrient agar plus 100mg/ml synthetic diaminopimelic acid (a mixture of LL-, DD- and meso isomers).

Davis and Mingioli medium A
K_2HPO_4	7.0g
KH_2PO_4	3.0g
$(NH4)2SO_4$	1.0g
Trisodium citrate	0.5g
$MgSO_4$	48.0mg
Distilled water	1.0 litre

Autoclave at 121°C for 15min, then add filter-sterilised solutions of the following to give the final concentrations indicated:-

Glucose	2.5g/l
L-histidine	20.0µg/ml
L-leucine	40.0µg/ml
L-methionine	20.0µg/ml

Deep liver broth

Liver infusion	1.0g
Yeast extract	5.0g
Tryptone	10.0g
K_2HPO_4	2.0g
Glucose	5.0g
Distilled water	to 1.0 litre

Adjust pH to 7.4 and autoclave at 121°C for 15min

Degryse medium 162

Macronutrients solution 10x	**mg/l**
Nitrilotriacetic acid	1000
$MgCl_2.6H_2O$	2000
$CaSO_4.2H_2O$	400
Micronutrients solution 100x	***mg/l***
$MnSO_4.4H_2O$	220
$ZnSO_4.7H_2O$	50
H_3BO_3	50
$CoCl_2.6H_2O$	4.6
$CuSO_4.5H_2O$	2.5
$Na_2MoO_4.2H_2O$	2.5

For liquid medium

Macronutrients solution 10x	100ml
Micronutrients solution 100x	5ml
Ferric citrate ($C_8H_5O_7Fe.5H_2O$) 0.01M	0.5ml
$Na_2HPO_4.12H_2O$ 0.2M	15ml
KH_2PO_4 0.2M	10ml
Yeast extract	2.5g
Tryptone	2.5g
Distilled water	to 1000ml

Adjust to pH with NaOH
Autoclave at 121°C for 15min

For solid medium

The same as liquid with 2% agar

Deleya halophila medium

NaCl	81.000g
$MgCl_2$	4.000g
$MgSO_4$	19.600g
$CaCl_2$	0.470g
KCl	2.000g
$NaHCO_3$	0.060g
NaBr	0.026g
Proteose peptone No.3 (Difco)	5.000g
Yeast extract (Difco)	10.000g
Glucose	1.000g
Distilled water	1.0 litre

$NaHCO_3$ is added from a filter sterilised stock solution when the medium has cooled. Adjust pH to 7.5. Agar is added at a concentration of 15g/l for solid medium.

Desulfococcus multivorans medium

Postgate's medium plus 1% NaCl

Desulfotomaculum thermosapovorans medium

Basal medium

K_2HPO_4	0.5g
NH_4Cl	1.0g
$CaSO_4$	1.0g
$MgSO_4.7H_2O$	2.0g
NaCl	15.0g
Yeast extract	1.0g
Distilled water	1.0 litre

Dissolve above and gas with oxygen free nitrogen for 10 - 15min, then add:-

Thioglycollic acid	0.1g
Ascorbic acid	0.1g
$FeSO_4.7H_2O$	0.5g
Sodium pyruvate	1.0g

Still gassing pH to 7.4, dispense and autoclave at 115°C for 10min

Dichloroacetic acid medium no 1

Basic cultivation medium plus less than 1 g/L 2,4 dichloroacetic acid.

Dichloroacetic acid medium no 2

Basic cultivation medium plus 10 mg/l 2,4 dichloroacetic acid.

Dilute peptone water

Peptone	1.0 g
NaCl	1.0 g

Adjust pH to 7.0. Autoclave at a 121°C for 15min

Dorset egg medium (Oxoid PM5)

Commercial formulation

Double strength crude *Lactobacillus* medium

Yeast extract (Basamine)	20.0g
Sucrose	20.0g
Casein hydrolysate	15.0g
Histidine HCl.H_2O	2.0g
Potassium acetate	3.0g
Ascorbic acid	1.0g
Pyridoxamine HCl	33.0µg
*Salts A	20.0ml
**Salts B	5.0ml
Distilled water	to 1.0 litre

Adjust pH to 5.4 with acetic acid.
This medium is used double strength for cultivation.

**Salts A:*

$KH_2PO_4.H_2O$	16.5g
$K_2HPO_4.3H_2O$	16.5g
Distilled water	1.0 litre

***Salts B:*

$MgSO_4.7H_2O$	8.0g

NaCl	0.4g
$FeSO_4.7H_2O$	0.4g
$MnSO_4.H_2O$	0.4g
HCl (concentrated solution)	0.1ml
Distilled water	1.0 litre

Dubos salts medium

$NaNO_3$	0.5 g
K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4.7H_2O$	10.0 mg
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 7.2. Autoclave at 121°C for 15min Dispense into slopes. When the agar has solidified, place a strip of sterile filter paper on to the surface of each slope. Inoculate on to the filter paper.

Dubos salts medium plus 1% NaCl

Dubos salts medium plus 1% NaCl.

DSM trypticase soy agar

Peptone from casein	15.0g
Peptone from soymeal	5.0g
NaCl	5.0g
Agar	15.0g
Distilled water	1000.0ml

Adjust pH to 7.3.

Enriched blood agar

Blood agar base	400ml
Brain heart infusion agar	400ml
Horse blood	25ml

Melt blood agar base and brain heart infusion agar. Pour a thin layer of blood agar base and leave to set. Then after the brain-heart infusion agar has cooled to 50°C, the blood is aseptically added to it, both are well mixed and then poured as a layer on top of the blood agar base.

Enriched *Cytophaga* agar

Tryptone	2.0g
Beef extract	0.5g
Yeast extract	0.5g
Sodium acetate	0.2g
Agar	15.0g
Distilled water	1.0 litre

Adjust pH to 7.2-7.4 and autoclave at 121°C for 15min

For soft agar for the maintenance of active cultures reduce agar content to 4.0g/L. Dispense in 3 to 4ml amounts in 7ml screw-capped bottles (bijou bottles).

Enriched *Cytophaga* medium

Enriched cytophaga agar without agar. Incubate broths at angle of 45 degrees.

Enriched nutrient agar

Heart infusion (Difco)	12.5g
Nutrient broth (Difco)	5.4g
Yeast extract (Difco)	2.5g
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 7.0. Autoclave at 121°C for 15min

Enterococcus faecium medium

Brain Heart Infusion	37.00g
Yeast extract	5.00g
NaCl	9.30g
Sucrose	97.30g
$MgSO_4$	0.25g
Agar (if needed)	13.30g
Deionized water up to	1.00 litre

Adjust pH to 7.4. Autoclave at 121°C for 15min.

To 900ml of solution aseptically add 100ml of horse serum, gamma globulin-free inactivated (30min at 56°C) and 500 units/ml of penicillin.

Erythromycin l broth medium

L (LURIA) broth plus 10µg/ml erythromycin.

Erythromycin lb medium

LB medium plus 300µg/ml erythromycin.

Erythromicrobium & *Roseococcus* medium

Yeast extract (Difco)	1.0g
Bacto Peptone (Difco)	1.0g
Sodium acetate	1.0g
KCl	0.3g
$MgSO_4.7H_2O$	0.5g
$CaCl_2.2H_2O$	0.05g
NH_4Cl	0.3g
K_2HPO_4	0.3g
Vitamin B_{12}	20µg
*Trace elements solution (see below)	1ml
Distilled water	1litre

pH 7.5-7.8.

**Trace elements solution:*

Erthyene diamine tetraacetic acid	500mg/l
$FeSO_4.7H_2O$	300mg/l
$MnCl_2.4H_2O$	3mg/l
$CoCl_2.6H_2O$	5mg/l
$CuCl_2.2H_2O$	1mg/l
$NiCl_2.6H_2O$	2mg/l
$Na_2MoO_4.2H_2O$	3mg/l
$ZnSO_4.7H_2O$	5mg/l
H_3BO	2mg/l

Dissolve each compound separately in distilled water, add to the solution of EDTA, adjust the pH to approximately 4, and make up to 1 litre.

Fastidious anaerobe broth (LABM71)

Commercial preparation

Fe(III)-lactate-nutrient agar

Mix 5ml of filter sterilised 5% $FeCl^3.6H_2O$ with 2.5ml of filter sterilised 5% sodium lactate AR. Add this mixture at the rate of 0.4ml per melted universal (12-14ml) of Nutrient agar and mix in.

Flexibacter medium

Tryptone (Difco 0123)	1.0g
Vitamin-free casamino acids	1.0g
Monosodium glutamate	0.1g
Sodium glycerophosphate	0.1g
Vitamin B_{12}	1.0 µg
*Ho-le trace element solution	1.0ml
Agar (if needed)	15.0 g
Filtered sea water to	1.0 litre

**Ho-le trace element solution:*

H_3BO_3	2.85g
$MnCl_2.4H_2O$	1.80g
$FeSO_4$	1.36g
Sodium tartrate	1.77g
$CuCl_2.2H_2O$	26.90mg
$ZnCl_2$	20.80mg
$CoCl_2.6H_2O$	40.40mg
$Na_2MoO_4.2H_2O$	25.20mg
Distilled water	1.00 litre

Fluid thioglycollate medium

Trypticase	15.0g
L-cystine	0.5g
Glucose	5.0g
Yeast extract	5.0g
NaCl	2.5g
Sodium thioglycollate	0.5g
Resazurin	1.0mg
Distilled water	to 1.0 litre

Add the trypticase and after dissolving add all the other ingredients before adjusting pH to 7.1. Finally add the resazurin and bottle in 15 - 20ml amounts in screw capped bottles and autoclave at 121°C for 15 min

Frateuria aurantia medium

Potato*	200g
Press yeast	30g
Liver, infusion from*	25g
Meat extract	5g
Thioglycolate medium dehydrated**	10g
Glucose	5g
Glycerol	15g
$CaCO_3$	15g
Distilled water to	1 litre
Agar	15g

Adjust pH to 7.0.

*Gently boil sliced potatoes in 500ml of water (or sliced liver in 150ml of water) for 30min and remove solids by filtration through cloth.
**Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

Glucose broth

Oxoid nutrient broth No. 2 (CM67)	2.5g
Glucose	1.0g
Distilled water	to 100.0ml

Dissolve and mix thoroughly. Distribute into 5"x 5/8" tubes about 7ml in each tube. Autoclaved at 121°C for 10min

Glucose broth buffered

Oxoid CM1 broth powder	13.00g
Glucose	1.00g
K_2HPO_4	3.68g
KH_2PO_4	1.32g
Distilled water	1.00 litre

Adjust pH to 7.2. Autoclaved at 121°C for 15min

Glucose nutrient agar

Nutrient agar plus 1% glucose Autoclave 115°C for 20min

Glucose salts medium

$(NH_4)2SO_4$	1.0g
NaCl	0.5g
$MgSO_4.7H_2O$	0.5g
$Na_2HPO_4.12H_2O$	0.7g
$NaH_2PO_4.2H_2O$	0.3g
Glucose	5.0g
*Trace element solution	0.5ml
Glass distilled water	1.0 litre

Adjust pH to 6.9. For solid medium add 15.0g agar. For soft agar add 3.0g agar.

**Trace element solution:*

H_3BO_3	2.85g
$MnCl_2.4H_2O$	1.8g
$FeSO_4$	1.36g
$CuCl_2.2H_2O$	26.9mg
$ZnCl_2$	20.8mg
$CoCl_2.6H_2O$	40.4mg
$Na_2MoO_4.2H_2O$	25.2mg
Sodium tartrate	1.77g
Distilled water	to 1 litre

Glucose yeast chalk medium

Yeast extract	10.0g
Glucose	50.0g
$CaCO_3$	30.0g
Agar	25.0g
Distilled water	1.0 litre

Dissolve the $CaCO_3$ separately in a portion of the total volume of distilled water and autoclave both solutions at 121°C for 15min. After sterilisation aseptically mix the solutions and dispense.

Glucose yeast peptone medium

Glucose	10.0g
Peptone	5.0g
Yeast extract	5.0g
Distilled water	1.0 litre

Adjust pH to 5.0 - 6.0.

Glycerol agar

Peptone	5.0g
Beef extract	3.0g
Glycerol	70.0ml
Agar	15.0g
Soil extract	250.0ml
Tap water	750.0ml

Adjust pH to 7.0.
Alternatively a nutrient agar (blood agar base) plus 7% (w/v) glycerol may be used.

Glycerol asparagine agar

L-asparagine	1.0 g
Glycerol	10.0 g
K_2HPO_4	1.0 g
Agar	20.0 g
*Trace salts solution	1.0ml
Distilled water	1.0 litre

Dissolve all except the agar in distilled water. Adjust pH to 7.0-7.4, add agar and steam to dissolve. Autoclave at 121°C for 15min. *Trace salts solution - see starch salts agar

Glycerol asparagine meat agar

Glycerol asparagine agar plus 1% beef extract.

Glycerol $CaCO_3$ agar - for luminous bacteria

Blood agar base	40.0g
NaCl	25.0g
$CaCO_3$	5.0g
Glycerol	10.0g
Distilled water	to 1.0 litre

Autoclave at 115°C for 20min

Glycerol nutrient agar

Nutrient agar plus 1% glycerol.

Gordona rubropertincus medium

Peptone	10.0g
NaCl	5.0g
Agar	20.0g
Beef water	1000.0ml

Adjust pH to 7.2-7.4. Sterilise at 121°C for 30min

GPY-salts medium

Glucose	1.0g
Peptone	0.5g
Yeast extract	0.1g
Modified Hutner's basal salts (see recipe)	20.0ml
Distilled water	1.0 litre

Grape juice medium

Yeast extract	10.0g
Tween 80	1.0g
Grape juice	170.0ml
Distilled water	1litre

For solid medium - 20.0gl^{-1} agar. pH to 5.5 with 1M NaOH before sterilising 20min at 121°C. Please note that growth is best in broth, but never as dense as for *Leuconostoc* species.

Half strength nutrient agar

Nutrient agar with all ingredients except agar at half concentration.

Halobacterium sodomense medium

NaCl	125.00g
$MgCl_2.6H_2O$	160.00g
$CaCl_2.2H_2O$	0.13g
K_2SO_4	5.00g
Peptone (Difco)	1.00g
Yeast extract (Difco)	1.00g
Soluble starch (BDH)	2.00g
Distilled water	1.00 litre

Adjust pH to 7.0 with NaOH before autoclaving.

Halobius medium

Vitamin-free casamino acid	7.5g
Yeast extract	10.0g
Sodium citrate	3.0g
KCl	2.0g
$MgSO_4.7H_2O$	20.0g
NaCl	116.0g
$FeCl_2$	23.0mg
Agar	20.0g
Distilled water	1.0 litre

Adjust pH to 6.2. Autoclave at 121°C for 15min

Halomonas magadii medium

Glucose	10.0g
Peptone (Difco)	5.0g
Yeast extract (Difco)	5.0g
KH_2PO_4	1.0g
$MgSO_4$ $7H_2O$	0.2g
NaCl	40.0g
Na_2CO_3	10.0g
Agar	20.0g
Distilled water	to 1 litre

Adjust pH to 10.0. NaCl and Na_2CO_3 are autoclaved separately and added to the organic components at 60°C before pouring the agar media.

Halophile medium

NaCl	156.0g
$MgCl_2.6H_2O$	13.0g
$MgSO_4.7H_2O$	20.0g

$CaCl_2.6H_2O$	1.0g
KCl	4.0g
$NaHCO_3$	0.2g
NaBr	0.5g
Yeast extract	10.0g
Agar (Difco)	20.0g

Adjust pH to 7.0 with 1M KOH and sterilise by autoclaving at 121°C for 15min

Halophilic chromatium medium

Chromatium/thiocapsa medium plus 6% NaCl.

Halovibrio variabilis medium

NaCl	95.0g
$MgSO_4.7H_2O$	81.0g
KCl	1.0g
Proteose peptone	2.5g
**Trace mineral solution SL-4	10.0ml
*Vitamin solution	10.0ml
Yeast extract	7.5g
Distilled water	980.0ml

Adjust pH to 7.5.

***Vitamin solution:**

Cyanocobalamine	0.01mg
Biotin	0.20mg
Folic acid	0.20mg
Nicotinic acid	0.50mg
Pyridoxine HCl	1.00mg
Riboflavin	0.50mg
Thiamine HCl	0.50mg
Pantothenic acid	0.50mg
p-Aminobenzoic acid	0.50mg
Lipoic acid	0.50mg
Distilled water	1.00 litre

Sterilise by filtration (0.2mm). Store at 4°C.

**Trace element solution SL-4:*

EDTA	0.5g
$FeSO_4.7H_2O$	0.2g
Trace element solution SL-6	100.0ml
Distilled water	900.0ml

Trace element solution SL-6:

See *Alicyclobacillus acidoterrestris* medium

Hartley digest broth (Oxoid)

Commercial preparation

Can be supplemented with 0.05% cysteine if required.

Histidans medium

KH_2PO_4	0.91g
Na_2HPO_4	0.95g
Yeast extract	10.00g
Glucose	10.00g
$MgSO_4.7H_2O$	0.50g
Agar	20.00g
Distilled water	to 1.00 litre

Adjust pH to 7.0 and autoclave at 121°C for 15min

p-hydroxybenzoate medium

NaCl	0.5g
p-hydroxybenzoic acid	3.0g
$(NH_4)_2HPO_4$	3.0g
K_2HPO_4	1.2g
$FeSO_4.7H_2O$	0.1g
$MgSO_4.7H_2O$	0.2g
Agar	20.0g
Distilled water	1.0 litre

Adjust pH to 7.0 and autoclave at 121°C for 15min

IE medium

Phosphate solution:

K_2HPO_4	78.0g
Distilled water	1.0 litre

Basal salts medium:

Phosphate solution	20.0ml
$(NH_4)SO_4$ (36%)	5.0ml
$MgSO_4$	0.5g
Distilled water to	1.0 litre

Adjust pH to 6.8-7.0.

Medium

Yeast extract	1.0g
Bacto peptone	1.0g
Lactose	10.0g
Agar	15.0g
Basal salts	1.0 litre

Autoclave 121°C for 15min

Add, aseptically, lactose and 1ml trace elements after sterilisation.

IFO medium 203

Peptone	10g
Yeast extract	5g
Liver, infusion from*	25g
Glucose	3g
Glycerol	15g
Distilled water, make up to	1 litre
Agar	15g

Adjust pH to 7.0.

*See *Frateuria aurantia* medium

Ionic medium with pipecolate

KH_2PO_4	2.26g
K_2HPO_4	4.10g
NaH_2PO_4	2.24g
Na_2HPO_4	3.34g
Salt solution	10.00ml
Distilled water	1.00 litre

To 200ml of hot ionic medium add 6.0g agar. Boil to dissolve and add 10ml of neutralised 0.25M pipecolic acid HCl (Sigma Chemical Co.). Autoclave at 121°C for 15min

**Salt solution:*

$MgSO_4.7H_2O$	14.80g

$FeSO_4.7H_2O$	0.55g
$MnSO_4$	45.00mg
Distilled water	1.00 litre
Concentrated H_2SO_4	2.00 drops

Janibacter medium

Bacto peptone (Difco)	10.0g
Yeast extract	5.0g
Casamino acids (Difco)	5.0g
Meat extract (Difco)	2.0g
Malt extract (Difco)	5.0g
Glycerol	2.0g
$MgSO_4.7\ H_2O$	1.0g
Tween 80	0.05g
Agar	20.0g
Distilled water	1000.0ml

Adjust pH to 7.2. Sterilise 121°C for 20min

Johnson's marine medium

Bacto peptone	5.0g
$FeSO_4.7H_2O$	0.2g
$Na_2S_2O_3$	0.3g
Yeast extract (Difco)	1.0g
Filtered, aged sea water	750.0ml
Distilled water	250.0ml

For solid medium add 15.0g agar.

Kanamycin brain heart infusion agar

Brain-heart infusion agar plus 25µg/ml kanamycin.

Kanamycin Luria agar

Luria agar plus 10µg/ml kanamycin.

Kanamycin l broth medium

L (Luria) broth plus 50µg/ml kanamycin.

Krebs' yeast lactate medium

Yeast extract	10.0 g
KH_2PO_4	1.0 g
$Na_2HPO_4.2H_2O$	3.0 g
Sodium lactate(70%)	40.0ml
Distilled water	960.0ml

Adjust pH to 7.0. Autoclave at 121°C for 15min

Kye medium

$NaNO_3$	2.50g
KH_2PO_4	1.00g
$CaCl_2.6H_2O$	0.15g
$MgSO_4.7H_2O$	0.30g
NaCl	0.10g
$FeCl_3$	10.00mg
Yeast extract	1.00g
Agar	15.00g
Distilled water	to 1.00 litre

Adjust pH to 6.5 and autoclave at 121°C for 15min

LAB 8 agar (LAB M)

Commercial preparation

LAB 8 starch agar

Lab 8 agar plus 1% starch.

Lactobacillus 8664 medium

Yeast extract	10.0g
Peptone	10.0g
Maltose	20.0g
Glucose	5.0g
Distilled water	1.0 litre

Autoclave/15min

Lactobacilli AOAC medium (Difco 0901)

Commercial preparation

Lactobacillus chloramphenicol medium no1

Lactobacillus AOAC medium plus 300mcg/ml chloramphenicol.

Autoclave at 121°C for 15min. When resuscitating NCIMB 10463 allow up to 72h incubation at 37°C and when subculturing use at least 1ml heavy suspension in a Pasteur pipette. Use of loops will result in weak cultures.

Lactobacillus chloramphenicol medium no.2

MRS medium plus 100mg/L chloramphenicol.

When resuscitating NCIMB 11295 allow up to 4 days incubation at 37°C to reach a suitable density.

Lactobacillus kefirgranum medium

Trypticase	5g
Tryptone	5g
Yeast extract	5g
KH_2PO_4	5g
Diammonium hydrogen citrate	2g
$MgSO_4.7H_2O$	0.5g
$MnSO_4.H_2O$	0.5g
Tween 80	1ml
Cheese whey	1 litre

Lactobacillus orotic acid medium

Lactobacilli AOAC medium with the following additions per 100ml.

Orotic acid	2.5mg
D-pantothine	20.0mcg

Distribute in screw-capped bottles in 20ml amounts, heat to boiling, add 0.15ml of 1.5% cysteine HCl per bottle, autoclave at 121°C for 15min. Immediately after autoclaving screw caps down to maintain the reduced form pantotheine.

Lactobacillus pontis medium

Tryptone	10.0g

Meat extract	5.0g
Yeast extract	5.0g
Glucose	7.0g
Fructose	7.0g
Maltose	7.0g
Sodium gluconate	2.0g
Sodium acetate $3H_2O$	5.0g
diammonium citrate	2.0g
$K_2HPO_4.3H_2O$	2.6g
$MgSO_4.7H_2O$	0.1g
$MnSO_4.4H_2O$	0.05g
cysteine HCl	0.5g
Tween 80	1.0g

Adjust pH to 6.3. If solid medium required add 15g/l agar.

L (Luria) agar

Bacto tryptone	10.0g
Yeast extract	5.0g
NaCl	0.5g
Glucose(10%	20.0ml
Agar	15.0g
Distilled water to	to 1.0 litre

Autoclave 121°C for 15min
Sterile glucose solution added aseptically after sterilisation.

LB (Luria-Bertani) medium

Tryptone	10.0g
Yeast extract	5.0g
NaCl	10.0g
Distilled water	to 1.0 litre

Adjust pH to 7.0. Autoclave at 121°C for 15min
For solid medium add 15.0g agar.

LBE medium

Lb (Luria-Bertani) medium plus 4ml/L 50X medium E and 10ml/L 20% glucose.

Medium E	(50X)
$MgSO_4.7H_2O$	10.0g
Citric acid.H_2O	100.0g
K_2HPO_4	500.0g
$NaNH_4HPO_4.4H_2O$	175.0g
Distilled water	670.0ml

Dissolve ingredients in the order listed.

L-broth DAP thymidine

Tryptone	10.00g
Yeast extract	5.00g
NaCl	5.00g
Diaminopimelic acid	0.10g
Thymidine	0.01g
Distilled water	to 1.00 litre

Make up without DAP and thymidine and autoclave at 121°C for 15min. Add filter-sterilised solutions of DAP and thymidine to give the final concentrations shown.

LB streptomycin medium

LB (Luria-Bertani) medium plus 200mcg/ml streptomycin.

Leucothrix mucor medium

NaCl	11.75g
$MgCl_2.6H_2O$	5.35g
Na_2SO_4	2.00g
$CaCl_2.6H_2O$	1.12g
KCl	0.35g
Tris buffer	0.50g
Na_2HPO_4	0.05g
Monosodium glutamate	10.00g

Lineola medium

Yeast extract (Difco)	0.5%
Peptone (Difco)	0.5%
Sodium acetate	0.1%
Agar (Difco)	1.5%

pH 7.4

Listeria selective agar base

Columbia blood agar base	39.0g
Aesculin	1.0g
Ferric ammonium citrate	0.5g
Lithium chloride	15.0g

pH 7.0 ± 0.2

L (Luria) broth

Tryptone	10.0g
Yeast extract	5.0g
NaCl	5.0g
Glucose	1.0g
Distilled water	to 1.0 litre

Autoclave at 121°C for 15min. For solid medium add 15.0g agar.

Lowenstein-Jensen medium (Oxoid PM1)

Commercial preparation

M9 salts medium

*10 x M9 salts (see below)	100.0ml
1 M $MgSO_4$	1.0ml
0.1 M $CaCl_2$	1.0ml
1 M Thiamine HCl (sterilised by filtration)	1.0ml
Glucose (20%)	10.0ml
Proline	20.0mg
Distilled water	900.0ml

The above solutions should be sterilised separately by filtration (thiamine, glucose) or autoclaving
**10 x M9 salts* (per l):

Na_2HPO_4	60.0g
KH_2PO_4	30.0g
NH_4Cl	10.0g
NaCl	5.0g

Adjust pH to 7.4
M10 - as per PPB but excluding rumen fluid

M13 medium

Glucose	0.25g
Peptone	0.25g
Yeast extract	0.25g
Tris/HCl (0.1 M, pH 7.5)	50.00ml
*Vitamin solution	10.00ml
Hutner's mineral salts(see brackish prosthecomicrobium medium)	20.00ml
Seawater or artificial seawater (optional)	250.00ml
Distilled water to	1.00 litre

Adjust pH to 7.2 before autoclaving.

**Vitamin solution:*

p-Aminobenzoic acid	5.0mg
Biotin	2.0mg
Calcium pantothenate	5.0mg
Folic acid	2.0mg
Nicotinamide	5.0mg
Pyridoxine HCl	10.0mg
Thiamine HCl	5.0mg
Riboflavine	5.0mg
Vitamin B_{12}	0.1mg
Distilled water	1.0 litre

Filter sterilise and store in refrigerator.

M56 medium

Na_2HPO_4	8.70g
KH_2PO_4	5.30g
$(NH_4)_2SO_4$	2.00g
$MgSO_4.7H_2O$	0.10g
$Ca(NO_3)_2$	5.00mg
$ZnSO_4.7H_2O$	5.00mg
$FeSO_4.7H_2O$	5.00mg
Glucose	4.00g
L-leucine	0.05g
L-histidine	0.05g
Uracil	0.03g
Agar	15.00g
Distilled water	to 1.00 litre

Adjust pH to 7.0. Autoclave at 121°C for 15min

Malt extract agar (Oxoid)

Commercial preparation

Malt yeast agar

Yeast extract	3.0g
Malt extract	3.0g
Peptone	5.0g
Glucose	10.0g
Agar	20.0g
Distilled water	1.0 litre

Adjust pH to 7.0. Autoclave at 115°C for 20min

Magnetic spirillum growth medium (MSGM)

Double glass distilled water	1.00 litre
Wolfe's vitamin solution	10.00ml
Wolfe's mineral solution	5.00ml
0.01M Ferric quinate	2.00ml
0.1% Resazurin	0.45ml
KH_2PO_4	0.68g
$NaNO_3$	0.12g
Ascorbic acid	35.00mg
Tartaric acid	0.37g
Succinic acid	0.37g
Sodium acetate	0.05g
Agar (for semi-solid media)	1.30g

Add components in the order given with stirring. Adjust pH to 6.75 with NaOH.

Liquid medium:

Sterilise medium at 121°C for 15min. Aseptically fill screw capped containers to full capacity with sterile medium. Inoculate heavily leaving no headspace of air, and screw down closures tightly.

Semi-solid medium:

Dispense into screw capped tubes/bottles and sterilise at 121°C for 15min

Wolfe's Vitamin Solution:

Biotin	2.0mg
Folic acid	2.0mg
Pyridoxine HCl	10.0mg
Thiamine HCl	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
Calcium pantothenate	5.0mg
Cyanocobalamine	100.0µg
p-Aminobenzoic acid	5.0mg
Thioctic acid	5.0mg
Distilled water	1.0 litre

Wolfe's Mineral Solution:

Nitrilotriacetic acid	1.50g
$MgSO_4.7H_2O$	3.00g
$MnSO_4.H_2O$	0.50g
NaCl	1.00g
$FeSO_4.7H_2O$	0.10g
$CoCl_2.6H_2O$	0.10g
$CaCl_2$	0.10g
$ZnSO_4.7H_2O$	0.10g
$CuSO_4.5H_2O$	0.01g
$AlK(SO_4)_2.12H_2O$	0.01g
H_3BO_3	0.01g
$Na_2MoO_4.2H_2O$	0.01g
Distilled water	1.00 litre

Add nitrilotriacetic acid to approximately 500ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1 L with remaining water and add remaining compounds one at a time.

0.01M Ferric Quinate:

$FeCl_3$	0.27g
Quinic acid (Sigma)	0.19g
Distilled water	100.00ml

Dissolve and autoclave at 121°C for 15min

Manganese sulphate nutrient agar

Nutrient agar plus 5mg per litre of $MnSO_4$

Manganous acetate agar

Manganous acetate	0.1g
Purified agar	10.0g
Distilled water	to 1.0 litre

Dissolve the manganous acetate in the water and adjust pH to approximately 7.0. Add agar and steam medium to dissolve it. Dispense the medium into screw-capped bottles and autoclave at 121°C for 15min and slope.

Mannitol agar

Yeast extract (Oxoid)	0.5%
Peptone (Oxoid)	0.3%
Mannitol	2.5%
Agar	1.5%

pH 6.8

Marine *Cytophaga* medium A

Enriched Cytophaga medium prepared with 70% sea water/30% distilled water.

Marine *Cytophaga* medium B

Enriched Cytophaga medium prepared with 50% sea water/50% distilled water.

Marine *Cytophaga* medium C

Enriched *Cytophaga* medium prepared with 100% sea water.

Sodium lactate	2.00g
Deionized water	1.00 litre

Adjust pH to 7.6. Autoclave at 121°C for 15min

Marine methylotroph medium

KH_2PO_4	0.14g
Bis-Tris	2.00g
Ferric ammonium citrate	0.06g
Sea water	1.00 litre

Adjust pH to 7.4 and autoclave at 121°C for 15min. For solid medium add 12.0g agarose. After cooling to 45°C, add 2.0ml sterile methanol and a filter sterilised solution of vitamin B_{12} to give a final concentration of 1.0µg/l.

Marine *Rhodopseudomonas* medium

Bacto yeast extract	2.5g
Bacto peptone	2.5g
NaCl	30.0g
Distilled water	1.0 litre

Adjust pH to 7.0-7.4. For solid medium add 15.0g Bacto agar. Distribute in 15ml amounts in 1oz screw-capped bottles and autoclave at 121°C for 15min. Before resuspending freeze-dried culture in the liquid medium re-heat to drive out O_2 and screw down the cap tightly. Incubate at 30°C in an internally illuminated incubator for several days.

10% marine salts medium

NaCl	81.000g
$MgCl_2$	7.000g
$MgSO_4$	9.600g
$CaCl_2$	0.360g
KCl	2.000g
$NaHCO_3$	0.060g
NaBr	0.026g
Proteose peptone No.3 (Difco)	5.000g
Yeast extract (Difco)	10.000g
Glucose	1.000g
Distilled water	1.0 litre

Adjust pH to 7.0 with KOH.

Medium for ammonia-oxidising bacteria

$(NH_4)_2SO_4$	235.0mg
KH_2PO_4	200.0mg
$CaCl_2.2H_2O$	40.0mg
$MgSO_4.7H_2O$	0.5mg
*NaEDTA	0.5mg
*Phenol red	0.5mg
Distilled water	1.0 litre

*Prepared as separate stock solution as follows:

$FeSO_4.7H_2O$	50.0mg
NaEDTA	50.0mg
Phenol red	50.0mg
Distilled water	100.0ml

Add 1ml/l medium and autoclave at 121°C for 15min. After autoclaving add sterile 5% Na_2CO_3 until medium turns pale pink. Add further Na_2CO_3 during incubation to restore pink coloration. When no further colour change occurs growth is complete. Grow in dark.

Medium for freshwater flexibacteria

$MgSO_4.7H_2O$	0.1g
KNO_3	0.1g
$CaCl_2.2H_2O$	0.1g
Sodium glycerophosphate	0.1g
*Trace elements solution	1.0ml
Tris buffer	1.0g
Thiamine	1.0mg
Cobalamin	1.0mcg
Casamino acids (Difco)	1.0g
Distilled water	1.0 litre

Adjust pH to 7.5. Add 1.0g glucose aseptically after autoclaving.

For solid medium add 10.0g agar.

* see media for marine *Flexibacter*

Medium for *Haemophilus piscium*

Tryptone soya agar + 1% NaCl. Add 1ml of a 2mg per cent filter sterilised solution of cocarboxylase per 10ml of medium.

Medium for marine flexibacteria

KNO_3	0.5g
Sodium glycerophosphate	0.1g
*Trace elements solution	1.0ml
Tris buffer	1.0g
Tryptone	5.0g
Yeast extract	5.0g
Filtered, aged sea water	1.0 litre

Adjust pH to 7.0. For solid medium add 10.0g agar.

**Trace elements solution:*

H_3BO_3	2.85g
$MnCl_2.4H_2O$	1.80g
$FeSO_4$	1.36g
$CuCl_2.2H_2O$	26.90mg
$ZnCl_2$	20.80mg
$CoCl_2.6H_2O$	40.40mg
$Na_2MoO_4.2H_2O$	25.20mg
Sodium tartrate	1.77g
Distilled water	1.00 litre

Medium for nitrite-oxidising bacteria

Medium for ammonia-oxidising bacteria except that $NaNO_2$ (0.247g/l) replaces $(NH_4)2SO_4$. Growth monitored by use of Griess-Ilosay's reagent (BDH) to determine removal of nitrite.

Medium L10

$CH_3COONa.3H_2O$	6.0g
Na_2SO_4	7.0g
NH_4Cl	0.25g
$KH_2PO_4.2H_2O$	1.0g
NaCl	10g
$MgCl_2.6H_2O$	3.0g
$CaCl_2.2H_2O$	0.15g
*Trace element solution (SL10)	1ml
Distilled water	1 litre

Glass vials or tubes, fitted with butyl or neoprene rubber septa should be used. Scrum vials with aluminium crimp seals and neoprene rubber septa are advised.

Prepare the above by dissolving in 1 litre of distilled water, then generously spurge with oxygen free nitrogen (OFN) to facilitate removal of dissolved oxygen for approximately 15min. Displace oxygen from appropriately chosen vials (see above) using a stream of OFN delivered via an hypodermic syringe needle and, while continuing to gas the bottles, dispense medium into them. Immediately seal the vials in such a way as to minimise the possibility of oxygen re-entering them. Sterilise by autoclaving at 121°C.

Reduce the medium in the vials by injecting 0.1ml filter-sterilised 1.25M $Na_2S.9H_2O$ solution per 10ml medium.

A final pH of 6.8 is required. If adjustment is necessary (as the vials are sterile and sealed, and to ensure anoxic conditions are maintained) this should be done by injecting an appropriate volume of sterile 1M Na_2CO_3 into each vial aseptically via a hypodermic syringe and needle. To determine the volume of 1M Na_2CO_3 required, open one vial and whilst monitoring pH measure the amount required to adjust the medium to pH 6.8 for a known volume of medium.

**Trace element solution SL-10:*

HCl (25%; 7.7 M)	10.0ml
$FeCl_2.4H_2O$	1.5g
$ZnCl_2$	70.0mg
$MnCl_2.4H_2O$	100.0mg
H_3BO_3	6.0mg
$CoCl_2.6H_2O$	190.0mg
$CuCl_2.2H_2O$	2.0mg
$NiCl_2.6H_2O$	24.0mg
$Na_2MoO_4.2H_2O$	36.0mg
Distilled water	990.0ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1.0 litre.

Medium L20

CH_3CH_2COONa	3.0g
Na_2SO_4	7.0g
NH_4Cl	0.25g
$KH_2PO_4.2H_2O$	1.0g
NaCl	20g
$MgCl_2.6H_2O$	3.0g
$CaCl_2.2H_2O$	0.15g
Trace element solution (SL10)	1ml
Distilled water	1 litre

Glass vials or tubes, fitted with butyl or neoprene rubber septa should be used. Scrum vials with aluminium crimp seals and neoprene rubber septa are advised.

Prepare the above by dissolving in 1 litre of distilled water, then generously spurge with oxygen free nitrogen (OFN) to facilitate removal of dissolved oxygen for approximately 15min. Displace oxygen from appropriately chosen vials (see above) using a stream of OFN delivered via an hypodermic syringe needle and, while continuing to gas the bottles, dispense medium into them. Immediately seal the vials in such a way as to minimise the possibility of oxygen re-entering them. Sterilise by autoclaving at 121°C.

Reduce the medium in the vials by injecting 0.1ml filter-sterilised 1.25M $Na_2S.9H_2O$ solution per 10ml medium.

A final pH of 6.8 is required. If adjustment is necessary (as the vials are sterile and sealed, and to ensure anoxic conditions are maintained) this should be done by injecting an appropriate volume of sterile 1M Na_2CO_3 into each vial aseptically via an hypodermic syringe and needle. To determine the volume of 1M Na_2CO_3 required, open one vial

and whilst monitoring pH measure the amount required to adjust the medium to pH 6.8 for a known volume of medium.

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.0ml
$FeCl_2.4H_2O$	1.5g
$ZnCl_2$	70.0mg
$MnCl_2.4H_2O$	100.0mg
H_3BO_3	6.0mg
$CoCl_2.6H_2O$	190.0mg
$CuCl_2.2H_2O$	2.0mg
$NiCl_2.6H_2O$	24.0mg
$Na_2MoO_4.2H_2O$	36.0mg
Distilled water	990.0ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1.0 litre

Meiothermus ruber medium

Universal peptone (Merck)	5.0g
Yeast extract	1.0g
Starch, soluble	1.0g
Agar	12.0g
Distilled water	1000.0ml

Adjust pH to 8.0

Methanol salts medium

Colby and Zatman medium containing 0.1% methanol instead of trimethylamine.

Methylomicrobium alcaliphilum medium

Mineral Base

Na_2CO_3	20g
$NaHCO_3$	10g
NaCl	3g
K_2HPO_4	1g
KNO_3	0.5g
Distilled water	1.0 litre

Agar Base

Agar	35g
Distilled water	1.0 litre

Stock Solutions

a) $MgSO_4.7H_2O$	2g/10ml
b) $CuSO_4.5H_2O$	0.025g/100ml

c) * Trace element solution (Pfennig & Lippert)

*(Pfennig, H G & Lippert (1966) Arch. Microbiol. **55**, 245 - 256)

Method

pH mineral base to 10.0. Autoclave mineral base and agar base separately at 121°C for 15min. Cool both solutions to 50°C and aseptically mix 1 part of mineral base with 1 part of agar base. Additionally aseptically add the following amounts of stock solutions to the final volume of mineral/agar base: a) 0.5ml/litre; b)1ml/litre; c) 2ml/litre. Dispense aseptically.

For liquid medium, as above but omit the agar base step (resultant increased concentration of solutes is acceptable).

Microcyclus-spirosoma agar

Glucose	1.0g
Peptone	1.0g
Yeast extract	1.0g
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 6.8-7.2. Autoclaved at 115°C for 10min

Microlunatus phosphovorus medium

Glucose	0.5g
Peptone	0.5g
Monosodium glutamate	0.5g
Yeast extract	0.5g
KH_2PO_4	0.44g
$(NH_4)_2SO_4$	0.1g
$MgSO_4.7H_2O$	0.1g

Adjust pH to 7.0 with a diluted NaOH solution. Growth is estimated by measuring the optical density at 600nm with a spectrophotometer

Micromonospora halophytica medium

Glucose	10.0g
Soluble starch	20.0g
Yeast extract	5.0g
N-Z Amine Type A (or casein hydrolysate)	5.0g
$CaCO_3$ (reagent grade)	1.0g
Agar	15.0g
Distilled water	1.0 litre

Milk agar

350ml blood agar base plus 150ml water containing 10g milk. Each sterilised separately at 121°C for 15min before combining, aseptically.

Mineral medium with crude oil

K_2HPO_4	0.100g
$MgSO_4.7H_2O$	0.020g
NaCl	0.010g
$CaCl_2$	0.010g
$FeCl_3$	0.002g
$(NH_4)_2SO_4$	0.100g
Crude oil.(v/v)	0.5%
Water	100.0ml

Adjust pH to 7.2 - 7.5.

MMA salts medium

Colby and Zatman medium containing 0.1% monomethylamine instead of trimethylamine.

Modified Anacter & Ordal's medium

Agar (Oxoid)	0.7%
or (Gibco)	1.0%
Tryptone	0.5%
Yeast extract	0.05%
Sodium acetate	0.02%
Beef extract	0.02%
Newborn calf serum	5%

Adjust pH to 7.2 - 7.4.

Modified CM+YE medium

Bacto vitamin assay casamino acids (Difco)	7.5g
Bacto yeast extract (Difco)	10.0g
$MgSO_4.7H_2O$	20.0g
Trisodium citrate.$2H_2O$	3.0g
KCl	2.0g
NaCl	150.0g
Fe_2+ solution	1.0ml
Agar	15.0g
Distilled water	1.0 litre

Adjust pH to 7.4.

Fe_2+ solution:

$FeSO_4.7H_2O$	4.98 g
Distilled water	

Modified Davis and Mingioli medium

KH_2PO_4	3.0g
K_2HPO_4	7.0g
$(NH_4)_2SO_4$	1.0g
$MgSO_4.7H_2O$	0.1g
Lactose	2.0g
Agar	15.0g

Medium is made up with 990ml distilled water and 10ml tap water. Dissolve $MgSO_4$ before adding to avoid precipitation. Adjust pH 7.0 and autoclave at 115°C for 20 min

Modified Palleroni and Doudoroff mineral base medium

$Na_2HPO_4.12H_2O$	6.00g
KH_2PO_4	2.40g
NH_4Cl	1.00g
$MgSO_4.7H_2O$	0.50g
$FeCl_3.6H_2O$	0.01g
$CaCl_2.6H_2O$	0.01g
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 6.8. Autoclave at 121°C for 15min

For autotrophic Alcaligenes spp. incubate in an atmosphere of H_2/CO_2/Air (4:1:5).

Modified rogosa broth

Trypticase	10.0g
Yeast extract	5.0g
Tryptose	3.0g
KH_2PO_4	3.0g
Ammonium citrate	2.0g
*Salt solution (see below)	5.0ml
Tween 80	1.0g
Sodium acetate	1.0g
Glucose	20.0g
Cysteine	0.2g
Distilled water	1.0 litre

**Salt solution*

$MgSO_4.7H_2O$	11.50g
$FeSO_4.7H_2O$	1.68g
$MnSO_4$	2.40g
Distilled water	100.00ml

Adjust pH to 6.8 and autoclave at 121°C for 15min

Modified Thorne medium

K_2HPO_4	0.5g
Ferric ammonium citrate	0.5g
$MgSO_4.7H_2O$	1.0g
Glycerol	20.0g
Citric acid	2.0g
L-glutamic acid	4.0g
Distilled water	to 1.0 litre

Adjust pH to 7.4 with NH_4OH (Not NaOH).

Autoclaved at 121°C for 15min

MRS chalk

MRS medium plus 3% $CaCO_3$

MRS cysteine medium

MRS medium plus 0.5g/l cysteine HCl.

MRS fructose medium

Peptone	10.0g
Beef extract	10.0g
Yeast extract	5.0g
Fructose	10.0g
Tween 80	1.0ml
K_2HPO_4	2.0 g
Triammonium citrate	2.0g
$MgSO_4.7H_2O$	0.2g
$MnSO_4.4H_2O$	0.2g
Distilled water	to 1.0 litre

Adjust pH to 6.2-6.6. Autoclave at 121°C for 15min

MRS medium

Commercial preparation

Can be modified with

1% arabinose + 1% maltose or 1% maltose or 1% lactose or 0.5% Panmede

MRS salt

MRS medium plus 10% salt.

Mycobacterium medium

$(NH_4)_2SO_4$	1.0g

Na_2HPO_4	0.5g
KH_2PO^4	0.5g
$MgSO_4$	0.2g
$FeSO_4.7H_2O$	5.0mg
$MnSO_4$	2.0mg
Liquid paraffin	5.0ml
Distilled water	1.0 litre

Homogenise, add 1.5% agar and autoclave at 121°C for 15min

Mycobacterium yeast extract medium

Yeast extract	2.5g
Tryptone	5.0g
Glucose	1.0g
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 7.0 and autoclave at 121°C for 15min

N-acetyl glucosamine medium

MRS medium with glucose replaced by an equivalent amount of N-acetyl glucosamine.

Naphthalene medium

Basic mineral media plus 5mM naphthalene.

NBY medium

Nutrient broth	0.800%
Yeast extract	0.200%
K_2HPO_4	0.200%
KH_2PO_4	0.050%
Glucose (5ml of 10% solution per 100ml)	0.500%
$MgSO_4.7H_2O$ (2.5ml of 1% solution per 100ml)	0.025%

Autoclave glucose and $MgSO_4$ solutions separately and add aseptically.
Add 1.5 % purified agar for solid medium.

Neomycin medium no 1

Nutrient agar plus 0.05% filter-sterilised sucrose and 1mg/ml neomycin sulphate.

Neomycin agar no 2

Blood agar base plus 50µg/ml neomycin.

Neomycin Luria agar

Luria agar plus 12µg/ml neomycin.

Nitrate mineral salts (NMS) medium

Solution 1. 10x NMS salts
Dissolve in approximately 700ml of distilled water (in this order):

KNO_3	10.0g
$MgSO_4.6H_2O$	10.0g
$CaCl_2$ (anhydrous)	2.0g
Dilute water	to 1 litre.

Solution 2. Iron EDTA

FeEDTA	3.8g

Made up to 100ml with distilled water.
Solution 3. Sodium molybdate

$Na_2MoO_4.2H_2O$	0.26g

Made up to 1 litre with distilled water.
Solution 4. Trace elements

$CuSO_4.5H_2O$	1.000g
$FeSO_4.7H_2O$	2.500g
$ZnSO_4.7H_2O$	2.000g
H_3BO_3	0.075g
$CoCl_2.6H_2O$	0.250g
EDTA Disodium salt	1.250g
$MnCl_2.4H_2O$	0.100g
$NiCl_2.6H_2O$	0.050g

Dissolve the above in the specified order in distilled water and dilute to 5 litres. Store in the dark.
Solution 5. Phosphate buffer.

$Na_2HPO_4.12H_2O$	71.6g
KH_2PO_4	26.0g

Dissolve the above in the specified order in 800ml of distilled water. Adjust pH to 6.8 and dilute to 1 litre.
Preparation of NMS Medium
1. Dilute 100ml of solution 1 (10x salts) to 1 litre.
2. Add 1ml of solution 3 (Na molybdate) and 1ml of solution 4 (trace elements).
3. Add 0.1ml of solution 2 (Fe EDTA).
4. Add 1.5% agar for plates.
5. Autoclave at 121°C for 15min
6. Autoclave separately 10ml of solution 5 (phosphate buffer) for every litre of NMS.
7. When the NMS is cool enough to hold in the hand, aseptically add the phosphate buffer. If this is done too early the phosphate will precipitate out.

Nitrate studies medium 1

NaCl	20g
KCl	0.5g
Na_2HPO_4	5.5g
NH_4Cl	0.0127g
K_2SO_4	1.75g
NaH_2PO_4	0.775g
Na_2EDTA	0.75g
$MgSO_4.7H_2O$	0.1g
Acetate	8.3mM
$NaNO_2$-	0.0759g (1.1mM)
Wolin's Trace Elements	7ml
Distilled water	1 litre

Nitrate studies medium 2

NaCl	20g
KCl	0.5g
Na_2HPO_4	5.5g
NH_4Cl	0.0127g
K_2SO_4	1.75g
NaH_2PO_4	0.775g

Na_2EDTA	0.75g
$MgSO_47H_2O$	0.1g
Glycerol	5.56mM
KNO_3	0.111g
Wolin's Trace Elements	7ml

Nitrococcus mobilis medium

(a)$NaNO_2$	10.0%
(b)K_2HPO_4	2.5%
(c)$NaHCO_3$	5.0%
(d) Chelated Metals Solution:	
$CoC_2.6H_2O$	4.00mg
$CuSO_4.5H_2O$	4.00mg
$FeCl_3.6H_2O$	1.00g
$ZnSO_4.7H_2O$	0.30g
$MnSO_4.H_2O$	0.60g
$Na_2MoO_4.2H_2O$	0.15g
EDTA	6.00g
Distilled water	1.00 litre

Adjust pH to 7.5 with NaOH. Add 1ml chelated metals solution to 1 litre seawater. Autoclave at 121°C for 15min. Add 1ml of solution a, b and c to the cooled seawater chelated metals solution.

Nitrogen free medium

K_2HPO_4	1.0g
$MgSO_4.7H_2O$	0.2g
$CaCO_3$	1.0g
NaCl	0.2g
$FeSO_4.7H_2O$	0.1g
$Na_2MoO_4.2H_2O$	5.0mg
Agar	15.0g
Distilled water	to 1.0 litre
Glucose	10.0g

The salts are dissolved in water and the pH is adjusted to approximately 7.0. The agar is then added, dissolved by steaming, and the medium is autoclaved at 121°C for 15min. The glucose is then added aseptically as 50ml of a filter-sterilised 20%(w/v) solution to 1.0 litre of medium.

Nitrosococcus oceanus medium

NH_4Cl	0.635g
$CaCl_2.H_2O$	20.000mg
$MgSO_4.7H_2O$	0.357g
K_2HPO_4	43.000mg
Phenol red	5.000g
*Chelated metals solution	1.000ml
Filtered sea water	1.000 litre

Adjust pH to 7.5. Autoclave at 121°C for 15min. Periodically during growth adjust pH of medium to 7.5 with sterile 0.1M K_2CO_3 (reappearance of original pink/red colour to medium).
* see *Nitrococcus mobilis* medium

Novobiocin agar

Nutrient agar plus 10μg/ml novobiocin.

Nutrient agar (Oxoid CM3)

Commercially available (Oxoid). Autoclave.115°C for 20min

Nutrient agar + 30 μg/ml chloramphenicol

Commercial preparation

N-Z amine with soluble starch and glucose

Glucose	10.0g
Soluble starch	25.0g
Yeast extract	5.0g
N-Z Amine Type A (Sigma C0626)	5.0g
Reagent grade $CaCO_3$	1.0g
Agar	15.0g
Distilled water	1.0 litre

Oatmeal agar

Oatmeal	20.0g
Agar	8.0g
*Trace salts solution	1.0ml
Distilled water	to 1.0 litre

Steam the oatmeal for 20min in the required volume of distilled water. Filter through cheese-cloth and make up the filtrate to its original volume with distilled water. Add trace salts, adjust the pH to 7.2, add the agar and steam to dissolve. Autoclave at 121°C for 15min. Mix well before pouring plates.
*Trace salts solution - see starch salts agar

One tenth nutrient agar

Nutrient Broth No.2 (Oxoid CM67)	2.5g
Agar	15.0g
Distilled water	to 1.0 litre

Autoclave at 121°C for 15min

OM-2

$(NH_4)_2C_2O_4$ (ammonium oxalate)	10-20g
$NaHCO_3$	10g
$NaH_2PO_4.2H_2O$	10g
NaCl	0.7g
KCl	0.57g
$MgCl_2.6H_2O$	0.1g
$CaCl_2.2H_2O$	0.01g
$Na_2S_2O_3.5H_2O$ (sodium thiosulfate)	1g
Deionized water	1000ml

Adjust pH to 6.8-7.0. The first three salts must be dissolved in the indicated sequence. After autoclaving for 15min at 121°C the pH increases to 8.5-9.0. Solid medium contained 1.5 to 2.0% (w/v) of Bacto-Agar.

OTTOW medium

Glucose	1.0g

Peptone	7.5g
Meat extract	5.0g
Yeast extract	2.5g
Casamino acids	2.5g
NaCl	5.0g
Tap water	1.0 litre

Adjusted pH to 8.5.

Peptone yeast glutamate medium

Peptone	20.0g
Yeast extract.	10.0g
Monosodium glutamate	4.0g
Sodium thioglycollate	1.0g
Distilled water	to 1.0 litre

Adjust pH to 7.0-7.2. Autoclave at 121°C for 15min

Pantothenate agar

KH_2PO_4	0.27g
$MgSO_4.7H_2O$	0.26g
$FeSO_4.7H_2O$	2.80mg
$MnSO_4$	1.50mg
Na_2MoO_4	2.10mg
Agar	25.00g
Distilled water	to 1.00 litre

Adjust to pH 6.8-7.0 with KOH and autoclave at 121°C for 10 min. Cool to 50°C and add concentrated, filter-sterilised solution of potassium pantothenate to give a concentration of 2.57g/litre

Pantothenate-free medium

Half strength Difco pantothenate medium AOAC USP(0816) with the following additions per litre:-

Folic acid	30.0µg
Asparagine	0.1g
D-pantethine	250.0µg
Casamino acids (vitamin free)	15.0g
10%K_2HPO_4	0.2ml

Adjust pH to 6.0 with NaOH. Autoclave at 121°C for 15min

Payne, Seghal & Gibbons medium

Casamino acids (Difco)	7.50g
Yeast extract	10.00g
Trisodium citrate	3.00g
KCl	2.00g
$MgSO_4.7H_2O$	20.00g
$FeCl_2.4H_2O$	36.00mg
$MnCl_2.4H_2O$	0.36mg
NaCl	250.00g
Distilled water	1.00 litre

Adjust pH to 7.4. For solid medium add 20.0g agar.

Pentachlorophenol medium (modified)

Basic mineral media plus 1mg/l pentachlorophenol.

Pentachlorophenol medium

K_2HPO_4	0.65g
KH_2PO_4	0.19g
$MgSO_4.7H_2O$	0.10g
$NaNO_3$	0.50g
Sodium glutamate	4.00g

Adjust pH to 7.3-7.4. Autoclave and add 2ml/l of filter sterilised 0.01M $FeSO_4$ stock solution.

To prepare pentachlorophenol stock (10,000ppm) add 1g pentachlorophenol to 100ml 0.5 N NaOH.
To induce for pentachlorophenol degradation inoculate media and place on shaker at 200 rpm at 25-30°C. Monitor growth on spectrophotometer at 560nm. When A560 = 0.5, add 5ml of pentachlorophenol stock (final concentration of 50ppm). To monitor pentachlorophenol degradation spin down 1ml of media and read in a spectrophotometer at 320nm. Degradation should begin within 1h and be complete with in 3-4h. The health of the culture decreases when A560 increases above 1.2.

Peptone succinate agar

$(NH_4)_2SO_4$	1.00g
$MgSO_4.7H_2O$	1.00g
$MnSO_4.H_2O$	2.00mg
$FeCl_3.6H_2O$	2.00mg
Succinic acid	1.68g
Peptone	5.00g
Agar	1.50g
Distilled water	1.00 litre

Adjust pH cautiously to 7.0 with KOH. Dispense in 20ml amounts into 1oz screw capped bottles and autoclave at 121°C for 15min

Peptone yeast medium

Peptone	1%
Yeast extract	0.2%
NaCl	0.2%
Glucose	0.2%

Adjust to pH 7.0.

Phenylobacterium medium

Antipyrin	1.00g
KH_2PO_4	0.30g
$Na_2HPO_4.12H_2O$	0.70g
$NH_4H_2PO_4$	0.30g
$(NH_4)_2HPO_4$	0.70g
$(NH_4)_2SO_4$	0.10g
$CaCl_2.6H_2O$	50.00mg
$MgSO_4.7H_2O$	0.25g
H_3BO_3	0.50mg
$CuSO_4.5H_2O$	0.10mg
KI	0.10mg

$FeCl_3.6H_2O$	0.20mg
$Mn_4.H_2O$	0.40mg
$ZnSO_4.7H_2O$	0.40mg
$(NH_4)_2MoO_4$	0.20mg
Biotin.	0.10mg
Vitamin B_{12}	30.00µg
Agar	15.00g
Distilled water	to 1.00 litre

Adjust pH to 6.8-7.0 and autoclave at 121°C for 15min

Plaice medium - for luminous bacteria

Fresh plaice, minced	200.0g
Peptone	20.0g
NaCl	120.0g
Tap water	4.0 litre

Soak plaice in water and allow to stand for 2 hours. Boil for 1 hour and filter. Add peptone and salt. Adjust pH to 7.3. Boil for a few min and filter.
For solid medium add 20.0g agar per litre. Steam to dissolve and autoclave at 121°C for 15min

Pluton medium

Glucose	1.0%
Starch	0.2%
Peptone (Oxoid)	0.25%
Yeast Extract Oxoid	0.25%
Malt extract (Oxoid)	0.5%
Neopeptone (Difco)	0.5%
Trypticase	0.2%
1 M potassium phosphate buffer pH 7.2	5.0%

Sterile cysteine HCl 2.5% is added 0.1ml per 10ml tube

Postgate's medium

Basal medium	
K_2HPO_4	0.5g
NH_4Cl	1.0g
$CaSO_4$	1.0g
$MgSO_4.7H_2O$	2.0g
Sodium lactate(70%)	3.5ml
Yeast extract	1.0g
Distilled water	1.0 litre

Dissolve above and gas with oxygen free nitrogen for 10 - 15min, then add:-

Thioglycollic acid	0.1 g
Ascorbic acid	0.1 g
$FeSO_4.7H_2O$	0.5 g

Still gassing pH to 7.4, dispense and autoclave at 115°C for 10 min

Postgate's salt medium

Postgate's medium plus 2.5% NaCl.

Postgate's seawater medium

Basal medium	
K_2HPO_4	0.5g
NH_4Cl	1.0g
$CaSO_4$	1.0g
$MgSO_4.7H_2O$	2.0g
Sodium lactate(70%)	3.5ml
Yeast extract	1.0g
Filtered aged sea water	1.0 litre

Dissolve above and gas with oxygen free nitrogen for 10 - 15min, then add:-

Thioglycollic acid	0.1g
Ascorbic acid	0.1g
$FeSO_4.7H_2O$	0.5g

Still gassing pH to 7.4, dispense and autoclave at 115°C for 10min

Potato dextrose agar

Commercial preparation

PPB

- Modification of Caldwell & Bryant 1966

Cellobiose	0.1%
Maltose	0.1%
Glucose	0.1%
Starch	0.1%
Difco Yeast Extract	0.2%
BBL trypticase	0.2%
Mineral I	7.5%
Mineral II	7.5%
Haemin Solution	1.0%
VFA mix (optional)	0.31%
Resazurin Solution	0.1%
Clarified Rumen Fluid	15.0%
Water	

Adjusted to pH 6.8 using 1N NaOH final volume 93%

Additions 2% of a 2.5% L-cysteine HCl solution
5% of an 8% sodium carbonate solution

Note: It is important that the carbonate is thoroughly equilibrated with CO_2 and also that the medium is thoroughly deep gassed with CO_2 before filling out otherwise the pH of the broth can be as high as 9.5.

1. Preparation of a sterile medium
A Medium prepared to 92% of final volume excluding cysteine and carbonate in flask plugged with cotton wool.
B Cysteine HCl 2.5% solution in screw capped bottles with little head space
C Sodium carbonate 8.0% solution in small flask, with large head space and plugged with cotton wool.
A, B, C are autoclaved at 121°C for 15min
2. When atmospheric pressure has been reached remove flasks and insert short sterile gassing jets and pass CO_2 over the surface of the medium and carbonate solution. Place flasks in ice bath. When temperature has cooled to 50°C or below add

cysteine followed by carbonate and insert long gassing jets and deep gas for at least 30min.
3. Fill out aseptically into sterile stoppered tubes using standard Hungate technique.
4. Incubate at 37°C at least overnight to check for contamination or oxidised tubes.

PPB can also be modified with + 1% sodium lactate

PPES-II medium

Polypeptone (Diago Eryokagakii Co.)	2.0 g
Proteose-peptone No.3 (Difco)	1.0 g
Bacto-soytone (Difco)	1.0 g
Bacto yeast extract (Difco)	1.0 g
Ferric phosphate, soluble (Merck)	0.1 g
Marine mud extract	100.0ml
Agar (Wako)	15.0 g
Aged sea water	900.0ml

Adjust pH to 7.6-7.8.

Prosthecobacter medium

$(NH_4)_2SO_4$	0.25g
Glucose	0.25g
Modified Hutner's basal salts	20.00ml
Na_2HPO_4	71.00mg
Distilled water to	1.00 litre

* See brackish prosthecomicrobium medium

Prosthecomicrobium and *Ancalomicrobium* medium

Ammonium sulphate	0.25g
Glucose	0.25g
*Vitamin solution	10.00ml
*Modified Hutner's basal salts	20.00ml
Na_2HPO_4	71.00mg
Distilled water to	1.00 litre

See brackish prosthecomicrobium medium

Pseudoamycolata halophobica medium

Peptone	5.0g
Glucose	5.0g
Yeast extract	3.0g
K_2HPO_4	0.2g
Distilled water	1.0 litre

Pseudomonas medium

Solution (a)

K_2HPO_4	2.56g
KH_2PO_4	2.08g
NH_4Cl	1.00g
$MgSO_4.7H_2O$	0.50g
Agar	20.00g
Distilled water	1.00 litre

Adjust pH to 6.8 and autoclave at 121°C for 15min

Solution (b)

Ferric ammonium citrate	1.0g
$CaCl_2$	0.1g
Distilled water	100.0ml

Sterilise by filtration.

Solution (c)

1M Succinic acid.
Adjust pH to 6.0 with NaOH and autoclave at 121°C for 15min
To solution (a) add 5ml solution (b) and 15ml solution (c)

Pseudomonas medium no 2

Modified Palleroni and Doudoroff mineral base medium plus 0.5 % succinic acid.
pH to 6.8, agar 2%.

Pseudomonas pickettii medium

1.0 litre of Nutrient agar plus K_2HPO_4 (0.45g) $Na_2HPO_4.12H_2O$ (2.39g) Adjusted to pH to 6.8.

Pseudomonas saccharophila medium

Solution (a)

KH_2PO_4	0.44g
Na_2HPO_4	0.48g
NH_4Cl	0.10g
$MgSO_4.7H_2O$	50.00mg
Distilled water	100.00ml

Solution (b)

Ferric ammonium citrate	0.1g
$CaCl_2$	10.0mg
Distilled water	10.0ml

Solution (c)

20% sucrose in distilled water.
Sterilise the three solutions separately. Aseptically add 0.5ml (b) and 1ml (c) to 100ml (a). If a solid medium is required, prepare (a) with 2% agar. For a completely inorganic medium, substitute ferric chloride for ferric ammonium citrate

PYGV medium

*Hutner's mineral salt solution	20.00ml
Bacto peptone	0.25g
Bacto yeast extract	0.25g
Bacto agar	15.00g
Distilled water	965.00ml

Autoclave at 121°C for 20min. After cooling down to 60°C add:

2.5% Glucose solution (filter sterilised)	10.0ml
Vitamin solution (See medium 249)	10.0ml

Adjust pH to 7.5 (carefully, only weakly buffered - approximately 10 drops of 6N KOH per litre of medium required). * see brackish prosthecomicrobium medium

PYS medium

KH_2PO_4	1.0g
$(NH_4)_2SO_4$	1.0g
NaCl	0.2g

$MgCl_2.6H_2O$	0.2g
$CaCl_2.2H_2O$	0.05g
*Trace element solution SL8	1.0ml
Sodium pyruvate	2.2g
Yeast extract	1.0g
Distilled water	1.0litre

**Trace Element Solution SL8*

EDTA-disodium salt	5.2g
$FeCl_2$ $4H_2O$	1.5g
$ZnCl_2$	70.0mg
$MnCl_2.4H_2O$	100.0mg
H_3BO_3	62.0mg
$CoCl_2.6H_2O$	190.0mg
$CuCl_2.2H_2O$	17.0mg
$NiCl_2.6H_2O$	24.0mg
$Na_2MoSO_4.2H_2O$	36.0mg
Distilled water	1.0 litre

Adjust pH to 6.8. Separately sterilise sodium pyruvate by filtration and aseptically add to the medium.

Quarter strength nutrient medium

Nutrient broth (Oxoid CM1) made to ¼ regular strength. For solid medium add 1.5% agar to ¼ strength nutrient broth.

R2A medium

Yeast extract	0.50g
Proteose peptone	0.50g
Casamino acids	0.50g
Glucose	0.50g
Soluble starch	0.50g
Sodium pyruvate	0.30g
K_2HPO_4	0.30g
$MgSO_4.7H_2O$	0.05g
Noble agar	15.00g
Distilled water	1.00 litre

Adjust pH to 7.2 with KH_2PO_4 or K_2HPO_4.
Autoclave at 121°C for 15min

R agar

Bactopeptone	10.0g
Yeast extract	5.0g
Malt extract	5.0g
Casamino acids	5.0g
Beef extract	2.0g
Glycerol	2.0g
Tween 80	50.0mg
$MgSO_4.7H_2O$	1.0g
Agar	20.0g
Distilled water	1.0 litre

Adjust pH to 7.2. Autoclave at 121°C for 15min

Reinforced Clostridial medium (Oxoid CML49)

Commercial Preparation
Can be supplemented with 0.1 or 1% Tween 80; 1% lactose; 5% serum or 5% serum +0.05% cysteine

Renibacterium KDM-2 medium

Peptone	10.0g
Yeast extract	0.5g
Cysteine HCl	1.0g
Agar	15.0g
Foetal calf serum	200.0ml
Distilled water	1.0 litre

Dissolve peptone, yeast extract and cysteine HCl in 800ml final volume distilled water. Adjust pH to 6.5 with NaOH. Add agar and dissolve by heating. Autoclave at 121°C for 15min. Cool to 45°C and add serum.

Rhamnose salts medium

K_2HPO_4	2.9g
KH_2PO_4	2.1g
NH_4Cl	2.0g
$MgSO_4.7H_2O$	0.4g
NaCl	30.0mg
$CaCl_2$	3.0mg
$FeSO_4.7H20$	1.0mg
Yeast extract	3.0g
Rhamnose	10.0g
Distilled water	1.0 litre

Adjust pH to 7.0. Autoclave at 121°C for 15min

Rhodococcus percolatus medium

Glucose	4.0g
Yeast extract	4.0g
Malt extract	10.0g
$CaCO_3$	2.0g
Agar	12.0g
Distilled water	1000.0ml

Adjust pH to 7.2 with KOH before adding agar (use pH-indicator paper).

Rhodocyclus medium

Yeast extract	0.2g
Di-sodium succinate	1g
Ferric citrate solution (0.1g per 100ml)	5ml
KH_2PO_4	0.5g
$MgSO_4.7H_2O$	0.4g
NaCl	0.4g
NH_4Cl	0.4g
$CaCl_2.2H_2O$	50mg
Ethanol	0.5ml
Yeast extract	0.8g
*Trace element solution (see below)	1ml
Distilled water up to	1 litre
pH 5.7	

**Trace element solution:*

$ZnSO_4.7H_2O$	0.1g
$MnCl_2.4H_2O$	30mg
H_3BO_3	0.3g
$CoCl_2.6H_2O$	0.2g
$CuCl_2.2H_2O$	10mg

$NiCl_2.6H_2O$	20mg
$Na_2MoO_4.2H_2O$	30mg
Distilled water	1 litre

Adjust pH to 6.8. Distribute 40ml medium into 50ml screw-capped bottles. Flush each bottle for 1 to 2min with nitrogen gas, then close immediately with rubber septa and screw-caps. Autoclave. Sterile syringes are used to inoculate and remove the samples. Incubate in light using a tungsten lamp. If needed, add 15g agar per litre medium.

Rhodospirillum salinarum medium

NaCl	100g
KCl	5g
$MgCl_2.6H_2O$	5g
NH_4Cl	5g
$MgSO_4.7H_2O$	5g
*Trace elements solution	5ml
1% Ferric citrate solution	10ml
Yeast extract solution (150g/l)	30ml
Peptone (150g/l)	30ml
Distilled water	925ml
**Trace elements solution:*	
$CuSO_4.5H_2O$	1.0mg
$ZnSO_4.7H_2O$	220.0mg
$CoCl_2.6H_2O$	10.0mg
$MgCl_2.4H_2O$	180.0mg
$Na_2MoO_4.H_2O$	6.3mg
Distilled water	1.0 litre

Rhodospirillaceae modified medium

Yeast extract	0.3g
Ethanol	0.5ml
Do-sodium succinate	1.0g
Ammonium acetate	0.5g
Ferric citrate solution (0.1% in H_2O)	5.0ml
KH_2PO_4	0.5g
$MgSO_4.7H_2O$	0.4g
NaCl	0.4g
NH_4Cl	0.4g
$CaCl_2.2H_2O$	0.05g
Vitamin B_{12} solution (10mg in 100ml H_2O)	0.4ml
*Trace element solution SL-6 (see below)	1.0ml
Distilled water	1050.0ml

Adjust pH to 6.8. Boil the medium under a stream of nitrogen gas for a few minutes and distribute 45ml medium into 50ml screw-capped bottles (already flushed with nitrogen gas). Bubble each bottle with nitrogen gas and close immediately with a rubber septum and screw tight. Autoclave at 121°C for 15min. Sterile syringes are used to inoculate and remove samples. Incubate in the light using a tungsten lamp. For brown and other oxygen sensitive *Rhodospirillaceae* add 300mg of L-cysteine (0.03% end concentration) to the boiling medium and readjust the pH to 6.8 or to the prepared medium in bottles inject neutralised sulphide solution (0.005 to 0.01% end concentration). The medium has been modified according to reference 3365.

**Trace element solution SL-6:*

$ZnSO_4.7H_2O$	0.1g
$MnCl_2.4H_2O$	0.03g
H_3BO_3	0.3g
$CoCl_2.6H_2O$	0.2g
$CuCl_2.2H_2O$	0.01g
$NiCl_2.6H_2O$	0.02g
$Na_2MoO_4.2H_2O$	0.03g
Distilled water	1000.0ml

***Neutralised sulphide solution:*

Distilled water	100.0ml
$Na_2S.9H_2O$	1.5g

The sulphide solution is prepared in a 250ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15min at 121°C. After cooling to room temperature the pH is adjusted to about 7.3 by adding sterile 2M H_2SO_4 drop-wise with a syringe without opening the bottle. Appearance of a yellow colour indicatesthe drop of pH to about 8. The solution should be stirred continuously to avoid precipitation of elemental sulphur. The final solution should be clear and is yellow in colour.

Rhodospirillum Medium

Acidic rhodospirillaceae medium plus 0.8g/L yeast extract and 0.5ml/L ethanol. Adjust pH to 6.8.

Rifampicin luria agar

Luria agar plus 30 μg/ml rifampicin.

Rose bengal

Commercial preparation

Ruminococcus pasteuri medium

KH_2PO_4	0.2g
NH_4Cl	0.25g
NaCl	1.0g
$MgCl_2.6H_2O$	0.4g
KCl	0.5g
$CaCl_2.2H_2O$	0.15g
*Trace Elements Solution SL-7 (see below)	1.0ml
Resazurin	1.0mg
$NaHCO_3$	2.5g
Sodium tartrate	2.0g
$Na_2S.9H_2O$	0.36g
Distilled water	1.0litre

Adjust medium for final pH 7.2. Boil medium without sodium bicarbonate, trace elements or sodium sulphide under 80% N_2 and 20% CO_2; dispense and tube with the same gas phase. Cool and aseptically add the filter-sterilised sodium bicarbonate and trace elements. Aseptically add the sodium sulphide, which has been separately autoclaved under N_2.

**Trace Elements Solution SL-7:*

Hydrochloric acid, 25%	10.0ml
$FeCl_2.4H_2O$	1.5g
$CoCl_2.6H_2O$	190.0mg
$MnCl_2.4H_2O$	100.0mg
$ZnCl_2$	70.0mg
$H3BO_3$	62.0mg
$Na_2MoO_4.2H_2O$	36.0mg
$NiCl_2.6H_2O$	24.0mg
$CuCl_2.2H_2O$	17.0mg
Distilled water	1.0litre

Dissolve the $FeCl_2.4H_2O$ in the concentrated HCl, then dilute. Use 1.0ml/litre of medium.

S-8 medium

Na_2HPO_4	1.20g
KH_2PO_4	1.80g
$MgSO_4.7H_2O$	0.10g
$(NH_4)_2SO_4$	0.10g
$CaCl_2$	0.03g
$FeCl_3.6H_2O$	0.02g
$MnSO_4.4H_2O$	0.02g
$Na_2S_2O_3.5H_2O$	10.00g
$NaHCO_3$	0.50g
KNO_3	5.00g
Agar, if required	15.00g
Distilled water	to 1.00 litre

Adjust pH to 7.0. Autoclave 121°C for 15 min

Salt medium

Tryptone	5.0g
Proteose peptone	5.0g
NaCl	58.4g
Distilled water	1000.0ml

Adjust pH to 6.9.

Salt nutrient agar

Nutrient agar plus 2% NaCl. Autoclave121°C for 15min

Salt nutrient agar no 2

Nutrient agar plus 10% NaCl.

Seawater agar

Beef extract (Lab-Lemco)	10.0g
Neutralised bacteriological peptone	10.0g
Filtered, aged sea water	750.0ml
Distilled water	250.0ml

Dissolve ingredients, heating if necessary. Adjust pH to 7.8, boil for 3-5min, filter. Readjust pH to 7.3. Autoclave at 121°C for 15min. For a solid medium add 15g/l agar after readjusting the pH and steam to dissolve the agar prior to autoclaving.

Seawater agar with foetal calf serum

Sea water agar plus 10% foetal calf serum 100.00ml

Sea water agar with horse blood

Seawater agar plus 10% horse blood

Sea water basal medium

Tris-HCl (pH 7.5)	50.0mM
NH_4Cl	10.0g
$K_2HPO_4.3H_2O$	75.0mg
$FeSO_4.7H_2O$	29.0mg
Lactate	2.0g
Artificial sea water	500.0ml
Distilled water	500.0ml
Artificial sea water:	
NaCl	23.37g
$MgSO_4.7H_2O$	24.65g
KCl	1.49g
$CaCl_2.2H_2O$	2.94g
Distilled water	1.00 litre

For solid medium add 20.0g agar or 10.0g purified agar.

Sea water blood agar

Tryptone soya broth plus 1.5% sea salts or NaCl plus 10% horse or sheep blood

Seawater spirillum medium

Spirillum medium made up with 750ml aged sea water and 250ml distilled water. Adjust pH to 7.0 and autoclave at 115°C for 20 min

Sea water yeast peptone medium

Yeast extract	3.0g
Peptone	5.0g
Filtered, aged sea water	750.0ml
Distilled water	250.0ml

Adjust pH to 7.3. Prepare in similar manner to seawater agar.

S-broth

Difco peptone	10.0g
Lab-Lemco meat extract	2.4g
NaCl	2.0g
Distilled water	1.0 litre

Adjust pH to 7.0-7.2 and autoclave at 121°C for 15min

SC medium

Corn meal agar	17.0g
Phytone	8.0g
K_2HPO_4	1.0g
KH_2PO_4	1.0g
$MgSO_4.7H_2O$	0.2g
Haemin (0.1% in 0.05M NaOH)	15.0ml
Distilled water	1.0 litre

Adjust pH to 6.6 with NaOH. Autoclave at 121°C for 15min and cool to 50°C. Aseptically add the following filter-sterilised components.
20% bovine serum albumin

fraction V (SigmaA-9647)	10.0ml
50% glucose	1.0ml
10% cysteine	10.0ml

Soap agar

Beef extract	0.30g
Yeast extract	0.60g
Peptone	1.50g
NaCl	1.50g
Stearic acid	30.00g
Agar	0.33g
NaOH(7M)	15.00ml
Distilled water	300.00ml

Adjust pH to 8.5-9.5. Autoclave at 121°C for 15 min

Sodalis glossinidius medium

Lactalbumin hydrolysate	8.1g
Yeast extract	6.2g
$CaCl_2.2H_2O$	0.25g
KCl	0.25g
$MgCl.6H_2O$	0.12g
NaCl	8.7g
$NaHCO_3$	0.15g
NaH_2PO_4 $2H_2O$	0.28g
D-glucose	5g
Distilled water	1 litre

Adjust pH to 8, then filter sterilise. To 4 parts of the above, add 1 part sterile FCS.

Sodium caseinate agar

Sodium caseinate	2.0g
Yeast extract	0.5g
Peptone	0.5g
K_2HPO_4	0.5g
Agar	15.0g
Distilled water.	to 1.0 litre

Dissolve all except the agar and adjust pH to 7.4-7.6. Add the agar, steam to dissolve, dispense in bottles or tubes. Autoclave at 121°C for 15min

Soil extract nutrient agar

Nutrient agar made up with soil extract instead of water. To prepare soil extract, autoclave 1Kg of soil in 1 litre of tap water at 121°C for 30 min. Add 2g $CaCO^3$, filter and make filtrate up to 1 litre with tap water.

Sorangium medium

KNO_3	1.0g
K_2HPO_4	1.0g
$MgSO_4.7H_2O$	0.2g
$CaCl_2.2H_2O$	0.1g
$FeCl_3.6H_2O$	20.0mg
Agar	10.0g
Tap water	to 1.0 litre

Autoclave121°C for 15min

Add sterile strip of filter paper aseptically to slants or broth. For plates add 4-6 strips of sterile filter paper. Inoculate on to filter paper.

Special mineral salts medium

$(NH_4)_2SO_4$	0.3g
NaCl	5.85g
$CaCl_2.2H_2O$	0.2g
K_2HPO_4	0.1g
$MgSO_4.7H_2O$	0.14g
$FeSO_4.7H_2O$	0.3mg
$CoSO_4.7H_2O$	0.11mg
H_3BO_4	0.6mg
$ZnCl_2$	0.22mg
$CuSO_4.5H_2O$	0.08mg
Distilled water	1.0 litre

Nutrient-enriched Agar

The nutritive agar contains 20g agar per litre of mineral salts solution. The carbon source is any *one* of the following added per litre of mineral salts.

Starch	2.4g
m-Toluic acid (neutralised)	1.35g
n-butanol	1.5ml
Lactic acid (85%)	1.35g
Ethanol (95%)	1.5ml
Glucose	2.4g

After the addition of the carbon source, the pH is adjusted to 7.0 with 0.5 N NaOH.

Special reinforced clostridial medium 1

Reinforced Clostridial medium without Agar + 0.05% cysteine

Special reinforced clostridial medium 2

Reinforced Clostridial medium + 0.3% Panmede (liver extract) + 0.05% Cysteine HCl

Special *Vibrio* medium

LB (Luria-Bertani) medium with agar + 1% NaCl + 2 mg/ml carbenicillin

Sphingomonas medium

5% PTYG

Peptone	0.25g
Tryptone	0.25g
Yeast extract	0.5g
Glucose	0.5g
$MgSO_4.7H_2O$	0.6g
$CaCl_2.2H_2O$	0.07g

Add agar in usual concentration for solid media.

Spirillum medium

Peptone	5.0g
Beef extract	3.0g
Yeast extract	10.0g
Agar (if required)	15.0g
Distilled water	to 1.0 litre

Adjust pH to 7.0. Autoclave at 115°C for 20 min There is no need to filter off the precipitate formed during preparation and sterilisation.

Spirillum nitrogen-fixing medium

KH_2PO_4	0.40g
K_2HPO_4	0.10g
$MgSO_4.7H_2O$	0.20g
NaCl	0.10g
$CaCl_2$	20.00mg
$FeCl_3$	10.00mg
$NaMoO_4.2H_2O$	2.00mg
Sodium malate	5.00g
Yeast extract	0.05g
Distilled water	1.00 litre

Adjust pH to 7.2-7.4. Autoclave at 121°C for 15min

Spiroplasma medium

PPLO broth (minus crystal violet) (Difco)	21.0g
Yeast extract	5.0g
Sorbitol	70.0g
Fructose	1.0g
Glucose	1.0g
Phenol red	20.0mg
Distilled water	800.0ml

Autoclave 121°C for 15min
Supplement, when cooled, with 100ml horse serum (previously heated at 60°C for 30min to sterilise). Incubate as static broth in conical flask at 32°C until medium begins to turn orange.
Add 1% agar for solid medium.

SP medium

Raffinose	1.0g
Sucrose	1.0g
Galactose	1.0g
Soluble starch	5.0g
Casitone (Difco 0259)	2.5g
$MgSO_4.7H_2O$	0.5g
K_2HPO_4	0.25g
Agar	15.0g
Distilled water	to1 litre

Sporomusa medium

K_2HPO_4	0.348g
KH_2PO_4	0.227g
NH_4Cl	0.500g
$MgSO_4.7H_2O$	0.500g
$CaCl_2.2H_2O$	0.250g
NaCl	2.250g
$FeSO_4.7H_2O$	0.002g
*Vitamin solution	10.000ml
*Trace element solution SL-10	1.000ml
$NaHSeO_3$	15.100µg
Yeast extract	2.000g
Casitone	2.000g
$NaHCO_3$	4.000g
Resazurin	1.000mg
Cysteine hydrochloride	0.300g
$Na_2S.9H_2O$	0.300g
Distilled water	1.00 litre

* see *Acetobacterium* medium
Adjust pH to 7.0. Prepare the medium anaerobically under 80% N_2 + 20% CO_2 gas mixture. Add fructose at 0.5% final concentration from a filter sterilised anaerobic stock solution.
**Trace element solution SL-10:*

HCl (25%; 7.7 M)	10.0ml
$FeCl_2.4H_2O$	1.5g
$ZnCl_2$	70.0mg
$MnCl_2.4H_2O$	100.0mg
H_3BO_3	6.0mg
$CoCl_2.6H_2O$	190.0mg
$CuCl_2.2H_2O$	2.0mg
$NiCl_2.6H_2O$	24.0mg
$Na^2MoO_4.2H_2O$	36.0mg
Distilled water	990.0ml

First dissolve $FeCl_2$ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1.0 litre

Sporulation medium for *Streptomyces*

Yeast Extract	1.0g
Beef extract	1.0g
Tryptose	2.0g
$FeSO_4$	Trace
Glucose	10.0g
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 7.2. For broth, eliminate agar and reduce concentration to 1/3 of the given quantities.

SSM medium

Solution (a)

$MgSO_4.7H_2O$	0.2g
Na_2HPO_4	6.0g
KH_2PO_4	3.0g
Purified agar	15.0g
Distilled water	850.0ml

Solution (b)

Glucose	5.0g
Distilled water	50.0ml

Solution (c)

Casein hydrolysate	20.0g
Distilled water	100.0ml

Filter solution (c) through Whatman no. 1 paper. Autoclave the three solutions separately at 121°C for 15 min, then mix.

Starch nutrient agar

Nutrient agar plus 1% starch.

Starch salts agar

Solution (a)

K_2HPO_4	1.0g
$MgSO_4.7H_2O$	1.0g
NaCl	1.0g
$(NH_4)_2SO_4$	2.0g
$CaCO_3$	2.0g
Trace salts	1.0ml
Distilled water	500.0ml

Solution (b)

Soluble starch	10.0g
Distilled water	500.0ml
Trace salts	
$FeSO_4.7H_2O$	0.1g
$MnCl_2.4H_20$	0.1g
$ZnSO_4.7H_2O$	0.1g
Distilled water	100.0ml

To prepare (b) make a paste of the starch with a little of the water and then gradually add the remaining water. Mix (a) and (b), adjust the pH to 7.2, add 20g agar and steam to dissolve the agar. Autoclave at 121°C for 15min. Before pouring slopes or plates, mix the medium thoroughly to ensure reasonable distribution of the chalk.

Streptomycin l broth medium

L (Luria) broth plus 25µg/ml streptomycin.

Streptomycin nutrient agar

Nutrient agar plus 50µg/ml streptomycin.

Streptomycin nutrient agar no.2

Nutrient agar plus 125µg/ml streptomycin sulphate.

Streptomycin nutrient agar no.3

Nutrient agar plus 500µg/ml streptomycin sulphate.

Streptomycin nutrient agar no. 4

Nutrient agar plus 80µg/ml streptomycin.

Sucrose peptone agar

Sucrose	20.00g
Difco peptone	5.00g
$MgSO_4.7H_2O$	0.25g
K_2HPO_4	0.50g
Agar	15.00g
Distilled water	1.00 litre

Dissolve ingredients and adjust pH to 7.0-7.2. Autoclave at 121°C for 15min

Sucrose yeast extract medium

Sucrose	20.0g
Yeast extract	4.0g
K_2HPO_4	2.5g
$MgSO_4.7H_2O$	1.0g
*Trace elements solution	4.0ml

**Trace elements solution:*

$MnSO_4.4H_2O$	3.0g
$FeSO_4.7H_2O$	9.0g
$ZnSO_4.7H_2O$	18.0g
$CuSO_4.5H_2O$	0.8g
$CoCl_2.6H_2O$	0.9g
Conc. H_2SO_4	5.0ml
Distilled water	1.0 litre

Sulfolobus medium

Yeast extract	1.00g
$(NH_4)_2SO_4$	1.30g
KH_2PO_4	0.28g
$MgSO_4.7H_2O$	0.25g
$CaCl_2.2H_2O$	0.07g
$FeCl_3.6H_2O$	0.02g
$MnCl_2.4H_2O$	1.80mg
$Na_2B_4O_7.10H_2O$	4.50mg
$ZnSO_4.7H_2O$	0.22mg
$CuCl_2.2H_2O$	0.05mg
$NaMoO_4.2H_2O$	0.03mg
$VOSO_4.2H_2O$	0.03mg
$CoSO_4$	0.01mg
Freshly distilled water	to 1.0 litre

Adjust pH to 2-3 with 5M H_2SO_4. Autoclave at 121°C for 15min. Warm medium to 70°C before inoculation with *Sulfolobus*.

Supplement yeast glucose broth

Yeast glucose broth supplemented with 10ug/ml tetracyclin and 50ug/ml erythromycin

Tetracycline l broth medium

L (Luria) broth plus 12.5µg/ml tetracycline.

Tetracycline luria agar no. 1

Luria agar plus 12µg/ml tetracycline.

Tetracycline luria agar no. 2

Luria agar plus 10µg/ml tetracycline.

Tetracycline luria agar no. 3

Luria agar plus 20 µg/ml tetracycline.

Tetracycline TY salt medium

TY salt medium plus 12.5µg/ml tetracycline.

TGYM medium

Tryptone	5.0g
Glucose	1.0g
Yeast extract	3.0g

DL-methionine	0.5g
Tap water	1.0 litre

Adjust pH to 7.3-7.5.

Thermoactinopolyspora medium

Phytone	15.0g
Maltose	20.0g
Yeast extract	2.0g
Agar	15.0g
Tap water	to 1.0 litre

Adjust pH to 7.2 and autoclave at 121°C for 15min

Thermocrispum medium

Standard I Broth (Merck)	25.0g
Malt extract	10.0g
$CaCO_3$	2.0g
Agar	12.0g
Distilled water	1000.0ml

Adjust pH to 7.2.

Thermodesulfobacterium medium

Na_2SO_4	3.0g
NH_4Cl	1.0g
$MgCl_2.6H_2O$	0.2g
KH_2PO_4	0.3g
$Na_2HPO_4.12H_2O$	2.0g
*Trace mineral solution	10.0ml
$FeSO_4.7H_2O$	1.5mg
**Vitamin solution	5.0ml
Resazurin	1.0mg
Yeast extract	1.0g
Sodium lactate	4.0g
$Na_2S.9H_2O$	0.5g
Distilled water	1.0 litre

**Trace mineral solution:*

Nitrilotriacetic acid	12.800g
$FeCl_3.4H_2O$	0.200g
$MnCl_2.4H_2O$	0.100g
$CoCl_2.6H_2O$	0.170g
$CaCl_2.2H_2O$	0.100g
$ZnCl_2$	0.100g
$CuCl_2$	0.020g
H_3BO_3	0.010g
$Na_2MoO_4.2H_2O$	0.010g
$NiCl_2.6H_2O$	0.026g
NaCl	1.000g
Na_2SeO_3	0.020g
Distilled water	1.00 litre

Firstly adjust nitrilotriacetic acid to pH 6.5 with KOH.

***Vitamin solution:*

Biotin	2.0mg
Folic acid	2.0mg
Pyridoxine-HCl	10.0mg
Thiamine-HCl	5.0mg
Riboflavin	5.0mg
Nicotinic acid	5.0mg
DL-Calcium pantothenate	5.0mg
Vitamin B_{12}	0.1mg
p-Aminobenzoic acid	5.0mg
Lipoic acid	5.0mg
Distilled water	1.0 litre

Adjust pH to 6.8-7.0. Prepare the medium anaerobically under 100% nitrogen. Prepare concentrated solutions each of yeast extract, sodium lactate and sodium sulphide anaerobically under nitrogen and autoclave separately. Before use, neutralise the sodium sulphide solution by drop-wise addition of 1N HCl.

Thermoleophilum medium

$NaNO_2$	2.00g
$MgSO_4.7H_2O$	0.20g
$FeSO_4.7H_2O$	1.00mg
Na_2HPO_4	0.21g
NaH_2PO_4	90.00mg
$CuSO_4.5H_2O$	5.00µg
H_3BO_3	10.00µg
$MnSO_4.5H_2O$	10.00µg
$ZnSO_4.7H_2O$	70.00µg
MoO_3	10.00µg
KCl	0.04g
$CaCl_2$	15.00mg
Distilled water	1.00 litre

Adjust pH to 7.0. Autoclave at 121°C for 15min Add 1.0ml n-heptadecane to 1.0 litre of medium.

Thermomonospora medium

Czapek (sucrose nitrate) agar plus 0.2% yeast extract and 0.6% casamino acids.
Adjust pH to 8.0.

Thermotoga elfii medium

NH_4Cl	1.0g
K_2HPO_4	0.3g
KH_2PO_4	0.3g
$MgCl_2.6H_2O$	0.2g
$CaCl_2.2H_2O$	0.1g
KCl	0.1g
NaCl	10.0g
*Trace element solution (see below)	10.0ml
Sodium acetate	0.5g
Yeast extract	5.0g
Trypticase	5.0g
Resazurin	0.5mg
L-Cysteine	0.5g
Na_2CO_3	2.0g
Sodium thiosulfate $5H_2O$	5.0g
Glucose	4.0g
$Na_2S.9H_2O$	0.5g
Distilled water	1000.0ml

Prepare medium anaerobically under 80% N_2 + 20% CO_2 gas atmosphere. Autoclave separately anaerobic (N_2) stock solutions of Na_2CO_3, thiosulfate, glucose and sulphide. The pH of the completed medium is 7.5.

**Trace element solution:*

Nitrilotriacetic acid	1.5g
$MgSO_4.7H_2O$	3.0g
$MnSO_4.2H_2O$	0.5g
NaCl	1.0g
$FeSO_4.7H_2O$	0.1g
$CoSO_4.7H_2O$	0.1g
$CaCl_2.2H_2O$	0.1g
$ZnSO_4.7H_2O$	0.18g
$CuSO_4.5H_2O$	0.01g
$KAl(SO_4)_2.12H_2O$	0.02g
H_3BO_3	0.01g
$Na_2MoO_4.2H_2O$	0.01g
$NiCl_2.6H_2O$	0.025g
$Na_2SeO_3.5H_2O$	0.3mg
Distilled water	1000.0ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

Thermus brockii medium

TY salts medium adjusted to pH 7.6.

Thermus enhanced medium

Yeast extract	2.5g
Tryptone	2.5g
Agar	28.0g
Nitrilotriacetic acid	100.0mg
$CaSO_4.2H_2O$	40.0mg
$MgCl_2.6H_2O$	200.0mg
0.01 M Ferric citrate	0.5ml
*Trace Element Solution (see below)	0.5ml
**Phosphate Buffer (see below)	100.0ml
Distilled water	900.0ml

Adjust pH to 7.2 with NaOH. Autoclave at 121°C for 15min. Autoclave the phosphate buffer separately and then add to the medium.

***Phosphate buffer:*

KH_2PO_4	5.44g
$Na_2HPO_4.12H_2O$	43.0g
Distilled water	1.0 litre

**Trace Element Solution:*

Nitrilotriacetic acid	12.8g
$FeCl_2.4H_2O$	1.0g
$MnCl_2.4H_2O$	0.5g
$CoCl_2.6H_2O$	0.3g
$CuCl_2.2H_2O$	50.0mg
$Na_2MoO_4.2H_2O$	50.0mg
H_3BO_3	20.0mg
$NiCl_2.6H_2O$	20.0mg
Distilled water	1.0 litre

Thiobacillus acidophilus medium

Glucose	10.00g
$(NH_4)_2SO_4$	3.00g
KH_2PO_4	0.50g
$MgSO_4.7H_2O$	1.00g
KCl	0.10g
$Ca(NO_3)_2.4H_2O$	18.00mg
$FeSO_4.7H_2O$	0.01mg
Agar	15.00g
Distilled water	to 1.00 litre

In liquid medium, the pH should be adjusted to 3.5 with H_2SO_4. In solid medium, the pH should be adjusted to 4.5 with H^2SO^4 after autoclaving the medium. Sterilise the glucose solution and the basal solution separately. Autoclave at 121°C for 15min

Thiobacillus agar (non-aciduric)

$(NH_4)_2SO_4$	0.1g
K_2HPO_4	4.0g
KH_2PO_4	4.0g
$MgSO_4.7H_2O$	0.1g
$CaCl_2$	0.1g
$FeCl_3.6H_2O$	2.0mg
$MnSO_4.4H_2O$	2.0mg
$Na_2S_2O_3.5H_2O$	10.0g
Purified agar	12.0g
Distilled water	to 1.0 litre

Dissolve all except the agar in distilled water and adjust the pH to 6.6. Add the agar and autoclave at 115°C for 20 min

Thiobacillus agar (aciduric)

NH_4Cl	0.1g
KH_2PO_4	3.0g
$MgCl.6H_2O$	0.1g
$CaCl_2$	0.1g
$Na_2S_2O_3.5H_2O$	5.0g
Purified agar	20.0g
Distilled water	to 1.0 litre

Dissolve all except the agar in distilled water and adjust the pH to 4.2. Add the agar and steam to dissolve. Autoclave at 121°C for 15min

Thiobacillus ferrooxidans medium

Solution I

$(NH_4)_2SO_4$	0.5g
K_2HPO_4	0.5g
$MgSO_4.7H_2O$	0.5g
1N H_2SO_4	5.0ml
Distilled water	1.0 litre

Solution II

$FeSO_4.H_2O$	167.0g
1N H_2SO_4	50.0ml
Distilled water	1.0 litre

Autoclave solution I at 121°C for 15min and sterilise solution II by filtration. After sterilisation 4 parts of solution I are added to 1 part of solution II.

Thiobacillus thermophilus medium

$Na_2S_2O_3$	5.0g
$NaHCO_3$	1.0g
$MgCl_2$	0.1g
NH_4Cl	0.1g
Na_2HPO_4	0.2g
Distilled water	to 1 litre

Adjust pH to 7.0-7.2. Autoclave 109°C for 20min or filter sterilise.

Thiobacillus thiooxidans medium

K_2HPO	3.500g
$(NH_4)_2SO$	0.300g
$MgSO_4.7H_2O$	0.500g
$FeSO_4.7H_2O$	0.018g
$CaCl_2$	0.250g
Flowers of sulphur	5.000g
Distilled water	1.0 litre

Dissolve the salts in distilled water and adjust the pH to 4.5. Add the sulphur aseptically after sterilisation.

Thiocyanate agar

Solution (a)

KH_2PO_4	1.0g
K_2HPO_4	1.0g
$MgSO_4.7H_2O$	0.2g
$CaCl_2$	20.0mg
$FeCl_3.6H_2O$ (60%)	0.1ml
Purified agar	30.0g
Distilled water	800.0ml
Solution (b)	
KCNS	3.6g
Distilled water	100.0ml
Solution (c)	
Disodium succinate	1.5g
Distilled water	100.0ml

Autoclave the three solutions separately at 121°C for 15min, then mix.

Thiosphaera medium

Na_2HPO_4	4.2g
KH_2PO_4	1.5g
NH_4Cl	0.3g
$MgSO_4.7H_2O$	0.1g
*Trace element solution	2.0ml
KNO_3	0.1g
Distilled water	1.0 litre

Adjust pH to 8.0 - 8.2. To avoid precipitation, filter sterilise broth. For agar prepare broth at double strength, filter sterilise and add aseptically to sterile 3% agar.

**Vishniac and Santer trace element solution:*

Ethylenediamine tetraacetic acid	50.00g
$ZnSO_4.7H_2O$	22.00g
$CaCl_2$	5.54g
$MnCl_2.4H_2O$	5.06g
$FeSO_4.7H_2O$	4.99g
$(NH_4)_6Mo_7O_{24}.4H_2O$	1.10g
$CuSO_4.5H_2O$	1.57g
$CoCl_2.6H_2O$	1.61g
Distilled water	1.00 litre

Adjust pH to 6.0 with KOH.

Thiosulphate salts broth

$Na_2S_2O_3.5H_2O$	24.8g
NH_4Cl	2.20g
KH_2PO_4	2.00g
*Artificial sea water	500.00ml
Deionized water	500.00ml
**Artificial sea water:*	
NaCl	23.476g
$MgCl_2$	4.981g
Na_2SO_4	3.917g
$CaCl_2$	1.102g
KCl	0.664g
$NaHCO_3$	0.192g
KBr	0.096g
H_3BO_3	0.026g
$SrCl_3$	0.024g
NaF	0.003g
Water to	1.00 litre

Adjust pH to 5.0.

Thymine nutrient broth

Nutrient broth (Oxoid CM1) plus 40µg/ml thymine.

Todd Hewitt broth (Oxoid)

Commercial preparation

Can be supplemented with5% serum; 1% Tween 80; 1% NaCl + 5% serum + 0.05% cysteine or 0.05% cysteine

Tomato juice agar

Commercial preparation

TPYG medium

Trypticase	10.0 g
Peptone	5.0 g
Yeast extract	5.0 g
Distilled water	to 1.0 litre

Bottle in 19ml amounts. Autoclave at 121°C for 15min. Add 1ml of filter sterilised 20% glucose solution aseptically to each 19ml of base.

Trypticase soya agar

Trypticase soya broth (BBL 11768)	30.0g
Agar	15.0g
Distilled water	1 litre

Trypticase soya yeast extract

Trypticase Soya Broth	30g
Yeast Extract	3g
Agar	15g
Distilled Water	1000ml

Adjust pH to 7.0 - 7.2.

Tryptone agar

Tryptone (Difco 0123)	8.0g
NaCl	8.0g
Agar	15.0g
Distilled water	1.0 litre

Tryptone bile agar (Oxoid CM595)

Commercial preparation

Tryptone glucose extract agar

Beef extract	3.0g
Tryptone	5.0g
Glucose	1.0g
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 7.0 and autoclave at 121°C for 15min

Tryptone soya agar

Commercial preparation

Tryptone soya broth

Commercial preparation
Can be supplemented with 1M KCl

Tryptone soya yeast extract broth

Tryptone soya broth plus 0.3% yeast extract.

Tryptone/yeast extract medium

Tryptone	10.0g
Yeast extract	1.0g
Distilled water	to 1.0 litre

Autoclave at 121°C for 15min

Tryptone yeast extract salt medium

Solution 1:

NaCl	125.0g
$MgCl_2.6H_2O$	50.0g
K_2SO_4	5.0g
$CaCl_2.6H_2O$	0.2g
Distilled water	500.0ml

Adjust pH to 6.8

Solution 2:

Tryptone (Oxoid)	5.0g
Yeast extract (Difco)	5.0g
Distilled water	500.0ml

Sterilise the two solutions separately and mix after sterilisation. After mixing, measure pH with a glass electrode and adjust pH to 6.8.

Tryptose blood agar base

Tryptone soya broth plus 7% sterile defibrinated horse blood added to cooled (50°C) but not solidified medium

TPY Medium

Tryptone	10g
Phytone peptone	5.0g
Glucose	5.0g
Yeast extract	2.5g
Tween 80	1.0ml
Cysteine Hydrochloride	0.5g
K_2HPO_4	2.0g
$MgCl_2.6H_2O$	0.5g
$ZnSO_4.7H_2O$	0.25g
$CaCl_2$	0.15g
$FeCl_3$	Traces
Agar	15.0g
Distilled water	1000ml

Make up salts in 100x stock solutions. Add 1ml of each salt to 100ml of media.
Autoclave media at 12°C for 25min

TSY medium

Trypticase Soy Broth (BBL 11768)	30.0g
Yeast extract	5.0g
Agar	20.0g
Distilled water	1.0 litre

Autoclave 121°C for 15min

TYG medium

Tryptone	20.0g
Glucose	5.0g
Yeast extract	0.5g
Sodium thioglycollate	0.5g
KH_2PO_4	4.0g
$MgSO_4.7H_2O$	0.2g
$FeSO_4.2H_2O$	5.0mg
$MnSO_4.4H_2O$	5.0mg
NH_4MoO_4	5.0mg
Distilled water	to 1.0 litre

Adjust pH to 7.4 with NaOH. Autoclave 116°C for 10min

TY medium

Bacto tryptone	5.0g
Yeast extract	3.0g
$CaCl_2.6H_2O$	15.0g
Distilled water	to 1.0 litre

Autoclave121°C for 15min

TY salt medium

Tryptone	10.0g

Yeast extract	5.0g
NaCl	10.0g
Distilled water	to 1.0 litre

Adjust pH to 7.0. Autoclave at 121°C for 15 min

TY salts medium

Tryptone	1.0g
Yeast extract	1.0g
*Salts solution	100.0ml
Distilled water	900.0ml

Autoclave121°C for 15min

**Salts solution:*

Nitrilotriacetic acid	1.00g
$CaSO_4.2H_2O$	0.60g
$MgSO_4.7H_2O$	1.00g
NaCl	80.00mg
KNO_3	1.03g
$NaNO_3$	6.89g
Na_2HPO_4	1.11g
$FeCl_3$ (0.028%)	10.00ml
** Trace elements solution	10.00ml
Distilled water	1.00 litre

Adjust pH to 8.2 with 1M NaOH.

***Trace elements solution:*

H_2SO_4	0.5ml
$MnSO_4.H_2O$	2.2g
$ZnSO_4.7H_2O$	0.5g
H_3BO_3	0.5g
$CuSO_4$	16.0mg
$Na_2MoO_4.2H_2O$	25.0mg
$CoCl_2.6H_2O$	46.0mg
Distilled water	

Universal beer agar (Oxoid CM651)

Commercial preparation

Urea nutrient agar

Nutrient agar plus 2% urea.

5ml filter-sterilised 20% urea solution are added aseptically to 100ml cooled molten sterile nutrient agar. The medium is then immediately dispensed aseptically.

Uric acid medium

Nutrient agar plus 0.5% uric acid

Make up the nutrient agar in seven eighths of the volume of distilled water normally required. Prepare a 4% suspension of uric acid in the remaining one eighth of the volume of distilled water. Autoclave both solutions at 121°C for 15min and mix aseptically immediately before pouring. Keep well mixed during pouring.

Vanillate medium

KH_2PO_4	0.40g
$(NH_4)_2SO_4$	1.00g
$MgSO_4.7H_2O$	0.01g
Yeast extract	0.10g
Agar	20.00g
*Trace element solution	10.00ml
Distilled water	1.00 litre

**Trace elements solution*

H_3BO_3	0.50mg
$CuSO_4.5H_2O$	0.04mg
KI	0.10mg
$FeCl_3$	0.20mg
$MnSO_4.4H_2O$	0.40mg
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.20mg
$ZnSO_4.7H_2O$	0.40mg
Distilled water	1.00 litre

Autoclave mineral salts-yeast extract medium at 121°C for 15 min

A solution of vanillic acid as sodium salt (to give final concentration of 1.5 g/l) is prepared separately and filter sterilised. Add aseptically to autoclaved mineral salts-yeast extract medium.

Van Niel's medium

K_2HPO_4	1.0g
$MgSO_4$	0.5g
Yeast extract (Difco)	10.0g
Agar	20.0g
Tap water	to 1.0 litre

Adjust pH to 7.0-7.2. Autoclave at 121°C for 15min

VCR medium

$NaNO_3$	2.00g
NH_4Cl	0.50g
KH_2PO_4	1.50g
K_2HPO_4	1.20g
$MgSO_4.7H_2O$	0.20g
$CaCl_2.2H_2O$	15.00mg
$FeCl_3$	0.01g
$CuSO_4.5H_2O$	1.00mg
Vitamin B_{12}	1.00µg
Purified agar	15.00g
Distilled water	to 1.00 litre

Adjust pH to 7.2. Autoclave all ingredients except vitamin B_{12} at 121°C for 15 min, then add vitamin B_{12} aseptically.

Vitamin B_{12} medium (Difco 0457-15-1) plus colistin

Add 500mg/litre of colistin sulphate (Pharmax) and 250nanograms/litre cyanocobalamin (Glaxo) to the prepared medium.

Von Hofsten & Malmqvist medium B

$NaNO_3$	2.00g

K_2HPO_4	0.50g
$MgSO_4.7H_2O$	0.20g
$CaCl_2.H_2O$	0.02g
$MnSO_4.H_2O$	0.02g
$FeSO_4.7H_2O$	0.02g
Carbon source	2.00g
Distilled water	1.00 litre

Adjust pH to 7.5. For solid medium substitute 15.0g agar for the carbon source.

Woods and Welton medium

Casein hydrolysate	17.0g
Glucose	5.0g
Glycerol	10.0g
NaCl	23.4g
Na_2SO_3	0.1g
Nutrient broth	8.0g
Soytone (soy digest)	3.0g
Tryptone	0.5g
Casamino acids (Vitfree)	0.5g
Yeast extract	2.0g
Agar	15.0g
Distilled water	1.0 litre

Adjust to pH 7.6.

Xanthobacter agilis medium

Solution I:

$Na_2HPO_4.12H_2O$	9.0g
KH_2PO_4	1.5g
NH_4Cl	1.0g
$MgSO_4.7H_2O$	0.2g
Trace elements solution	1.0ml
Sodium proprionate or 3-hydrobutyrate	1.0g
Agar	15.0g
Distilled water	1.0 litre

Adjust pH to 7.0.

Solution II:

Ferric ammonium citrate	50mg
$CaCl_2.2H_2O$	100ml
Distilled water	100ml

Trace elements in 2ml:

H_3BO_4	560µg
$NiCl_2.H_2O$	160µg
$CuSO_4.5H_2O$	16µg
$MnCl_2.4H_2O$	16µg
$ZnSO_4.7H_2O$	350µg
$Na_2MoO_4.2H_2O$	100 µg

Sterilise solutions I and II separately. Mix aseptically after sterilisation. This prevents formation of a precipitate.

Xanthobacter tagetidis medium

NH_4Cl	0.4g
$MgSO_4.7H_2O$	0.1g
*Trace metal solution	10ml
$Na_2HPO_4.2H_2O$	7.9g
KH_2PO_4	1.5g
Oxoid bacteriological agar	15g
Sodium acetate	0.4g
Distilled water	to 1 litre

Adjust pH to 7.3. Sterilise at 115°C for 10min. Can add phenol red to observe pH change if desired. Produces shiny yellow colonies.

*Trace metal solution

Dissolve 50g EDTA (disodium salt) in about 400ml of water. Dissolve 9g NaOH in the EDTA solution. Best to do this in a 1-2L beaker on a magnetic stirrer.

Dissolve the following salts individually in 30-40ml lots water and add to the EDTA - NaOH solution (plus 5-10ml washings).

$ZnSO_4.7H_2O$	11g
$CaCl_2$ or $CaCl_2$ $2H_2O$	5g or 7.34g
$MgCl_2.4H_2O$	2.5g
$CoCl_2.6H_2O$	0.5g
Ammonium molybdate	0.5g
$FeSO_4.7H_2O$	5g
$CuSO_4.5H_2O$	0.2g

Adjust pH to pH6 with N NaH (20-40ml approx. Add gradually with mixing).

Make up to 1 litre with distilled water. Store in a dark bottle. Do NOT autoclave the stock solution.

Yeast dextrose agar

Nutrient Broth No 2	2.5%
Glucose	0.5%
Agar	1.5%
Yeast Extract	0.3%

pH 6.8

Can be supplemented with 10% serum if desired

Yeast glucose agar

Glucose	20.0g
Yeast extract	10.0g
Agar	15.0g
Distilled water	to 1.0 litre

Autoclave121°C for 15min

Yeast glucose broth

Yeast Glucose agar without the agar.

Yeast glucose cysteine medium

Yeast glucose broth + 0.05% cysteine

Yeast glucose urea agar

Yeast glucose agar + 2% urea

Yeast extract agar 1

Yeast extract	3g
Agar	15g
Distilled water	to 1 litre

Yeast extract water

Yeast extract	10.0g

Distilled water	1.0 litre

Autoclave 121°C for 15min

Yeast malate medium

Yeast extract	1.0g
$KH_2PO_4.12H_2O$	1.0g
$NaHCO_3$	1.0g
$(NH_4)_2SO_4$	0.5g
Sodium malate	1.0g
*Trace elements solution	1.0ml
Distilled water	1.0 litre

Adjust pH to 6.8-7.0. Autoclave 121°C for 15 min

*Trace elements solution:

$ZnSO_4.7H_2O$	0.10g
$MnCl_2.4H_2O$	0.03g
H_3BO_3	0.30g
$CoCl_2.6H_2O$	0.20g
$CuCl_2.2H_2O$	0.01g
$NiCl_2.6H_2O$	0.02g
$Na_2MoO_4.2H_2O$	0.03g
Distilled water	1.00 litre

Yeast malt agar

Yeast extract	4.0g
Malt extract	10.0g
Glucose	4.0g
Agar	20.0g
Distilled water	1.0 litre

Adjust pH to 7.2. Autoclave at 121°C for 15 min
Can be supplemented with 5% NaCl if desired

Yeast mannitol medium

Mannitol	10.00g
Yeast extract	0.25g
$MgSO_4.7H_2O$	0.25g
$CaSO_4.2H_2O$	30.00mg
$Na_2HPO_4.12H_2O$	1.20g
KH_2PO_4	0.55g
NaCl	0.25g
$FeSO_4.7H_2O$	3.50mg
$ZnSO_4.7H_2O$	160.00µg
$CuSO_4.5H_2O$	80.00µg
H_3BO_3	500.00µg
$MnSO_4.4H_2O$	400.00µg
Agar	20.00g
Distilled water	1.00 litre

Adjust pH to 7.0 and autoclave at 121°C for 15min

Yeast mannitol extract medium

Yeast extract	1.0g
Soil extract	200.0ml
Mannitol	10.0g
Agar	20.0g
Tap water	to 1.0 litre

Adjust pH to 7.0-7.2.

Yeast nutrient agar

Nutrient agar plus 0.2% yeast extract.

Yeast peptone medium

Yeast extract.	2.5g
Peptone	2.5g
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 7.0-7.4. Autoclave at 121°C for 15min

Yeast peptone salt medium

Yeast peptone plus 0.125% NaCl.

Yeastrel agar (388)

Lab-lemco (Oxoid)	5.0g
Yeastrel*	7.0g
Peptone (Difco)	9.5g
NaCl	5.0g
Agar	15.0g
Distilled water	1.0 litre

Adjust pH to 7.0.

*Yeastrel is produced by Mapleton's Foods Ltd, Moss Street, Liverpool and is available from health food shops.

YGCB salt medium

Peptone	10.0g
Beef broth	10.0g
Yeast extract	5.0g
Glucose	10.0g
Triammonium citrate	5.0g
Sodium acetate	2.0g
$MgSO_4.7H_2O$	0.2g
$MnSO_4.4H_2O$	50.0mg
NaCl	50.0g
Tween 80	1.0ml

Adjust pH to 6.7 and autoclave at 121°C for 15min

YGLPB medium

Peptone (Oxoid)	1.00g
Lemco (Oxoid)	0.80g
Yeast extract (Oxoid)	0.30g
KH_2PO_4	0.25g
K_2HPO_4	0.25g
$MgSO_4.7H_2O$	0.02g
$MnSO_4.4H_2O$	5.00mg
Glucose	0.50g
Lactose	0.50g
Distilled water	100ml

Adjust pH to 6.8.

YGLM

Litmus milk (LM) Skimmed, separated milk or reconstituted powdered separated milk. 0.75g/litre of Difco Powdered Litmus (code 0209-13) is added

to the milk and thoroughly stirred for 15-20min. Autoclave at 110 °C for 10 min. Incubated for 1 week at 30°C to check for sterility before use.

Yeast Glucose Litmus Milk (YGLM/YDLM)

Prepared as for Litmus Milk plus 1.0% Glucose and 0.3% Yeast Extract (Oxoid).

LM + Chalk, UDLM + Chalk Approximately 2.0% $CaCO_3$ (thick layer in bottom of tube or bottle) is pre-sterilised in situ at 121°C for 15min before the milk media is added. This reduces the risk of contamination by spore formers surviving the lower heat treatment for the milk. Sterilised at 110°C for 10min then incubated as for LM.

YMA medium

Mannitol	10.0g
KH_2PO_4	0.5g
$MgSO_4.7H_2O$	0.2g
NaCl	0.1g
Yeast extract	0.4g
$CaCO_3$	4.0g
Agar	15.0g
Distilled water	to 1.0 litre

Adjust pH to 6.8-7.0 and autoclave at 121°C for 15min

Ymomonas medium

Yeast extract	10.0g
Glucose	10.0g
Tap water	to 1.0 litre

Dissolve the above ingredients in tap water and adjust the pH to 4.8. Autoclave at 115°C for 20min Boil the medium immediately before use.

YT medium

Tryptone	8.0g
Yeast extract	5.0g
NaCl	5.0g
Distilled water	1.0 litre

Autoclave121°C for 15 min

9K medium

Solution (a)

$(NH_4)_2SO_4$	3.0g
KCl	0.1g
K_2HPO_4	0.5g
$MgSO_4.7H_2O$	0.5g
$Ca(NO_3)_2$	10.0mg
10N H_2SO_4	1.0ml
Distilled water	700.0ml

Solution (b)

$FeSO_4.H_2O$	44.0g
Distilled water	300.0ml

Prepare solutions (a) and (b) separately. Dispense (a) as 70ml in 250ml screw-capped bottles, and (b) as 30ml amounts in 1oz screw-capped bottles. Autoclave (a) and (b) separately at 121°C for 15min. Immediately before use add (b) aseptically to (a).

Media for fungi

Corn Meal Agar (CMA)

Maize	30g
Oxoid Agar Nº 3	20g
Tap Water	to 1 litre

Place the maize and water in a saucepan (if meal is not available break up 30-35g of grain and pass through a coffee mill). Heat over a double saucepan until boiling, continue heating for one hour stirring occasionally. Filter the decoction through muslin, add the agar, and boil until it is dissolved. Autoclave at 121°C for 20min

Czapek Dox Agar (CZ)

Stocks

Made with stock Czapek solutions

Solution A

$NaNO_3$	40.0g
KCl	10.0g
$MgSO_4.7H_2O$	10.0g
$FeSO_4.7H_2O$	0.2 g
Distilled water	to 1 litre

Stored in a refrigerator.

Solution B

K_2HPO_4	20 g
Distilled water	to 1 litre

Stored in a refrigerator.

Medium

Stock solution A	50ml
Stock solution C	50ml
Distilled water	900ml
Sucrose (Analar)	30g
Oxoid Agar Nº 3	20g

Dissolve agar in distilled water then add sucrose and stock solutions just before autoclaving.

To each litre add 1ml of following stock solutions:

$ZnSO_4.7H_2O$ Analar 1.0g in 100ml distilled water

$CuSO_4.5H_2O$ Analar 0.5g in 100ml distilled water

Autoclaved at 121°C for 20min

Czapek Yeast Autolysate Agar (CYA)

K_2HPO_4	1.0g
Czapek concentrate	10.0ml
Oxoid Yeast extract or autolysate	5.0g
Sucrose (Analar)	30.0g
Oxoid Agar Nº 3	15.0g
Distilled water	to 1.0 litre

Autoclave at 121°C for 15min

See Pitt, 1973.

Glucose yeast medium (GYM)

Glucose	10g
$NH_4H_2PO_4$	1g
KCl	2g
$MgSO_4.7H_2O$	0.5g
1% w/v $ZnSO_4.7H_2O$ solution	1ml
0.5% w/v $CuSO_4.5H_2O$ solution	1ml
Distilled water	to1 litre

Autoclaved at 121°C for 15min

Malt broth (MB)

Malt extract (Amersham)	30g
Mycological Peptone (Oxoid)	5g
Distilled water	to1litre

Autoclaved at 121°C for 20min

Malt Extract Agar (MA)

White bread malt extract (EDME Ltd)	20g
Oxoid Agar Nº 3	20g
Tap water	to 1 litre

Add agar to water and dissolve over a double saucepan, add malt and dissolve. Adjust pH to 6.5. Autoclave at 121°C for 20min

Malt-Czapek Agar (MCZ)

Stock Czapek solution A	50ml
Stock Czapek solution C	50ml
Sucrose	30g
Toffee malt extract	40g
Oxoid Agar Nº 3	20g
Distilled water	900ml

Dissolve malt extract and agar in water. Heat over a double saucepan until dissolved. Then add sucrose, when dissolved add stock solutions. Adjust pH to 5.0 and autoclave at 121°C for 20min

Malt Extract Agar plus Sucrose

For organisms requiring high osmotic pressure for sporulation.

	M_{20}	M_{40}	M_{60}
Malt extract	20g	20g	20g
Sucrose	200g	400g	600g
Agar	20g	20g	20g
Tap water	1 litre	1 litre	1 litre

Prepare in the same way as Malt Extract Agar but add sugar last to reduce caramelisation.

Malt Extract Agar (MEA)

Blakeslee's formulation.

Malt extract (powdered, Difco or Oxoid)	20g
Peptone, bacteriological	1g
Glucose (Analar)	20g
Oxoid Agar Nº 3	15g
Distilled water	to 1 litre

Autoclaved at 121°C for 15min

See Raper & Thom, 1949; Pitt, 1973.

Oat Agar (OA)

Oat Meal (ground)	30 g
Oxoid Agar Nº 3	20 g
Tap water	to 1 litre

Add oat meal to 500ml of water in a saucepan. Heat for 1h. To the other 500ml water add agar and dissolve in a double saucepan. Pass cooked oat meal through a fine strainer and add to agar mixture. Stir thoroughly. Autoclaved at 121°C for 20min

Potato Carrot Agar (PCA)

Avoid new potatoes, which do not make good media. Red Désirée potatoes have been found to be best. Wash, peel and grate vegetables.

Grated potato	20g
Grated carrot	20g
Oxoid Agar Nº 3	20g
Tap water	to 1 litre

Boil vegetables for about 1 h in 500ml tap water, then pass through a fine sieve keeping the liquid. The agar is added to 500ml of water in a double saucepan. When the agar has dissolved add the strained liquid and stir. Pour through a funnel into bottles. Autoclave at 121°C for 20min

Potato Dextrose Agar (PDA)

Avoid new potatoes. Red Désirée have been found to be best.

Potatoes	200g
Oxoid Agar Nº 3	20g
Dextrose	15g
Tap water	to 1 litre

Scrub potatoes clean and cut into 12mm cubes (do not peel). Weigh out 200g and rinse rapidly under a running tap, and drop into 1 l of tap water in a saucepan. Boil until potatoes are soft (about 1h) then put through blender. Add 20 g of agar, and heat in a double saucepan until dissolved. Then add 15g of dextrose and stir until dissolved. Make up to 1 litre. Place into 250ml bottles, stirring occasionally to ensure that each bottle has a percentage of solid matter. Autoclave at 121°C for 20min. (3lb of potatoes will make 7 litres of agar).

Potato Sucrose Agar (PSA)

Medium

*Potato water	500ml
Sucrose	20g
Oxoid Agar Nº 3	20g
Distilled water	500ml

Heat in double saucepan until agar is dissolved. Autoclave at 121°C for 15min. 5lb potatoes makes 7 litres. Adjust to pH 6.5 with calcium carbonate if necessary.

***To make 2 litres of potato water:**

Tap water	1125g
Potato	450g

Peal and dice potatoes, suspend in double cheesecloth and boil in the tap water until almost cooked.

PSA using powdered potato

Powdered potato	5g
Sucrose	20g
Agar	20g
Distilled water	to 1 litre
Calcium carbonate	5g

Autoclaved at 121°C for 15min

Rabbit Dung Agar (RDA)

The rabbit dung must be from wild rabbits.
5 pellets in medical flats for plates
3 pellets in universals for slopes.

Oxoid Agar Nº 3	15g
Tap water	to 1 litre

Heat agar to dissolve. Pour into medical flats or universals with the pellets. Autoclaved at 126°C for 20min

Sabouraud's Agar

Glucose	20g
Peptone	10g
Agar	15g
Water	to 1 litre

Autoclaved 114°C for 15min

Sabouraud`s Dextrose Agar (SDA)

Mycological Peptone (Oxoid)	10g
Dextrose	20g
Agar No3 (Oxoid)	15g
Distilled Water	to 1 litre

Autoclaved at 121^{o}C for 15min

Soil Extract Agar (SEA)

(Flentje's formula for *Corticium praticola*, promotes formation of basidia).

Stock

To make extract:

Soil	1 kg
Water	1 l

Agitate frequently for a day or two; pour extract through glass wool, and make up to 1 litre again. Prepare SEA as follows.

Medium

Extract	1.0 litre
Sucrose	1.0g
KH_2PO_4	0.2g
Dried yeast	0.1g
Oxoid Agar Nº 3	25.0

NB. It may be advisable to test pH before pouring into bottles.

Starch Agar (SA)

Soluble starch	40g
Marmite	5g
Oxoid Agar Nº 3	20g
Tap water	to 1 litre

Place all the constituents in water and heat in a double saucepan until dissolved. Bottle and sterilise (pH is 6.5 - 7 and requires no adjustment). Autoclaved at 121°C for 20 min

Syntheyic Nutrient Agar (SNA)

KH_2PO_4	1g
KNO_3	1g
$MgSO_4.7H_20$	0.5g
KCl	0.5g
Glucose analar	0.2g
Sucrose analar	0.2g
Agar No 3 (Oxoid)	20g
Distilled water	to1 litre

pH 6.5 (HCl/NaOH)

Autoclaved at 121^{o}C for 20 min

Tap Water Agar (TWA)

Tap water	to 1 litre
Oxoid Agar Nº 3	15g

Dissolve agar in water. Autoclaved at 121°C for 20min

Tap Water and Glycerol (TWA+G)

Tap water	to 1 litre
Oxoid Agar Nº 3	15g
Glycerol	25ml

Dissolve agar in water then add glycerol. Autoclaved at 121°C for 20min

V8 Agar (V8A)

V8 Vegetable juice	200ml
Oxoid Agar Nº 3	20g
Distilled water	800ml

Dissolve agar in water and add vegetable juice. Adjust pH to 6.0 with 10% sodium hydroxide. Autoclave at 121°C for 20 min. (pH after autoclaving should be 5.8).

V8 Agar (as recommended for Actinomycetes)

V8 Vegetable juice	200ml
Calcium carbonate	4g
Oxoid Agar Nº 3	20g
Water	800ml

Adjust to pH 7.3 with KOH Autoclaved at 121°C for 20min

Yeast extract sucrose (YES)

Yeast extract	20g
Sucrose	150g
Agar No3 (Oxoid)	20g
Distilled water	to 1 litre

Autoclaved at 121°C for 20 min

Yeast Phosphate Soluble Starch (YPSS)

Difco yeast extract	4.0g
Soluble starch	15.0g
K_2HPO_4	1.0g
$MgSO_4.7H_2O$	0.5g
Oxoid Agar Nº 3	20.0g
Distilled water	to 1.0 litre

Mix together, dissolve and dispense. Autoclaved at 121°C for 15min

Media for yeasts

Yeast Malt (YM)
Broth Medium

Yeast Extract	3g
Malt extract	3g
Peptone	5g
Glucose	10g
Distilled water	to 1 litre

Autoclaved at 121^{o}C for 15min
For agar medium add agar to a final concentration of 1.5-2%
YM both is also commercially produced by Difco (Difco 0711-01)

Yeast Extract Peptone-glucose (YEP-glucose)
Broth Medium

Yeast Extract	5g
Peptone	5g
Glucose	10g
Distilled water	to 1 litre

Autoclaved at 121^{o}C for 15min
For agar medium add agar to a final concentration of 1.5-2%

Malt Extract (ME)
Broth Medium

Malt Extract	3g
Peptone	5g
Distilled water	to 1 litre

Autoclaved at 121^{o}C for 15min
For agar medium add agar to a final concentration of 1.5-2%

Sabouraud's Glucose (SG)
Broth Medium

Glucose	40g
Peptone	10g
Distilled water	to 1 litre

Autoclaved at 121^{o}C for 15min
For agar medium add agar to a final concentration of 1.5-2%

Yeast Peptone Dextrose (YPD)
Broth Medium

Yeast Extract	10g
Peptone	20g
Glucose	40g
Distilled water	to 1 litre

Autoclaved at 121^{o}C for 15min
For agar medium add agar to a final concentration of 1.5-2%

Yeast Nitrogen Base
Broth Medium

Yeast Nitrogen Base (Difco 0392-15-9): a chemically defined medium to which a carbon source must be added
For agar medium add agar to a final concentration of 1.5-2%

Yeast Nitrogen Base without amino acids
Broth Medium

Yeast Nitrogen Base without amino acids (Difco 0919-15-3): a chemically defined medium to which a carbon source must be added. Can be supplemented with appropriate amino acids or other source of nitrogen. Useful for genetically defined strains.
For agar medium add agar to a final concentration of 1.5-2%

Media for animal cell lines

Ready to use Media

Ready to use media are suppled with shipments of new cell lines, either frozen or growing, to avoid any risk that the customer's existing stock of medium and serum may not be optimal for the new cell line. The medium and serum provided by ECACC will be the same as that used to cultivate your cell shipment so that the cells may be established in your laboratory with minimum change of culture conditions. Medium is in a ready to use format – serum and glutamine added so you can be sure that your cells get the start they are used to. Subsequently the cells can be switched to your regular medium and serum supplies while maintaining security stocks in ECACC medium until the switch is validated. Ready to use medium is available in most types with 10 or 20% serum added (500ml).

ECCAC Media

DMEM: Dulbecco's modified eagles medium
EMEM (HBSS): Eagles minimum essential medium with Hank's balanced salt solution
EMEM (EBSS): Eagles minimum essential medium with Earles's balanced salt solution
F12K: ModifiedHam F12 (Coon's Medium)
GMEM: Glasgow's modified minimum essential medium
IDMEM: Iscove's modified DMEM medium
MEM: Minimum essential Medium
McCOYS'S 5A
RPMI 1640 Medias: Roswell Park Memorial Institute media's
SDM: Schneiders drosophila medium

Premium Grade Serum

ECACC routinely propogates a large variety of diverse cell types, many of which are fastidious and place exacting demands on the medium and its components. Consequently ECACC's primary supplies of culture medium and medium supplements, particularly foetal bovine serum, are subjected to rigorous incoming Quality Control and batch selection.

Foetal Bovine Serum (FBS) can demonstrate significant variation between different suppliers, which probably reflects differences in national/ regional animal husbandry, collection and processing practices and the final supplier's inventory policies. Significant differences are also observed between different batches of bovine serum from the same supplier. Evidence of these qualitative differences will depend on the tolerance of the cell lines and the stringency of the culture conditions. ECACC selects batches of bovine serum using more stringent conditions than most of its customers. This is additional to the detailed certificate of analysis provided by the original supplier.

ECACC only sources serum, for its general and hybridoma collection, which is guaranteed to be of USA origin, supported by relevant documentation that can be supplied upon request.

ECACC now offers the facility for its customers to use the same reserved serum batches as we use. Our Premium Grade selected serum will be from the same reserved stock as that used to cultivate the cells we ship to you. Premium Grade Foetal Bovine Serum can be supplied in 100ml or 500ml plastic bottles at a very competitive price. A reservation and call off system can be arranged. This batch of serum will only be available via ECACC as the whole batch has been reserved solely for ECACC's use.

Appendix C Useful addresses and contacts

FOR UKNCC MEMBER COLLECTION ADDRESSES: SEE PAGE iii

UKNCC (United Kingdom National Culture Collection)
Secretariat: Dr. David Smith
CABI Bioscience UK Centre (Egham), Bakeham Lane, Egham, Surrey, UK
Tel: +44-1491-829080 Fax: +44-1491-829100
Email: D.Smith@Cabi.org
URL: http://ukncc.co.uk/

i. Databases

WDCM (World Data Center for Micro-organisms)
Center for Information Biology, National Insitute of Genetics, 1111 Yata, Mishima, Shizuoka 411, Japan
Tel: +81-559-81-6895; Fax: +81-559-81-6896;
URL: http://wdcm.nig.ac.jp/
SUGAWARA Hideaki
<hsugawar@GENES.NIG.AC.JP>

MSDN (Microbial Strains Data Network)
The secretariat is based at 63 Wostenholm Road, Nether Edge, Sheffield S7 1LE (Dr Sunil Nandi)
Tel: +44- 114 258 3397; Fax: +44- 114 258 3402
Email: s.nandi@sheffield.ac.uk.

CABRI
Common Access to Biological Resources and Information
Dr W Hominick c/o CABI Bioscience UK Centre (Egham), Bakeham Lane, Egham, Surrey, UK Tel: +44-1491-829080; Fax: +44-1491-829100
Email: w.hominick@cabi.org

ii. Federations and Organisations

UKFCC (United Kingdom Federation of Culture Collections), Dr John Day, Secretary, Centre for Ecology & Hydrology, Institute of Freshwater Ecology, Windermere Laboratory, The Ferry House, Far Sawrey, Ambleside, Cumbria LA22 0LP.
Tel: +44-15394-42468; Fax: +44-15394-46914
Email: JGD@ceh.ac.uk

ECCO (European Culture Collections Organizations), Dr Maija-Liisa Suitiko, Secretary, VTT, Biotechnology and Food Research, PO Box 1501, FIN-02044 VTT, Finland, Tel: +358 0 456 5133, Fax: +358 0 455 2028, E-mail: maija-liisa.suitiko@VTT.FI

WFCC (World Federation for Culture Collections), Dr David Smith, Secretary, CABI Bioscience UK Centre (Egham), Bakeham Lane, Egham, Surrey, UK
Tel: +44-1491-829080 Fax: +44-1491-829100
Email: D.Smith@Cabi.org

MIRCEN (Microbial Resource Centres), The MIRCEN Secretariat
Division of Scientific Research and Higher Education, United Nations Educational Scientific, and Cultural Organization (UNESCO), 7 Place de Fontenoy, 75700 Paris, France, Tel: +331 4568 3883; Fax: +331 4306 1122

iii. UK based Culture Collections not in the National Service Collection Network

Aquatic Peronosporomycete Culture Collection
Department of Botany, School of Plant Sciences, 2 Earley Gate, Whiteknights, Reading, Berks RG6 2AU. Tel: 0118 93181864. Holdings of 600 strains.

BEG (La Banque Européenne des Glomales) see IIB

CABI Insect Pathology Culture Collection
CABI Bioscience, Silwood Park, Ascot, Berks. Tel: 01344 872999
Holdings of 800 filamentous fungi

CABI Weed Pathology Culture Collection
CABI Bioscience, Silwood Park, Ascot, Berks. Tel: 01344 872999
Holdings of 3800 filamentous fungi

CEH Merlewood Research Station
74 Jutland Avenue, Flookburgh, Grange-over-Sands LA11 7LQ. Tel: 015395 58366
Holdings of 1000 bacteria, filamentous fungi & yeasts (including flax retting & humic acid/peat related strains)

CSL York Food Microbiology Collection
Central Science Laboratory, Sand Hutton, York YO4 1LZ. Tel: 01904 462624
Holdings of 900, mainly pathogenic bacteria

Don Whitley Scientific Culture Collection
14 Otley Road, Shipley, West Yorkshire BD17 7ES. Tel: 01274 595728
Holdings of 2957, mainly bacterial, some filamentous fungi

HRI Microbiology Culture Collection
Horticulture Research International, Wellesbourne, Warwick CV35 9EF. Tel: 01789 470382
Holdings of 5000, mainly filamentous (edible) fungi, but includes bacteria, actinomycetes and yeasts.

IGER Rhizobium Collection
IGER, Plas Gogerddan, Aberystwyth, Ceredigion SY23 3EB. Tel: 01970 823000
Holdings of 375 bacteria (rhizobia)

IIB (Biotechnology MIRCEN) / BEG Collection of Arbuscular Mycorrhizal Fungi
International Institute of Biotechnology, 1/13 Innovation Buildings, Sittingbourne Research Centre, Sittingbourne, Kent ME9 8HL
U.K. http://wwwbio.ukc.ac.uk/beg/
E-Mail:j.c.dodd@ukc.ac.uk
Holdings of 500, non-axenically culturable symbiotic fungi, maintained on living plants

John Innes Centre Streptomyces Collection
John Innes Centre, Norwich Reserch Park, Colney, Norwich NR4 7UH. Tel: 01603 452571
Holdings of 4000 actinomycetes

Logan Bacillus Collection
School of Biological and Biomedical Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow G4 0BA. Tel: 0141 3313207
Holdings of 1500 *Bacillus* spp and related strains

Manchester University Collection of Bacteria
University Dept of Medical Microbiology, 2nd floor, Clinical Sciences Building, MRI, Oxford Road, Manchester M13 9WL. Tel: 01612768825/31
Holdings of 350 mainly bacteria, but includes actinomyces

Marine Laboratory Bacterial and Viral Culture
Marine Laboratory, PO Box 101, Victoria Road, Aberdeen AB11 9DB
Holdings of 3000 bacteria and fish viruses

Mast Cryobank Culture Collection
Mast, Mast House, Derby Road, Bootle, Liverpool L20 1EA. Tel: 0151 933 7277
Holdings of 1300 mainly bacteria but includes actinomycetes and fungi

Newcastle Actinomycete Culture Collection
Dept of Agricultural & Environmental Science, University of Newcastle, Newcastle upon Tyne, NE1 7RU. Tel: 0191 2227706
Holdings of 3000 strains of actinomycetes

North Manchester General Gospital Collection
Department of Microbiology, Hope Hospital, Eccles Old Road, Salford M6 8HD
Holdings of 6000 mainly yeasts, but including filamentous fungi

Philip Harris Education Culture Collection
Gazelle Road, Weston-super-Mare, North Somerset BS24 9BJ. Tel: 01934413606
Holdings of 85 (wide range of teaching strains)

Plymouth Algal Culture Collection
Plymouth Marine Laboratory, Citadel Hill, Plymouth PL1 2PB. Tel: 01752633100
Holdings of 500 marine algal flagellates

Reading Scientific Services Culture Collection
25/27 Robert Cort Estate, Britten Road, Reading RG2 0AU. Tel: 0118 9868541
Holdings of 500 algae

Rhizobium mutants and cloned DNA Collection
John Innes Centre, Colney, Norwich NR4 7UH
Holdings of 2000 *Rhizobium* mutants and cloned DNA

Rowett Research Institute Collection of rumen
Bucksburn, Aberdeen AB21 9SB. Tel: 01224 712751
Holdings of 150 mainly bacteria including anaerobic rumen

SAC, Inverness
Drummonhill, Stratherrick Road, Inverness

IV2 4JZ. Tel: 01463 243030
Holdings of 2500 bacteria, mainly of veterinary origin

Sheffield Microbiology Culture Collection
Department of Molecular Biology & Biotechnology, University of Sheffield, Forth Court, Western Bank, Sheffield. Tel: 01142224409
Holdings of 150 mainly bacteria, some yeasts

Tik-borne Amboviruses
London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT.
Tel: 0207 927 2293
Holdings of 45 viruses

University of Bradford Culture Collection
Dept of Biomedical Sciences, Bradford BD7 1DP. Tel: 01274 235523
Holdings of 1202 actinomycetes

University of Portsmouth Culture Collection
School of Biological Sciences, University of Portsmouth, King Henry Building, Portsmouth PO1 2DY. Tel: 01705 848484
Holdings of 6000 mainly marine fungi (some marine protozoa)

Veterinary Laboratories Agency
Salmonella Stock Culture Collection & Field Strain Collection, Woodham Lane, Newham, Addleston, Surrey KT15 3NB.
Tel: 01932 357462
Holdings of 80000

VLA Culture Collection
Veterinary Laboratories Agency, Rougham Hill, Bury St. Edmunds IP33 2RY. Tel: 01284 724499
Holdings of 12500 bacteria of veterinary origin

Zeneca Billingham Culture Collection
Zeneca Life Science Molecules, PO Box 2, Billingham, Cleveland TS23 1YN.
Tel: 01642 364567
Holdings of 1500, mainly bacteria

Zeneca Plant Pathology Culture Collection
Zeneca Agrochemicals, Jealotts Hill, Bracknell, Berks RG42 6EY. Tel: 01344 414443
Limited distribution of their 3500 strains, mainly filamentous fungi, but includes bacteria

iv. European based belonging to the European Culture Collection Organisation (ECCO)

Belgium

BCCM/IHEM, Scientific Institute of Public Health - Louis Pasteur, Mycology Section, Rue J. Wytsman 14, B-1050 Brussels, Belgium, Tel +32-2-6425630, Fax +32-2-6425519, Email bccm.ihem@ihe.be

BCCM/LMBP, Universiteit Gent (RUG), Laboratorium voor Moleculaire Biologie, Plasmid Collection, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium, Tel +32-9-2645347, Fax +32-9-2645348, Email bccm.lmbp@lmb.rug.ac.be

BCCM/LMG, Universiteit Gent (RUG), Laboratorium voor Microbiologie, Bacteria Collection, K.L. Ledeganckstraat 35, B-9000 Gent, Belgium, Tel +32-9-2645108, Fax +32-9-2645346, Email bccm.lmg@rug.ac.be

BCCM/MUCL, Mycotheque de l'Universite Catholique de Louvain, Faculté des Sciences Agronomiques (UCL), Place Croix du Sud 3 Bte 6, B-1348 Louvain-la-Neuve, Belgium, Tel +32-10-473742, Fax +32-10-451501, Email bccm.mucl@mbla. ucl.ac.be

Bulgaria

NBIMCC, National Bank for Industrial Micro-organisms and Cell Cultures, P.O. Box 239, 1113 Sofia, Bulgaria, (visiting address: 125 Tsarigradsko chausse blvd., block 2), Tel +359-2-720865, Fax +359-2-9733058, Email nbimcc@yellowpages-bg.com

Czech Republic

CAPM, Collection of Animal Pathogenic Micro-organisms, Veterinary Research Institute, Hudcova 70, CZ-62132 Brno, Czech Republic, Tel +42-5-41321241, Fax +42-5-41211229, Email kahr@vuvel.anet.cz

CCF, Culture Collection of Fungi, Charles University, Faculty of Science, Benátská 2, CZ-12801 Prague 2, Czech Republic, Tel +420-2-24915520 (+420-2-66053076), Fax +420-2-21953125, Email vanova@prfdec.natur.cuni.cz (kubatova@ prfdec.natur.cuni.cz

CCM, Czech Collection of Micro-organisms, Masaryk University, Tvrdého 14,CZ-60200 Brno, Czech Republic, Tel +420-5-43247231, Fax +420-5-43247339, Email ccm@ sci.muni.cz

CNCTC, Czech National Collection of Type Cultures, National Institut of Public Health, Srobárova 48, CZ-10042 Prague 10, Czech Republic, Tel +42-2-67310578, Fax +42-2-746024

Denmark

IBT, IBT Culture Collection of Fungi, Technical University of Denmark, Department of Biotechnology, Building 221, DK-2800

Lyngby, Denmark, Tel +45 4593 3066, Fax +45 4588 4922, Email ut@ibt.dtu.dk

Finland

VTT, VTT Biotechnology and Food Research, Culture Collection, P.O. Box 1501, FIN-02044 VTT, Finland, (visiting address: Tietotie 2, Espoo), Tel +358-9-4565133, Fax +358-9-4552028, Email maija-liisa.suihko@vtt.fi

France

CFBP, Collection Francaise des Bacteries Phytopathogenes, INRA Station de Pathologie Végétale et Phytobactériologie, 42 rue G. Morel, B.P. 57, F-49071 Beaucouzé Cedex, France, Tel +33-41225729, Fax +33-41225705

CIP, Collection des Bactéries de l´Institut Pasteur, B.P. 52, 25 rue du Docteur Roux, F-75724 Paris Cedex 15, France, Tel +33-1-45688775, Fax +33-1-40613007

CNCM, Collection Nationale de Cultures de Micro-organismes, Institut Pasteur, 25 rue du Docteur Roux, F-75724 Paris Cedex 15, France, Tel +33-1-45688251, Fax +33-1-45688236

LCP, Museum National d'Histoire Naturelle, Laboratoire de Cryptogamie, 12 rue Buffon, F-75005 Paris, France, Tel+33-1-40793194, Fax +33-1-40793594, Email roqueber@frmnhn.fr

Germany

DSMZ, DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany, Tel +49-531-26160, Fax +49-531-2616418, Email dsmz@gbf-braunschweig.de

Greece

ACA-DC, Dairy Collection, Agricultural University of Athens, Iera Odos 75, Botanikos, GR-11855 Athens,Greece, Tel +30-1-5294661, Fax +30-1-5294651, Email kalatz@auadec.aua.ariadne-t.gr

ATHUM, Collection of Fungi, University of Athens, Department of Biology, Section of Ecology and Systematics, Panepistimiopolis, GR-15784 Athens, Greece, Tel +30-1-7244380, Fax +30-1-7243325, Email ekapsan@ atlas.uoa.ariadne-t.gr

BPIC, Collections of Phytopathogenic Fungi and Bacteria, Benaki Phylopathological Institute, 8 St. Delta Street, Kiphissia, GR-14561 Athens, Greece, Tel +30-1-8078832, Fax +30-1-8077506, Email ppsall@leon. nrcps.ariadne-t.gr

Hungary

HNCMB, Hungarian National Collection of Medical Bacteria, "B. Johan" National Center for Epidemiology, Gyali ut 2-6, H-1097 Budapest, Hungary, Tel +36-1-2152250, Fax +36-1-2150731, Email konkoly@oki1.joboki.hu

NCAIM, National Collection of Agricultural and Industrial Micro-organisms, Somlói út. 14-16, H-1118 Budapest, Hungary, Tel/Fax +36-1-2095304, Email jtornai@hoya.kee.hu, Internet http://ncaim.kee.hu

Italy

ICLC, Interlab Cell Line Collection, CBA - ABC Advanced Biotechnology Center, Biotechnology Department, Largo Rosanna Benzi 10, I-16132 Genova, Italy, Tel +39-10-5737283, Fax +39-10-5737295, Email iclc@ist.unige.it

DBVPG, Collezione dei Lieviti Industriali, Dipartimento di Biologia Vegetale, Università di Perugia, Borgo 20 Giugno 74, I-06121 Perugia, Italy, Tel +39-75-5856457, Fax +39-75-5856470

NCB, National Culture Bank, Università di Udine, Dip. Biologia Applicata Difesa Piante, Area Rizzi, Via delle Scienze 208, I-33100 Udine, Italy, Tel +39-432-558503, Fax +39-432-558501, Email micol@pldef.uniud.it

Latvia

MSCL, Microbial Strain Collection of Latvia, University of Latvia, Faculty of Biology, Kronvalda Blvd. 4, LV-1586 Riga, Latvia, Tel +371-7322914, Fax +371-7325657, Email collect@lanet.lv

The Netherlands

CBS, Centraalbureau voor Schimmelcultures, P.O. Box 273, NL-3740 AG Baarn, The Netherlands, (visiting address: Oosterstraat 1, Baarn), Tel +31-355481211, Fax +31-355416142, Email stalpers@cbs.knaw.nl

Norway

NIVA, Culture Collection of Algae, Norwegian Institute for Water Research, P.O. Box 173 Kjelsås, N-0411 Oslo, Norway, (visiting

address: Brekkeveien 19, Oslo), Tel +47-22-185100, Fax +47-22-185200

Poland

IBA, Collection of Micro-organisms Producing Antibiotics, Institute of Biotechnology and Antibiotics, Staroscinska 5, PL-02-516 Warsaw, Poland, Tel +48-22-496051, Fax +48-22-494207

IPF, Collection of Industrial Micro-organisms, Institute of Agricultural and Food Biotechnology (IAFB), Rakowiecka 36, PL-02-532 Warsaw, Poland, Tel +48-22-6063691, Fax +48-22-490426, Email misiewicz@ibprs.waw.pl

KOS, Collection of Salmonella Micro-organisms, Institute of Maritime and Tropical Medicine, National Salmonella Centre, Powstania Styczniowego 9 b, PL-81-519 Gdynia, Poland, Tel +48-58-6223011, Fax +48-58-6223354, Email renglo@immt.gdynia.pl

PCM, Polish Collection of Micro-organisms, Polish Academy of Sciences, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Czerska 12, PL-53-114 Wroclaw, Poland, Tel +48-71-679424, Fax +48-71-679111, Email mordarsk@immuno.pan.wroc.pl

Portugal

PYCC, Portuguese Yeast Culture Collection, SA Biotecnologia, Fac. Ciências e Tecnologia/UN, Quinta da Torre, 2825 Monte de Caparica, Portugal, Tel +351-1-2948530, Fax +351-1-2954461/2948530, Email ism@mail.fct.unl.pt

Russia

IPPAS, Collection of Microalgae of the Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya 35, Moscow 127276, Russia, Tel +7-095-4824491, Fax +7-095-4821685, Email vladimir@ad.plantphys.msk.ru

RACCC, Russian Animal Cell Culture Collection, Institute of Cytology, Russian Academy of Sciences, Department of Cell Culture, Tikhoretsky av. 4, 194064 St. Petersburg, Russia, Tel +7-812-2474296, Fax +7-812-2470341, Email root@cell.spb.su

SRC CCM, Collection of Cultures of Micro-organisms, VECTOR - State Research Center of Virology and Biotechnology, 633159 Koltsovo, Novosibirsk region, Russia, Tel +7-383-2-647098, Fax +7-383-2-328831, Email repin@vector.nsk.su

VKM, Russian Collection of Micro-organisms, Institute of Biochemistry and Physiology of Micro-organisms, Prospekt Naoki No 5, 142292 Pushchino (Moscow Region), Russia, Tel +7-095-9257448, Fax +7-095-9233602, Email vkm@stack. serpukhov.su

VKPM, Russian National Collection of Industrial Micro-organisms, VNII Genetika, I Dorozhny proezd 1, Moscow 113545, Russia, Tel +7-095-3151210, Fax +7-095-3150501, Email vkpm@vnigen.msk.su

Slovakia

CCY, Culture Collection of Yeasts, Slovak Academy of Sciences, Institute of Chemistry, Dúbravská cesta 9, 842 38 Bratislava, Slovakia, Tel +42-7-3782625, Fax +42-7-373811, Email chemvad@savba.sk

Slovenia

MZKI, Culture Collection of Fungi, National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia, Tel +386-61-1760333, Fax +386-61-1259244, Email nina.gunde.cimerman@ki.si

Spain

CECT, Coleccion Espanola de Cultivos Tipo, Universidad de Valencia, Edificio de Investigacion, Campus de Burjasot, E-46100 Burjasot (Valencia), Spain, Tel +34-6-3864612, Fax +34-6-3983187, Email cect@uv.es

Sweden

CCUG, Culture Collection University of Göteborg, Department of Clinical Bacteriology, Guldhedsg. 10, S-41346 Göteborg, Sweden, Tel +46-31-604625, Fax +46-31-825484, Email ccugef@ccug.gu.se

FCUG, Fungal Cultures University of Göteborg, Department of Systematic Botany, Carl Skottsbergs Gata 22, S-41319 Göteborg, Sweden, Tel +46-31-7732659, Fax +46-31-7732677, Email nils.hallenberg@systbot.gu.se

UPSC, Uppsala University Culture Collection of Fungi, Botanical Museum, Uppsala University, Villavägen 6, S-75236 Uppsala, Sweden, Tel +46-18-182794, Fax +46-18-508702, Email ovidiu.constantinescu@fyto.uu.se

Turkey

HÜKÜK, Culture Collection of Animal Cells, Foot and Mouth Disease Institute, Sap Enstitüsü, P.K. 714, TR-06044 Ankara, Turkey, Tel +90-312-2873600, Fax +90-312-2873606

KÜKENS, Centre for Research and Application of Culture Collections of Micro-organisms, Istanbul Faculty of Medicine, Department of Microbiology, Temel Bilimler Binasi, TR-34390 Capa-Istanbul, Turkey, Tel +90-212-5348640, Fax +90-212-5348640, Email gurle-ed@mam.net.tr

v. Some public service collections based in the rest of the world

ARS Culture Collection
Fermentation Laboratory, US Department of Agriculture, Peoria, Illinois, Washington DC 20250, USA.

ATCC (American Type Culture Collection)
10801 University Blvd., Manassas, VA 20110-2209, USA.
Tel: 800-638-6597; Fax: 703-365-2750
Email: sales@atcc.org

CSIRO (Council for Scientific & Industrial Research Organization)
Dehli Road, North Ryde, New South Wales, PO Box 52, NSW 2113, Australia.

DAOM (Centre for Land and Biological Resources Research (CLBRR) Canada)
Agriculture, Central Experimental Farm, Ottawa, Ontario, Canada K1A 0C6.

FAT (Faculty of Agriculture, University of Tokyo)
Faculty of Agriculture, University of Tokyo, Tokyo, Japan.

FGSC (Fungal Genetic Stock Center)
California State University, Humnboldt, Arcata, California 95521, USA.

FRR Division of Food Research, Food Research Laboratory, CSIRO, Dehli Road, North Ryde, New South Wales, PO Box 52, NSW, Australia.

HAC (formerly Hyogo University of Agriculture, Sasayama).
Faculty of Agriculture, Kobe University, Kobe, Japan.

HACC1 (Hindustani Antibiotics Culture collection India)
Hindustani Antibiotics Ltd, Pimpri, Poona, India.

HUT, Faculty of Engineering, Hiroshima University, Hiroshima 730, Japan.

IAM (Institute of Applied Microbiology)
University of Tokyo, Tokyo, Japan.

IARI (Indian Agricultural Research Institute)
Indian Agricultural Research Institute, New Delhi 11012, India.

IFO (Institute for Fermentation, Osaka)
Institute for Fermentation, Osaka 532, Japan. Tel: +81 3 5814 5811 Fax: +81 3 5814 5822

IJMARI (Indian Jute Mills Association Research Institute), Calcutta, India.

IMUR (Instituto de Micologia Universidade), Recife, Brazil.

ITCC (Indian Type Culture Collection)
IARI, New Delhi 11012, India.

JCM (Japan Collection of Micro-organisms)
Japan Collection of Micro-organisms, RIKEN, Wako-shi, Saitama 351-01 Japan.
Tel: 0484 62 1111, Fax: 0484 64 5651

MR (Mycological Herberium of Federation of Rhodesia & Nyasaland)
(now Mycological Herbarium of Federation of Zimbabwe and Malawi).

NHL (National Institute of Hygienic Sciences)
National Institute of Hygienic Sciences,Tokyo, Japan.

NRRL (formerly Northern Regional Research Laboratory)
Northern Utilization Research Branch USDA, Peoria, Illinois 61604, USA.

OAC Herbarium, Department of Botany and Genetics, University of Guelph, Guelph,
Ontario, Canada N1G 2W1.

PRL (Praire Regional Laboratory)
Praire Regional Laboratory, Saskatoon, Saskatchewan, Canada.

QM (US Army Natick Laboratories)
Natick, Massachusetts 01760, USA (formerly Quartermaster Research & Engineering
Center).

TRTC University of Toronto, Toronto, Ontario, Canada M5S 1AI.

UAMH (University of Alberta Mold Herbarium & Culture Collection)
Devonian Botanic Garden, Edmonton, Alberta, Canada T6G 3GI.

WB (Wisconsin Bacteriology)
University of Wisconsin College of Agriculture, Madison, Wisconsin, USA.

Appendix D UKNCC controls on the distribution of dangerous organisms

1. The UKNCC is committed to preventing dangerous or hazardous pathogens from falling into the hands of non-legitimate users. This document describes the policy of the collections on this matter. It is based on procedures currently used by the collections and the current legislation governing the distribution of organisms both inside and outside the 'Australia Group' of countries, and also the current MAFF regulations governing the distribution of animal and plant pathogens.

2. The organisms governed by this policy are placed in three categories and the UKNCC Collections refer to current lists before organisms are supplied. In addition collections staff assess the dangers presented by an organism and subsequently restrict distribution accordingly.
   Category 1. Hazardous organisms, including Advisory Committee on Dangerous Pathogens (ACDP) category 3 & 4 pathogens, included in the 'Australia Group' regulations.
   Category 2. Hazardous organisms including ACDP category 3 & 4 pathogens and others not included in the Australia Group regulations.
   Category 3. Animal and plant pathogens controlled by MAFF, and other, legislation.

3. The policy governing the distribution of organisms in each of these categories is outlined below.

Category 1: Hazardous organisms, including ACDP category 3 & 4 pathogens, included in the 'Australia Group'regulations.

4. The export of organisms specified by the 'Australia Group' of countries is governed by legislation. The Export of Goods (Control) Order 1994 specifies that a licence is required for all exports of these organisms. Exports of the listed organisms to countries outside the Australia Group require an Individual Export Licence (IEL). Only individuals who are registered with the DTI may submit an IEL application. Exports to countries within the 'Australia Group' require an Open General Export Licence (OGEL) which removes the need for an individual export licence provided there are no grounds for knowing or suspecting that goods are going to be used for biological weapons purposes. An OGEL is granted only to organizations registered with the DTI.
5. Failure to comply with these requirements is a criminal offence. Any enquiries should be directed to the DTI's Export Control Organization.
6. In addition to possessing the appropriate licence, collections supplying organisms in this category take all practicable steps to ensure that these organisms are going to legitimate users. Sales of listed organisms may continue to existing users however the request should be signed by the Head of Department/Division (or the safety officer or other person authorized by the Head of Department) and the registered user. The signatures are matched against those held on record in

the collection on the appropriate form. New users submitting requests for these organisms should register through the UKNCC registration procedure.

7. The Biological Weapons Act 1974 includes the clause that "No person shall develop, produce, stockpile, acquire or retain any biological agent or toxin of a type and in a quantity that has no justification for prophylactic, protective or other peaceful purposes". Contravention of this act is a criminal offence with a maximum penalty of life imprisonment.

Category 2: Hazardous organisms including ACDP category 3 & 4 pathogens and others not included in the Australia Group regulations.

8. All other ACDP group 3&4 organisms, and selected other organisms offering potential dangers to humans, animals or plants, are also included in this policy. Sales of listed hazardous organisms may continue to existing users but the request should be signed by the Head of Department/Division or the registered user. The signatures are matched against those held on record in the collection on the appropriate form. New users submitting requests for these organisms should register through the UKNCC registration procedure.

9. ACDP hazard group 4 viruses are only supplied to known established scientists with a track record of legitimate work with such organisms and who are known to have appropriate containment facilities.

Category 3: Animal and plant pathogens controlled by MAFF

10. The regulations governing the distribution of animal and plant pathogens are shown below. Some animal viral pathogens and some plant bacterial and fungal pathogens are also included in the 'Australia Group' of organisms. Distribution arrangements for these organisms conforms to both sets of regulations.

Animal Pathogens

11. The Specified Animal Pathogens Order 1998 makes it an offence to possess or spread a listed animal pathogen within Great Britain without a license. It is supplemented by the Importation of Animal Pathogens Order 1980 which makes it an offence to import any animal pathogen, or potential or actual carrier, of an animal pathogen from a non-EC country, except under license. Enquiries should be made to the Animal Health Division of MAFF. The culture collection and the customer must hold the appropriate licenses to hold these organisms. Orders will be refused where the customer is unable to produce a copy of the appropriate license.

Plant Pathogens

12. The Plant Health Order 1993 regulates the import, movement and keeping of plant pests including plant pathogens within Great Britain. The order notes that licenced pathogens may be provided to persons or organizations that hold a relevant current MAFF licence. Licenced pathogens may also

be sent to persons or organizations overseas that have authority from their national plant health service to receive such material. However material must not be released to other persons or organizations without the written agreement from Plant Health Division who would make arrangements for the issue of phytosanitary certificates or plant passports or for endorsement of letters of authority. Enquiries should be directed to the Plant Health Division of MAFF.

13. Collections include in this category any other pathogen that may not be indigenous to, and which may cause damage to plants within, Great Britain. If there is any uncertainty over the status of any organism the matter is referred to the Plant Health Division at MAFF.

Legislation in other countries

14. Other countries have different regulations and lists of restricted organisms, collections ensure, as far as is possible, that the export of organisms does not contravene local legislation.

Record keeping

15. Collections maintain records of all requests for all controlled organisms including those requests which are refused for any reason. Records are kept for a period of 25 years. The DTI requires registered organizations to maintain separate records of the supply of 'Australia Group' organisms whether under an IEL or an OGEL.

Conditions of supply

16. All controlled organisms are supplied on the basis that they are not passed on to third parties. Recipients of these cultures are cautioned that some third party transfers, such as the unlicenced export of 'Australia Group' organisms and the transfer of some MAFF controlled pathogens, may be a criminal offence. Users may also be liable under civil law for disease or damage following third party transfer. Collection users receiving requests for third party transfer of controlled organisms should refer such requests back to the collection.

17. Collection staff assess user registrations and reserve the right to refuse any order which does not show clear evidence that it is for the safe and legitimate use of an organism. A collection accepts no obligation to give reasons for this refusal.

Appendix E Forms

Form 1: UKNCC culture order form

NAME OF UKNCC COLLECTION (CABI (IMI), NCIMB, ECCAC etc):

Customer details

Name:
Company / organisation:
Address:
Country:
Post/zip code:
Contact number(s):
Fax:
E-mail:
Customer order/reference id:

Shipping address if different from above

Name:
Company/organisation:
Address:
Country:
Post/zip code:
Contact number(s):

Authorised signature:
Print name:
Position held:
Date: / /

Continued overleaf……

	CULTURE NAME	**Collection number (i.e. IMI, NCIMB)**	**UKNCC number (not necessary)**	**Quantity**
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				

NOTES
CABI Bioscience was formerly known as the International Mycological Institute (IMI). Cultures obtained from CABI retain their IMI prefix.
NCWRF cultures are obtained from CABI; NCFB cultures are obtained from NCIMB.

Form 2: Compliance with Convention on Biological Diversity (CBD)

CABI Bioscience, in the spirit of the terms of the Convention on Biological Diversity (CBD) and along with other major international culture collections, require that customers sign the declaration below.

Declaration under the terms of the Convention on Biological Diversity

*I/we agree not to claim ownership over the micro-organisms or cell lines received from *……*, nor to seek intellectual property rights over them or related information. If we wish to utilise or exploit such organisms commercially, suitable and adequate sharing of benefits in the spirit of the Convention on Biological Diversity will first be discussed with the relevant UKNCC collection.*

I/we also agree to ensure that any subsequent person or institution to whom I/we make samples of the micro-organism available, is bound by the same provision.

* Enter name of culture collection*

Authorised signature:	**Date:** / /
Print name:	
Position held:	
Address:	

At the time of writing only CABI require the completion of this form. Once completed and supplied to CABI it covers all subsequent requests unless otherwise instructed. For mechanisms of compliance with the CBD please contact the appropriate collection direct.

Form 3: Accessions form to accompany deposits

	Name & authority: Principal Synonyms/Name Change: Identified by:	UKNCC collection accession Nº: UKNCC collection accession date: Type of deposit:

Depositor: please complete as much as possible:

Name of isolate: Date sent to centre: Name & address of depositor: Designation: Previous history: (other collection/owners/isolate designation): Other collections where held: (give collection number and acronym):
Isolated from (substratum/genus & species of organism): Anatomical part / substratum part: Geographical location: Isolated by: Date of isolation: Isolation method (soil plate, damp chamber, surface sterilisation, micromanipulation etc.): ***Continued over leaf***…….

Special features & usage (metabolic products, culture derived from type, etc.):

References (journal, volume, page, year) please attach copies/reprints if available:

Recommendations For Maintenance & Preservation:

i) Growth medium:
ii) Incubation temperature:
iii) Incubation time:
iv) Light requirements/pH etc.:
v) Period between transfer:

Please tick appropriate method(s)

i) Freeze-drying ○
ii) Liquid nitrogen storage ○
iii) Water storage ○
iv) Soil storage ○
v) Oil storage ○
vi) Silica gel storage ○
vii) Other - please specify ○

For UKNCC collection use only:

Permission to deposit in ukncc member collections and distribute to third parties under the Convention on Biological Diversity (CBD).

Was the organism collected after December 1993?…….Yes or No

If the answer to the above question is YES please answer the following questions and provide the requested information.

From whom did you receive prior informed consent to collect the material?

Land owner:

National Authority:

To the best of your knowledge do the above have the authority to grant this permission?…… .Yes or No

Do you have the authority to deposit them in a UKNCC living and dead dried collections for further use and distribution?……Yes or No

If so under what terms:

Appendix F Cryopreservation protocols

Cryopreservation protocols for microalgae and cyanobacteria

Species	Growth conditions or pretreatment before freezing	Cryoprotective Additives	Cooling protocol/ rate	Thaw (°C min^{-1})	Recovery	Storage temp. (°C)	Reference
Actinocyclus subtilis	-	Methanol (5% v/v)	Two step 15 (-40)	Rapid warming	2%	-196	McLellan, 1989
Anabaena cylindrica	-	Me_2SO (5% w/v)	Two step 20min (-30)	Rapid warming	100%	-196	Day, 1998
Ankistrodesmus angustus	-	Me_2SO (5% w/v)	Two step 15min (-30)	Rapid warming	93%	-196	Day, 1998
Attheya decora	-	Me_2SO (10% v/v)	Two step 15 (-40)	Rapid warming	54%	-196	McLellan, 1989
Brachiomonas submarina	Encapsulation in calcium alginate with 0.5M sucrose, dessicated at 30°C	-	Rapid cooling	Rapid warming	33%	-196	Hirata *et al.*, 1996
Calothrix brevissima	Encapsulation in calcium alginate with 0.5M sucrose, dessicated at 30°C	-	Rapid cooling	Rapid warming	12%	-196	Hirata *et al.*, 1996
Chaetoceros gracilis	-	Me_2SO (15% v/v)	Two step 0.5-4 (-35)	Rapid warming	9-29%	-196	Canavate & Lubian, 1995 a, b
Chaetoceros neogracile	-	Me_2SO (10% v/v)	Two step 15 (-40)	Rapid warming	56%	-196	McLellan, 1989
Chlamydomonas	Encapsulation in calcium alginate with 0.5M sucrose, dessicated at 30°C	-	Rapid cooling	Rapid warming	34%	-196	Hirata *et al.*, 1996
C. reinhardtii	18h in Me_2SO (1% v/v)	Me_2SO (5% v/v)	Two step 1 (-20)	Rapid warming	0.1-10%	-196	Gresshoff, 1977
C. reinhardtii	-	Me_2SO (10% v/v)	Two step 1 (-30)	Rapid warming	+	<-180	Hwang & Hudock, 1971
C. reinhardtii	-	Me_2SO (5% v/v)	Two step 1 (-55)	Rapid warming	<10%	-150	McGarth & Daggett, 1977

Chlorella	-	Me_2SO (5% v/v)	200 (-196)	Rapid warming	<30%	-196	Morris, 1976a
C. emersonii	-	Me_2SO (5% v/v)	Plunge into liquid nitrogen	Rapid warming	70%	-196	Day *et al.*, 1997
C. emersonii	-	Methanol (1.5M)	One step 10 (-196)	Rapid warming	87%	-196	Morris *et al.*, 1980
C. protothecoides	-	Me_2SO (5% v/v)	Plunge into liquid nitrogen	Rapid warming	85%	-196	Day *et al.*, 1997
C. pyrenoidosa	Encapsulation in calcium alginate with 0.5M sucrose, dessicated at 30°C	-	Rapid cooling	Rapid warming	30%	-196	Hirata *et al.*, 1996
Chlorocloster engadinensis	-	Me_2SO (5% w/v)	Two step 20min (–30)	Rapid warming	70%	-196	Day, 1998
Chlorococcales (250 strains)	-	Glycerol (10% v/v) Me_2SO (5% v/v)	Two step 15min (–30)	Rapid warming	>50%	-196	Morris, 1977, 1978
Chroomonas placoidea	-	Me_2SO (5% w/v)	Two step 15min (–30)	Rapid warming	<10%	-196	Day, 1998
Cricosphaera roscoffensis	Encapsulation in calcium alginate with 0.5M sucrose, dessicated at 30°C	-	Rapid cooling	Rapid warming	8	-196	Hirata *et al.*, 1996
Cyanobacteria 150 strains	Cultured in cryotube on agar	Me_2SO (8% w/v)	Two step ~1 (-70)	Rapid warming	+	-196	Bodas *et al.*, 1995
Cyclotella cryptica	-	Me_2SO (10% v/v)	Two step 15 (-40)	Rapid warming	52%	-196	McLellan, 1989
C. cryptica	-	Me_2SO (5% w/v)	Two step 15 (-40)	Rapid warming	57%	-196	Day, 1998
Cylindrocystis brebissonii	-	Me_2SO (5% v/v)	Two step 1 (-60)	Rapid warming	55%	-196	Morris *et al.*, 1986
Dunaliella salina	3 days at 4°C in dark	Glycerol added at 20μL min^{-1} until concentration of 3.5 M. Then 1 hour at 4° C	Two step 1 (-40)	Rapid warming	78%	-196	Mortain-Bertrand *et al.*, 1996

D. tertiolecta	Encapsulation in calcium alginate with 0.5M sucrose, dessicated at 30°C	-	Rapid cooling	Rapid warming	30	-196	Hirata *et al.*, 1996
Eisenia bicyclis		Proline (10% w/v) + Ethyleneglycol (10% v/v)	Two step 1 (-40)	Rapid warming	52%	-196	Kono, *et al.*, 1998
Enteromorpha intestinalis	-	Me_2SO (15% v/v) + 5% proline	Two step 1 (-40)	Rapid warming	71%	-196	Kono *et al.*, 1997
Enteromorpha intestinalis	-	Me_2SO (5% v/v)	Two step 1 (-35)	Rapid warming	100%	-196	Day *et al.*, 1998
Enteromorpha intestinalis	-	PVS 2 Vitrification solution	plunge	Rapid warming	100%	-196	Day *et al.*, 1998
Euglena gracilis	-	Methanol 10% (v/v)	Two step 0.3 (-60)	Rapid warming	34%	-196	Day, 1998
Euglena gracilis	Encapsulation in calcium alginate 3h air drying	Methanol 10% (v/v)	1 step plunge	1min room temperature then rapid warming	37%	-196	Day *et al.*, 2000
Euglena gracilis	Pre culture 48h in 0.75 M sucrose Encapsulation in calcium alginate 2h air drying	Methanol 10% (v/v)	Two step 0.5 (-60)	1min room temperature then rapid warming	24%	-196	Day *et al.*, 2000
Euglena gracilis	Pre culture 24h in 0.5 M sucrose and then Pre culture 48h in 0.75 M sucrose Pre culture 24h in 0.75 M sucrose Encapsulation in calcium alginate	Methanol 10% (v/v)	Two step 0.5 (-60)	1min room temperature then rapid warming	48%	-196	Day *et al.*, 2000
Euglena gracilis (26 strains)	-	Methanol (10% v/v)	Two step 1 (-30)	Rapid warming	~30%	-196	Morris & Canning, 1978

Eukaryotic algae (16 strains)	Cultured in cryotube on agar	Polyethylene glycol 10% (w/v) in agar Me_2SO (5% w/v) + methanol (5% v/v)	Two step ~1 (-70)	Rapid warming	+	-196	Bodas *et al.,* 1995
Eukaryotic Algae (365 strains)	Low light, 1% agar media, 20 ^oC	Me_2SO (5% v/v)	Two step 1 (-40), then -40 for 15 min	Rapid warming	+	-196	Beaty & Parker, 1992
Fragilaria pinnata	-	Me_2SO (10% v/v)	Two step 15 (-40)	Rapid warming	57%	-196	McLellan, 1989
Geitlerinema amphibium	Stationary phase culture	Me_2SO (10% v/v)	Rapid cooling	Rapid warming	+	<80	Romo & Bécares, 1992
Heterococcus caespitosus	-	Me_2SO (5% w/v)	Two step 20min at –30	Rapid warming	65%	-196	Day, 1998
H. protonematoides	-	Me_2SO (5% w/v)	Two step 30min at –30	Rapid warming	34%	-196	Day *et al.*, 1997
Isochrysis sp.	-	Me_2SO (5% w/v)	Two step 15min (-30)	Rapid warming	<10%	-196	Day, 1998
Marine microalgae (12 strains)	-	Me_2SO (5% v/v)	Two step 15min (-30)	Rapid warming	20-87%	-196	Ben Amotz & Gilboa, 1980
Microcystis aeruginosa	-	Me_2SO (5% v/v)	DI	Rapid warming	60%	-196	Box, 1988
Microcystis aeruginosa	-	Me_2SO (5% v/v)	DI	Rapid warming	+	-196	Box, 1988
Microcystis aeruginosa f. aeruginosa	Stationary phase culture	Me_2SO (3% v/v), 15min at room temp.	Two step 1 (-30) then –30 for 15 min	Rapid warming	>50%	-196	Wantanabe & Sawaguchi, 1995
Microcystis auruginosa	-	Me_2SO (5% w/v)	Two step 20 min (-30)	Rapid warming	100%	-196	Day, 1998
Monodopsis subtteranea	-	Me_2SO (5% w/v)	Two step 15min (-30)	Rapid warming	100%	-196	Day, 1998

Nannochloropsis	Encapsulation in calcium alginate with 0.5M sucrose, dessicated at 30°C	-	Rapid cooling	Rapid warming	42%	-196	Hirata *et al.*, 1996
Nitzschia ovalis (axenic)	-	Me_2SO (10% v/v)	Two step 15 (-40)	Rapid warming	82%	-196	McLellan, 1989
Nitzschia ovalis (non-axenic)	-	Me_2SO (10% v/v)	Two step 15 (-40)	Rapid warming	59%	-196	McLellan, 1989
Nostoc commune	Encapsulation in calcium alginate with 0.5M sucrose, dessicated at 30°C	-	Rapid cooling	Rapid warming	76%	-196	Hirata *et al.*, 1996
N. muscorum	-	None	Rapid cooling	Rapid warming	Good	-196	Holm-Hansen, 1963
N. muscorum	-	Glycerol (10% v/v) Me_2SO (5% v/v)	Rapid cooling	Rapid warming	60-70%	-196	Tsuru, 1973
N. spongiaeforme	Encapsulation in calcium alginate with 0.5M sucrose, dessicated at 30°C	-	Rapid cooling	Rapid warming	21%	-196	Hirata *et al.*, 1996
Pediastrum duplex	-	Me_2SO (10% v/v)	Two step 1 (-30)	Rapid warming	96%		Watanabe *et al.*, 1992 Day *et al.*, 1997
Phaeodactylum tricornutum	-	Me_2SO (10% v/v)	Two step 15 (-40)	Rapid warming	68%	-196	McLellan, 1989
Phaeodactylum tricornutum	-	Me_2SO (5% w/v)	Two step 15 (-40)	Rapid warming	65%	-196	Day, 1998
Phormidium foveolarum	Encapsulation in calcium alginate with 0.5M sucrose, dessicated at 30°C	-	Rapid cooling	Rapid warming	44%	-196	Hirata *et al.*, 1996
Porphyra minata	-	Me_2SO (5% w/v)	Two step 15min at –30	Rapid warming	+	-196	Day, 1998
Prorocentrum micans	-	Me_2SO (5% w/v)	Two step 15min at –30	Rapid warming	<10%	-196	Day, 1998
Prototheca (5 species)	Precultured for 14 days at 4°C	-	Two step 0.3 (-60)	Rapid warming	34-89%	-196	Morris, 1976b

Pseudanabaena galeata Böcher	Stationary phase culture	Me_2SO (10% v/v)	Rapid cooling	Rapid warming	+	<80	Romo & Bécares, 1992
Scenedesmus acutus	-	Me_2SO (10% v/v)	Two step 1 (-30)	Rapid warming	75%	-196	Watanabe *et al.*, 1992 Day *et al.*, 1997
S. basiliensis	-	Me_2SO (5% v/v)	Two step 15min at -30	Rapid warming	97%	-196	Morris, 1978 Day *et al.*, 1997
Selenastrum capricornutum	-	Me_2SO (5% w/v)	Two step 15min at –30	Rapid warming	90%	-196	Day, 1998
Tetraselmis (4 strains)	Grown at room temp.	Glycerol (10% v/v)	Two step 1 (-30)	Rapid warming	>50%	-196	Day & Fenwick, 1993
T. suecica	-	Glycerol 10% (v/v)	Two step 1 (-30)	Rapid warming	70%	-196	Day, 1998
T. suecica	Room temp.	Glycerol (5% v/v)	Two step 5 (-30)	Rapid warming	>65%	-196	Fenwick & Day, 1992

DI, direct immersion in liquid coolants; Two/ multi step cooling Protocols, intermediate temperatures given in parenthesis. Unless otherwise stated final step is a plunge into liquid nitrogen.

See also; Day & Deville (1995) and Taylor & Fletcher (1999).

Cryopreservation protocols for bacteria

Species	Growth conditions or pretreatment before freezing	Cryoprotective Additives	Cooling (°C min^{-1})	Thaw (°C min^{-1})	Recovery	Storage period (years) & temp. (°C)	Reference
Actinomyces	-	Blood serum	Rapid	Not specified	100%	Immediate thaw	Bradshaw *et al*., 1989
Aegyptianella pullmorum	Blood serum	Glycerol (10% v/v)	30min (4°C) -0.4-5°Cmin^{-1}	Rapid	Infectious for vector	At least 7 years	Gothe & Hartman, 1979
Alcalgenes eutrophus	Not specified	Glycerol (10%v/v) Polyvinylethanol (10%v/v)	Rapid	Rapid warming	Good viability; plasmids retained	Immediate thaw	Beyersdorf-Radeck *et al*., 1993
Azotobacter vinelandii	-	Glycerol (10% v/v)	Rapid	Rapid	60-99%	Immediate thaw	Thompson, 1987
Bacteria not amenable to freeze-drying	Mid to late log phase to a concntration of (2-6) x10^6 cells/ml	Glycerol (5% v/v) Me_2SO (5% w/v)	-1 to -3°C min^{-1} to -30°C, -15 to –30°C min^{-1} to -100°C or lower, store at -196°C	Water bath 30-37°C	95%	10 years	Nakamura, 1996
Bacterioides intermedius	Not specified	Blood serum	Rapid	Rapid warming	100%	Immediate thaw	Bradshaw *et al*., 1989
Borrelia burgdorferi	Infected dog, bull, ram semen, citrate – saccharose – egg yolk in BSK medium	Growth medium	Rapid	Rapid warming	79-91%	12 weeks	Kumi-Diaka & Harris, 1995
Clostridium chauvoei	Beef liver medium	Beef liver medium + glucose (1%w/v) + peptone (1%w/v)	Rapid	Rapid warming	No loss in infectivity	Immediate thaw	McCrindle, 1979
Cytophaga	-	No additive	Rapid	Not specified	Not provided		Sanfilippo & Lewin, 1970
Flexibacter	-	No additive	Rapid	Not specified	Not provided		Sanfilippo & Lewin, 1970
Lactobacillus casei	Collected on Millipore membrane	No additive	Ultrarapid on membrane	Rapid warming	100%	Immediate thaw	Albrecht *et al*., 1973

Leptospira	Grown and preserved in Ellinghausen & McCulloughs medium	Ellinghausen & McCulloughs medium	Placed in the vapour phase of nitrogen tank	Rapid warming	10-20%	2 years	Waitkins, 1991
Medical bacteria	Growth on non-selective solid media e.g., nutrient or blood agar; $10^8 – 10^{10}$ cells/mL in glass beads (Microbank Pro-Lab Diagnostics)	2.5g nutrient broth pwder N°2, 15mL glycerol, 85mL distilled water	Placed in a –70°C freezer or direct into liquid nitrogen vapour	Streak a bead directly onto solid medium	Not indicated	Not provided	Perry, 1995
Methanococcoides, Methanosarcina, Methanophilus, Methanobolus	Growth in a $H_2 + CO_2$ gas mixture	Glycerol (10% v/v) Me_2SO (5% w/v)	-50°C min^{-1} gas phase of liquid nitrogen tank	Water bath 30-37°C	Not indicated	Not provided	Hippe, 1984, 1991
Methanotrophic bacteria	Growth in a $H_2 + CO_2$ gas mixture; in glass capillary tubes	Glycerol (10% v/v) Me_2SO (5% w/v)	Ice bath and directly into storage	Warm water: +1000°C min^{-1}	Not indicated	15 years	Hippe, 1991
Microsilla	-	No additive	Rapid	Not specified	Not provided	Immediate thaw	Sanfilippo & Lewin, 1970
Mycoplasma	Hayflick medium + horse serum and yeast extract	Growth medium	Rapid	Rapid warming	Decreased by 1 log value	Not provided	Ritter & Ulrich, 1986
Obligate methanotrophic bacteria	Normal grow conditions for these bacteria	PVP dialysed mol.wt. 40000	plunge	Rapid warming	Not quantified	Not provided	Green & Woodford, 1992
Obligate methanotrophic bacteria	Normal grow conditions for these bacteria	methanol	plunge	Rapid warming	Not quantified	Not provided	Green & Woodford, 1992
Phototrophic bacteria	anaerobic conditions, under a nitrogen blanket	Me_2SO (5% w/v)	plunge	Rapid warming	Not quantified	Not provided	Malik, 1984
Streptococcus	Blood serum	Blood serum	Rapid	Rapid warming	100%	Immediate thaw	Bradshaw *et al.*, 1989
Streptomyces griseus	Nutrient broth	Growth medium	Rapid	Rapid warming	100%	5 years	Fortney & Thoma, 1977

Cryopreservation protocols for fungi

Species	Growth conditions before freezing	Cryoprotective Additives	Cooling (°C min^{-1})	Thaw (°C min^{-1})	Recovery	Storage period (years) & temp. (°C)	Reference
Achyla spp. (9 strains)	Mature cultures washed from agar slopes or mature liquid cultures	Glycerol (10% v/v)	1	c. 400	+	1-3 at -196	Hwang , 1966
Achyla spp. (7 strains)	14d culture mycelium washed from agar surface	Glycerol (10% v/v)	1	Rapid warming	+	1-8 at -196	Smith, 1982
Agaricus spp	Agar blocks cut from growing colony	Glycerol (10% v/v) Me_2SO (5% v/v)	Slow cooling	Rapid warming	+	9 at -196	Elliot & Challen, 1981 Challen & Elliot, 1986
Allomyces spp. (5 strains)	14d culture mycelium washed from agar surface	Glycerol (10% v/v)	1	Rapid warming	+	3-8 at -196	Smith, 1982
Albugo occidentalis	On infected spinach leaves	None	Slow cooling	Rapid warming	+	0.5 at -23	O'Brien & Webb, 1958
Aphanomyces spp., 6 strains)	14d culture mycelium washed from agar surface	Glycerol (10% v/v)	1	Rapid warming	+	4-7 at -196	Smith, 1982
Aphanomyces spp. (2 strains)	Mycelium washed from agar or liquid culture	Glycerol (10% v/v)	1	c. 400	+	3 at -196	Hwang, 1966
Ascomycota (62 strains)	Spores or mycelium washed from agar surface	Glycerol (10% v/v) Me_2SO (5% v/v)	1	Rapid warming	+	2-8 at -196	Butterfield *et al.*, 1974
Ascomycota (44 strains)	Spores or mycelium washed from agar surface	Glycerol (10% v/v) Me_2SO (5% v/v)	1	Rapid warming	+	0.5-3 at -196	Hwang, 1966
Basidiobolus (239 strains)	Spores or mycelium washed from agar surface	Glycerol (10% v/v) Me_2SO (5% v/v)	1	Rapid warming	+	1-8 at -196	Butterfield *et al.*, 1974
Basidomycota (5 strains)	Spores or mycelium washed from agar surface	Glycerol (10% v/v) Me_2SO (5% v/v)	1	Rapid warming	+	1-3 at -196	Hwang, 1966
Bremia lactucae	Spores washed from infected plant tissue	Skimmed milk (8.5% w/v) + Glycerol (10% v/v)	1	Rapid warming	80%*	5-9 at -196	Dahmen *et al.*, 1983
Bremia lactucae	Spores washed from infected plant tissue	Me_2SO (15% v/v)	1	Rapid warming	80%*	5-9 at -196	Dahmen *et al.*, 1983
Brettanomyces	-	Me_2SO (10% v/v) + 6%	1	Rapid	79%	-196	Smentek & Windisch,

anomalus		Ficoll400 + 0.24% yeast extract + 0.5% glucose		warming			1982
Bullera alba	-	Me_2SO (10% v/v) + 6% Ficoll400 + 0.24% yeast extract + 0.5% glucose	1	Rapid warming	24%	-196	Smentek & Windisch, 1982
Candida albicans	-	Me_2SO (10% v/v) + 6% Ficoll400 + 0.24% yeast extract + 0.5% glucose	1	Rapid warming	97%	-196	Smentek & Windisch, 1982
Coprinus cinereus	Spores or mycelium washed from agar surface	Glycerol (10% v/v) Me_2SO (5% v/v)	1	Rapid warming	+	2 at -196	Butterfield *et al.,* 1974
Entomophthorales (19 strains)	Spore or mycelium washed from agar surface	Glycerol (10% v/v) Me_2SO (5% v/v)	1	Rapid warming	+	1-5 at -196	Hwang, 1968
Entophlyctis cinfervaeglomerata	14d culture mycelium washed from agar surface	Glycerol (10% v/v)	1	Rapid warming	+	4-8 at –196	Smith, 1982
Ganoderma oregonense	Mycelium	Glycerol, 10% v/v)	1	Rapid warming	+	-196	Butterfield *et al.,* 1974
Ganoderma spp (36 strains)	Cultures grown on millet grains	Glycerol (5-10% v/v)	Slow cooling	Rapid warming	+	1 in nitrogen vapour	Wang *et al.,* 1990
Helminthosporium (12 strains)	Spores or mycelium washed from agar surface	None	DI	Rapid warming	+	2 at 189	Joshi *et al.,* 1974
Hyphochytrium catenoides	14d culture mycelium washed from agar surface	Glycerol (10% v/v) Me_2SO (5% v/v)	1	Rapid warming	+	4-10 at -196	Smith, 1982
Mitotic fungi** (33 strains)	Spores or mycelium washed from agar surface	Glycerol (10% v/v) Me_2SO (5% v/v)	1	c. 400	+	1-3 at -196	Hwang, 1966
Mitotic fungi** (239 strains)	Spores or mycelium washed from agar surface	Glycerol (10% v/v) Me_2SO (5% v/v)	1	Rapid warming	+	2-8 at –196	Butterfield *et al.,* 1974
Neocallimastix patricarum	10^5/ml zoospores	Me_2SO (0.68M)	Placed at -80	Rapid warming	40%	1 at -80	Yarlett *et al.,* 1986
Peronospora effusa	On infected spinach leaves	None	Slow cooling	Rapid warming	+	0.5 at -23	O'Brien & Webb, 1958
Peronospora tabacina	Harvested & filter concentrated conidia	Me_2SO (15% v/v)	Two step	Rapid warming	61%	1 at -196	Bromfield & Schmitt, 1967
Phlyctochytrium	14d culture mycelium	Glycerol (10% v/v)	1	Rapid	+	4-10 at -196	Smith, 1982

acuminatum	washed from agar surface			warming			
Phytophthora boehmeriae	Agar discs	Glycerol (10% v/v) Me_2SO (5% v/v)	Two step 24min –20	Rapid warming	75-100%	-	Tooley, 1988
Phytophthora palmivora	7-14d culture on agar plugs	Glycerol (10% w/v)	1	Rapid warming	100%	0.5 at -196	Smith, 1982
Phytophthora phaseoli	Infected lima bean epicoyl	None	DI	Air	+	1 at -196	Antonio & Blount, 1973
Phytophthora spp. (3 strains)	14-21d spores or mycelium washed from agar surface	Skimmed milk (8.5% w/v) & glycerol (10% v/v)	1	Rapid warming	100%*	5.9 at -196	Dahmen *et al.*, 1983
Phytophthora spp. (104 strains)	14d culture mycelium washed from agar surface	Glycerol (10% v/v)	1	Rapid warming	+	2-11 at -196	Smith, 1982
Plasmopara viticola	Spores harvested from infected leaves	Agar powder	Two step	Rapid warming	c. 20%	1 at -196	Tetsuka & Katsuya, 1983
Plasmopara viticola	Spores harvested from infected leaves	Polyvinyl alcohol	Two step	Rapid warming	c. 20%	1 at -196	Tetsuka & Katsuya, 1983
Plasmopara viticola	Spores harvested from infected plant tissue	Skimmed milk (8.5% w/v) & glycerol (10% v/v)	1	Rapid warming	51%	5-9 at -196	Dahmen *et al.*, 1983
Plasmopara viticola	Spores harvested from infected plant tissue	Me_2SO (15% v/v)	1	Rapid warming	50% & 96% infection	5-9 at -196	Dahmen *et al.*, 1983
Pseudoperonospora cubensis	Spores washed from infected plant tissue	Skimmed milk (8.5% w/v) & glycerol (10% v/v)	1	Rapid warming	50% & 100% infection	5-9 at -196	Dahmen *et al.*, 1983
Pseudoperonospora humuli	Spores harvested from infected leaves	Agar powder	Two step	Rapid warming	c. 22% (control 37.4%)	1 at -196	Tetsuka & Katsuya, 1983
Pseudoperonospora humuli	Spores harvested from infected leaves	Polyvinyl alcohol	Two step	Rapid warming	c. 29% (control 37.4%)	1 at -196	Tetsuka & Katsuya, 1983
Puccinia graminis	Uredospores	None	Rapid warming	Rapid warming	62-74%	Immediate	Kilpatrick *et al.*, 1971
Puccinia (12 strains)	Uredospores from infected plants	None	DI	Rapid warming	75-90%	2 at -189	Joshi *et al.*, 1974
Pythium sylvaticum (2 strains)	7-14d culture on agar plugs	Glucose (8% w/v) & Me_2SO (10% v/v)	1	Rapid warming	100%	0.5 at -196	Smith, 1983b

P. ultimum	14-21d spores or mycelium washed from agar surface	Skimmed milk (8.5% w/v) + glycerol (10% v/v)	1	Rapid warming	100%**	5-9 at -196	Dahmen *et al.*, 1983
Pythium spp. (10 strains)	Culture washed from agar or mature liquid culture	Glycerol (10% v/v)	1	c. 400	+	3 at -196	Hwang, 1966
Pythium spp. (28 strains)	14d culture; mycelium washed from agar surface	Glycerol (10% v/v)	1	Rapid warming	+	2-9 at -196	Smith, 1982
Rhizophydium bisporum	14d culture; mycelium washed from agar surface	Glycerol (10% v/v)	1	Rapid warming	+	4 at -196	Smith, 1982
Saprolengnia spp. (4 strains)	14d culture; mycelium washed from agar surface	Glycerol (10% v/v)	1	Rapid warming	+	1-6 at -196	Smith, 1982
Sclerospora philippinensis	Conidia harvested from infected host	Me_2SO (10% v/v)	1	Rapid warming	76%	2 at -196	Long *et al.*, 1978
S. sacchari	Conidia harvested from infected host	Me_2SO (10% v/v)	1	Rapid warming	10%	2 at -196	Long *et al.*, 1978
S. sorghi	Conidia harvested from infected host	Me_2SO (10% v/v)	1	Rapid warming	14-20%	2 at -196	Long *et al.*, 1978
S. sorghi	Conidia washed from leaves of *Sorghum bicolor*	Me_2SO (15% v/v)	1	Rapid warming	100%	7d at -196	Gale *et al.*, 1975
S. sorghi (9 strains)	Infected tissue taken from host	Glycerol (10% v/v)	1	Rapid warming	+	1 at -196	Smith, 1982
S. sorghi (9 strains)	Infected tissue taken from host	None	1	Rapid warming	+	1 at -196	Smith, 1982
Sterile mycelia (20 strains)	Spores or mycelium washed from agar surface	Glycerol (10% v/v) Me_2SO (5% v/v)	1	Rapid warming	+	0.5-4 at -196	Hwang, 1966
Thraustotheca clavata	14d culture; mycelium washed from agar surface	Glycerol (10% v/v)	1	Rapid warming	+	1 at -196	Smith, 1982
Ustilago nuda tritici	Chlamydospores from wheat	None	DI	Rapid warming	+	2 at -189	Joshi *et al.*, 1974

*, Germination and infection; *, fungi not linked to a perfect state; DI, direct immersion in liquid coolants; Two step, cooling at -22°C for 2 h and stored at -196°C.

Cryopreservation protocols for nematodes

Species	Growth conditions before freezing	Cryoprotective Additives	Cooling (°C min^{-1})	Thaw (°C min^{-1})	Recovery	Storage period (years) & temp. (°C)	Reference
Dictyocaulus viviparus	Exsheathment of third stage lavae with sodium hypochlorite	None	10-70	6800	30%	Immediate thaw	James & Peacock, 1986
Dictyocaulus viviparus	Exsheathment of third stage lavae with sodium hypochlorite	None	1°C min^{-1} to -10°C plunged into liquid nitrogen	6800	50-75%	Immediate thaw	James & Peacock, 1986
Heterorhabditis bacteriophora	Preincubation in 15% glycerol	10min in cold 70% methanol	Rapid	Rapid	+	24h	Popiel & Vasquez, 1991
Heterorhabditis megidis	Preincubation for 6-8 days in Me_2SO	10min in cold 70% methanol	Plunge into liquid nitrogen	Rapid	81%	24h	Nugent *et al.*, 1994
Heterorhabditis megidis	Preincubation for 4 days in 11% glycerol	10min in cold 70% methanol on filter paper strips	Plunge into liquid nitrogen	Rapid	75%	24h	Nugent *et al.*, 1994
Heterorhabditis zealandica	Preincubation for 4 days in 15% glycerol	10min in cold 70% methanol on filter paper strips	Plunge into liquid nitrogen	Rapid	28%	24h	Nugent *et al.*, 1994
Heterorhabditis spp	Preincubation for 6 days in 15% glycerol	10min in cold 70% methanol on filter paper strips	Plunge into liquid nitrogen	Rapid	88%	24h	Nugent *et al.*, 1994
Heterorhabditis spp	72h at 23°C in 17% glycerol	Rinse with ice cold 70% methanol, incubate for 10 min, absorb onto filter paper strips	Plunge into liquid nitrogen	Rapid	+	6 years	CABI Bioscience Collaborating Research Group Method
Steinernema carpocapsae	Preincubation in Me_2SO	10min in 0.5% methanol	Rapid	Rapid	+	24h	Popiel & Vasquez, 1991
Steinernema feltiae	No pretreatment	60% methanol for 45 sec at	5100	10000	12.3%	Immediate	Smith *et al.*, 1990

		0°C			motility	thaw	
Steinernema feltiae	Surface sterilized in 0.5% hyamine for 30min, desiccated to 97%RH at 25°C over 24h	70% methanol for 10min at 0°C	330-4800	600	84% motility infectivity not quantified	Immediate thaw	James, 1990
Steinernema spp	72h at 23°C in 22% glycerol	Rinse with ice cold 70% methanol, incubate for 10 min, absorb onto filter paper strips	Plunge into liquid nitrogen	Rapid	+	6 years	CABI Bioscience Collaborating Research Group Method

DI, direct immersion in liquid coolants; Two step, cooling at -22°C for 2 h and stored at -196°C (unless cited otherwise).

Cryopreservation protocols for protozoa

Species	Growth conditions or pretreatment before freezing	Cryoprotective Additives	Cooling (°C min^{-1})	Thaw (°C min^{-1})	Recovery	Storage temp. (°C)	Reference
Free living pathogenic and nonpathogenic amoeba	-	Me_2SO (10% v/v)	Three step -20 (60 min), -70 (60 min)	Hold at room temp for 3 min, then rapid warming	8 - 49%	-196	Kilvington, 1995
Pathogenic protozoa	Chill harvested cultures on ice, centrifuge 3min resuspend pellet in fresh medium, harvest resuspend pellet in cryoprotectant equilibriate at RT 15 min	Basal broth 6ml; Inactivated adult bovine serum 2ml; glucose (2.5M) 0.8ml; DMSO 1ml; cystein/ascorbic acid sol. (Cystein monohydrochloride 1g; ascorbic acid 0.1g dist H_2O 9.3ml; 10N NaOH 0.7ml) 0.2ml	Four step 8-10 (~-8); 1.5 (-10); 1–2 (-40); plunge	Rapid warming	+	-160/ -196	Diamond, 1995
Aegypian pullorum	30min 4^0C	Glycerol (10% w/v)	Plunge into LN_2	Rapid warming	+	-196	Raether & Seidenath, 1977
Anaplasma marginale	-	Me_2SO (2M)	Plunge into LN_2	Rapid warming	+	-196	Love, 1972
Babsia bigemina	30min 0^0C	Me_2SO (14% v/v)	Two step 82 (-60)	Rapid warming	+	-196	Dalgliesh & Mellors, 1974
B. bovis	-	PVP(15% v/v)	Two step 20 (–70), slow (-196)	Rapid warming	+	-196	Vega *et al.*, 1985
B. rodhaini	0^0C	Me_2SO (10% v/v)	Two step 100 (–100), fast (-196)	Rapid warming	+	-196	Dalgliesh *et al.*, 1980

Cryptosporidium	-	Me_2SO (5% v/v)	Two step 0.3 (–80), fast (-196)	Rapid warming	+	-196	Rossi *et al.*, 1990
Cryptothecodinium cohnii (16 strains)	-	Glycerol (7.5%)	Two step 1 (–40), fast (-196)	Rapid warming	20-76	-150	Simione & Daggett, 1977
Cytilophosis mucicola	-	Me_2SO (5% v/v)	2-3	Rapid warming	+	-196	Simon & Schneller, 1973
Dientamoeba fragilis	-	Me_2SO (2.75% v/v)	Three step 5.5 (–6), 3.5 (–60), fast (-196)	Rapid warming	+	-196	Dwyer & Honigberb, 1971
Eimeria	-	Me_2SO (2.75% v/v)	Two step 1 (–70), fast (-196)	Rapid warming	+	-196	Norton *et al.*, 1968
Entamoeba histolytica	-	Me_2SO (5% w/v)	Two step 1 (–100), fast (-196)	Rapid warming	+	-196	Farri *et al.*, 1983
Entodinium caudatum	-	Me_2SO (4% w/v)	Two step 45min at -25, fast (-196)	Rapid warming	36%	-196	Kisidayova, 1995
Giardia intestinalis	-	Me_2SO (7.5-10% v/v)	Two step 1 (–25), 5 (–196)	Rapid warming	+	-196	Phillips *et al.*, 1984
G. lamblia	-	Me_2SO (6.5% w/v)	Two step 1.2 (–40), fast (-196)	Rapid warming	+	-196	Lyman & Marchin, 1984
Leishmania tropica	-	Me_2SO (10.6% w/v)	Two step 1.9 (–65), fast (-196)	Rapid warming	+	-196	Callow & Farrant, 1973
Naegleria	-	Me_2SO (5% v/v)	Two step 1.3 (–55),	Rapid warming	+	-196	Simione & Dagett, 1976

			fast (-196)				
N. gruberi	-	Me_2SO (5% w/v)	Three step 2 h at -29, 2 h at –70 plunge	Rapid warming	>40%	-196	Brown & Day, 1993
Paramecium aurelia	-	Me_2SO (5% v/v)	2-3	Rapid warming	1.3-16	-196	Simon & Scheller, 1973
Plasmodium chabaudi	-	Glycerol (10% v/v)	Plunge to LN_2	Rapid warming	+	-196	Mutetwa & James. 1984
P. falciparum		Glycerol (10% v/v)	One step 300 to -196	Rapid warming	+	-196	Mutetwa & James. 1985
P. galinaceum	-	Glycerol (10% v/v)	Two step 1 (–70), fast (-196)	Rapid warming	+	-196	Mutetwa & James. 1985
P. berghei	-	Glycerol (10% v/v)	Two step 50 (–80), fast (-196)	Rapid warming	+	-196	Hollingdale *et al.*, 1985
Tetrahymena pyriformis	-	Me_2SO (5% v/v)	2-3	Rapid warming	+	-196	Simon & Scheller, 1973
T. pyriformis	-	Me_2SO (1.4 M)	Two step 1 (–53), fast (–196)	Rapid warming	0.4	-196	Osbourne & Lee, 1975
Theileria parva	-	Glycerol (7.5% v/v)	Two step 1 (–80), fast (-196)	Rapid warming	+	-196	Cunningham *et al.*, 1973
Toxoplasma gondii	-	Glycerol (10% v/v)	1 (-160)	Rapid warming	+	-196	Bollinger *et al.*, 1974
T. gondii	-	Glycerol (10% v/v)	0.3 (-79)	Rapid warming	+	-79	Eyles *et al.*, 1956
Trichomonas vaginalis	-	Me_2SO (5% v/v)	Two step 1 (–35), fast (-196)	Rapid warming	+	-196	Ivey, 1975
Trypanosoma brucei spp	-	Glycerol (7.5 v/v)	Two step 0.7 (–60),	Warming in air 20°C	+	-196	Lumsden *et al.*, 1973

			fast (-196)				
Trypanosoma brucei congolense	-	Glycerol (12 v/v)	Two step 1-2 (–59), fast (-196)	Rapid warming	+	-196	Diffley *et al*., 1976
T. cruzi	-	Me_2SO (10% v/v)	Two step 3 (–60), fast (-196)	Rapid warming	+	-196	Ribiero dos Santos *et al*., 1978
T. cruzi	-	Glycerol(7% v/v)	Two step 2 (–60), fast (-196)	Rapid warming	+	-196	Engel *et al*., 1980
Zoa entodinium simplex	-	Me_2SO (38% w/v)	Two step 0.5 –2.2 (-100), fast (-196)	Rapid warming	15%	-196	Marcin *et al*., 1989

DI, direct immersion in liquid coolants; Two/ multi step cooling Protocols, intermediate temperatures given in parenthesis. Unless otherwise stated final step is a plunge into liquid nitrogen.

See also Lee & Soldo (1992).

References

Albrecht, R.M., Orndorff, G.R. & MacKenzie, A.P. (1973). Survival of certain microorganisms subjected to rapid and very rapid freezing on membrane filters. *Cryobiology* **10**, 233-239.

Antonio, J.P. San- & Blount, V. (1973). Use of liquid nitrogen to preserve downy mildew (*Phytophthora phaseoli*) inoculum. *Plant Disease Reporter* **57**, 724.

Beaty, M. H. & Parker, B. C. (1992). Cryopreservation of eukaryotic algae. *Virginia Journal of Science* **43**, 403-410.

Ben-Amotz, A. & Gilboa, A. (1980). Cryopreservation of marine unicellular algae. II. Induction of freezing tolerance. *Marine Ecology Progress Series* **2**, 221-224.

Beyersdorf-Radeck, B., Schmid, R.D. & Malik, K.A. (1993). Long-term storage of bacterial inoculum for direct use in a microbial biosensor. *Journal of Microbiological Methods* **18**, 36-39.

Bodas, K., Brennig, C., Diller, K. R. & Brand, J. J. (1995). Cryopreservation of blue-green and eukaryotic algae in the culture collection at the University of Texas at Austin. *Cryo-Letters* **16**, 267-274.

Bollinger, R.O., Musallam, N. & Stulberg, C.S. (1974). Freeze preparation of tissue and propagated *Toxoplasma gondii. Journal of Parasitol*ogy **60**, 368-369.

Box, J.D. (1988). Cryopreservation of the blue-green alga *Microcystis aeruginosa*. *British Phycology Journal* **23**, 385-386.

Bradshaw, D.J., McKee, A.S. & Marsh, P.D. (1989). The use of defined inocula stored in liquid nitrogen for mixed culture chemostat studies. *Journal of Microbiological Methods* **9**, 123-128.

Bromfield, K.R. & Schmitt, C.G. (1967). Cryogenic storage of conidia of *Peronospora tabacinae*. *Phytopathology* **57**, 1133-1134.

Brown, S. & Day, J.G. (1993). An improved method for the long-term preservation of *Naegleria gruberi*. *Cryo-Letters* **14**, 347-352.

Butterfield, W., Jong, S.C. & Alexander, M.J. (1974). Preservation of living fungi pathogenic for man and animals. *Canadian Journal of Microbiology* **20**, 1665-1673.

Callow, L.L. & Farrant, J. (1973). Cryopreservation of the promastigote form of *Leishmania tropica* var. *major* at different cooling rates. *International Journal of Parasitology* **3,** 77-88.

Canavate, J.P. & Lubian, L.M. (1995a). Some aspects on the cryopreservation of microalgae used as food for marine species. *Aquaculture* **136**, 277-290.

Canavate, J.P. & Lubian, L.M. (1995b). Relationship between cooling rates, cryoprotectant concentrations and salinates in the cryopreservation of marine microalgae. *Marine Biology* **124**, 325-334.

Cunningham, M.P., Brown, C.G.D., Burridge, M.J. & Purnell, R.E. (1973). Cryopreservation of infective particles of *Theileria parva*. *International Journal of Parasitology* **3**, 583-587.

Challen, M.P. & Elliott, T.J. (1986). Polypropylene straw ampoules for the storage of micro-organisms in liquid nitrogen. *Journal of Microbiological Methods* **5**, 11-23.

Dahmen, H., Staub, T. & Schwinn, F.T. (1983). Technique for long-term preservation of phytopathogenic fungi in liquid nitrogen. *Phytopathology* **73**, 241-246.

Dalgliesh, R.J. & Mellors, L.T. (1974). Survival of the parasitic protozoan, *Babesia bigemina*, in blood cooled at widely different rates to -196°C.*International Journal of Parasitology* **4**, 169-172.

Dalgliesh, R.J., Mellors, L.T. & Blight, G.W. (1980). Comparisons of glucose, sucrose and dimethyl sulfoxide as cryoprotective agents for *Babesia rodhaini*, with estimates of survival rates. *Cryobiology* **17**, 410-417.

Day, J.G. (1998). Cryoconservation of microalgae and cyanobacteria. *Cryo-Letters suppl.* **1**, 7-14.

Day, J.G. & DeVille, M.M. (1995). Cryopreservation of algae. *Methods in Molecular Biology* **38**, 81-89.

Day, J.G. & Fenwick, C. (1993) Cryopreservation of members of the genus Tetraselmis used in Aquaculture *Aquaculture* **118**, 151-160.

Day, J.G., Fleck, R.A. & Benson, E.E. (1998). Cryopreservation of multicellular algae: Problems and perspectives. *Cryo-Letters* **19**, 205-206.

Day, J.G., Fleck, R.A. & Benson, E.E. (2000). Cryopreservation-recalcitrance in microalgae: novel approaches to identify and avoid cryo-injury. *Journal of Applied Phycology* **12,** 369-377.

Day, J.G., Watanabe, M.M., Morris, G.J., Fleck, R.A. & McLellan, M.R. (1997). Long-term viability of preserved eukaryotic algae. *Journal of Applied Phycology* **9**, 121-127.

Diamond, L.S. (1995) Cryopreservation and storage of parasitic protozoa in liquid nitrogen. *Journal of Eukaryotic Microbiology* **42**, 585-590.

Diffley, P., Honigberg, B.M. & Mohn, F.A. (1976). An improved method of cryopreservation *Trypanosoma (Nannomonas) congolense* Borden in liquid nitrogen. *Journal of Parasitology* **62**, 136-137.

Dwyer, D.M. & Honigberg, B.M. (1971). Freezing and maintenance of *Dientamoeba fragilis* in liquid nitrogen. *Journal of Parasitology* **57**, 190-191.

Elliott, T.J. & Challen, M.P. (1981). The storage of mushroom strains in liquid nitrogen. *Annual Report of the Glasshouse Crops Research Institute* **1979**, 194-204.

Engel, J.C., Perez, , A.C. & Wynne de Martini, G. (1980). *Medicina* **40**, 103-108.

Eylees, D.E., Coleman, N. & Cavanaugh, D.J. (1956). Preservation of *Toxoplasma gondii* by freezing**.** *Journal of Parasitology* **42**, 408-413.

Farri, T.A., Warhurst, D.C & Marshall, T.F.D. (1983). The use of infectivity titrations for measurement of viability of *Entamoeba histolytica* after cryopreservation. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **77**, 259-266.

Fenwick, C. & Day, J. G. (1992). Cryopreservation of *Tetraselmis suecica* cultured under different nutrients regimes. *Journal of Applied Phycology* **4**, 105-109.

Fortney, K.F. & Thoma, R.W. (1977). Stabilization of culture productivity. *Developmental.and Industrial Microbiol*ogy **18**, 319-325.

Gale, A.W., Schmitt, C.G. & Bromfield, K.R. (1975). Cryogenic storage of *Sclerospora sorghi*. *Phytopathology* **65**, 828-829.

Gothe, R. & Hartman, S. (1979). The viability of cryopreserved *Aegyptianella pullorum* Carpano, 1928 in the vector *Argas* (*Persicargas*) *walkerae* Kaiser and Hoogstraal, 1969. Z. Parasitenk. **58**, 189-190.

Gresshoff, P.M. (1977). *Chlamydomonas reinhardi* - A model plant system. II Cryopreservation. *Plant Science Lett*ers **9,** 23-25.

Hirata, K., Phunchindawan, M., Tukamoto, J., Goda, S. & Miyamoto, K. (1996). Cryopreservation of microalgae using encapsulation/dehydration. *Cryo-Letters* **17**, 321-328.

Hollingdale, M.R., Leland, P., Singer, C.I. & Leef, J.L. (1985). *In vitro* infectivity of cryopreserved *Plasmodium berghei* sporozoites to cultured cells. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**, 206-208.

Holm-Hansen, O. (1963). Viability of blue-green and green algae after freezing. *Physiology of Plants* **16**, 530-539.

Hwang, S-W. (1966). Long term preservation of fungus cultures with liquid nitrogen refrigeration. *Applied Microbiology* **14**, 784-788.

Hwang, S-W. (1968). Investigation of ultra-low temperature for fungal cultures. I. An evaluation of liquid nitrogen storage for preservation of selected fungal cultures. *Mycologia* **60**, 613-621.

Hwang, S-E. & Hudock, G.A. (1971). Stability of *Chlamydomonas reinhardi* in liquid nitrogen storage. *Journal of Phycology* **7**, 300-303.

Ivey, M.H. (1975). The use of solid medium techniques to evaluate factors affecting the ability of *Trichomonas vaginalis* to survive freezing. *Journal of Parasitology* **61**, 1101-1103.

James, E.R. (1990). Cryopreservation of helminth parasites. *In*: *In vitro* cultivation of parasitic helminths (ed Smyth, J.D.). Boca Raton, FL, USA: CRC Press Inc.

James, E.R. & Peacock, R. (1986). Studies on the cryopreservation of *Dictyocaulus viviparus* (Nematoda) third stage larvae. *Journal of Helminthoogy* **60**, 65-73.

Joshi, L.M., Wilcoxson, R.D., Gera, S.D. & Chatterjee, S.C. (1974). Preservation of fungal cultures in liquid air. *Indian Journal of Experimental Biology* **12**, 598-599.

Kilpatrick, R.A., Harmon, D.L., Loegering, W.Q. & Clark, W.A. (1971). Viability of uredospores of *Puccinia graminis* f. sp. *tritici* stored in liquid nitrogen. *Plant Disease Reporter* **55**, 871-873.

Kilvington, S. (1995). Cryopreservation of pathogenic and non-pathogenic free-lving amoebae. *Methods in Molecular Biology* **38**, 63-70.

Kisidayova, S. (1995). 2-step freezing of the rumen ciliate protozoan *Entodinium caudatum. Journal of Microbiological Methods* **22**, 185-192.

Kono, S., **Kuwano, K. & Saga N.** (1998). Cryopreservation of *Eisenia bicyclis* in liquid nitrogen. *Journal of Marine Biotechnology* **6**, 220-223.

Kono, S., **Kuwano, K., Ninomiya, M., Onishi, J. & Saga, N.** (1997). Cryopreservation of *Enteromorpha intestinalis* in liquid nitrogen. *Phycologia* **36**, 76-78.

Kumi-Diaka, J. & Harris, O. (1995). Viability of *Borrelia burgdorferi* in stored semen. *British Veterinary Journal* **151**, 221-224.

Lee, J.J. & Soldo, A.T. (1992). "*Protocols in Protozoology"* Society of Protozoologists, Kansas, USA.

Long, R.A., Wood, J.M. & Schmitt, G.C. (1978). Recovery of viable conidia of *Sclerospora philippinensis, S. sacchari* and *S. sorghi* after cryogenic storage. *Plant Disease Reporter* **62**, 479-481.

Love, J.N. (1972). Cryogenic preservation of anaplasma marginale with dimethyl sulfoxide.*American Journal of Vetinerary Research* **33**, 2557-2560.

Lumsden, W.H.R., Herbert, W.J. & McNeillage, G.J.C. (1973). Techniques with trypanosomes. Edinburgh, Churchill Livingstone, 183p

Lyman, J.R & Marchin, G.L. (1984). Cryopreservation of *Giardia lamblia* with dimethylsulfoxide using a dewer flask. *Cryobiology* **21**, 170-176.

Marcin, A Gyulai, F., Vardy, M. & Sorokova, M. (1989). A simple technique for cryopreservation of the rumen protozoa *Entodinium simplex*. *Cryo-Letters* **10**, 89-104.

McCrindle, C.M.E. (1979). The preservation of *Clostridium chauvoei* in liquid nitrogen. Journal of the South African Veterinary Association **50**, 221-222.

McGrath, M.S. & Daggett, P.M. (1977). Cryopreservation of flagellar mutants of *Chlamydomonas reinhardii. Canadian Journal of Botany* **55**, 1794-1796.

McGrath, M. S., Daggett, P. M. & Dilworth, S. (1978). Freeze-drying of algae: Chlorophyta and Chrysophyta. *Journal of Phycology* **14**, 521-525.

McLellan, M.R. (1989). Cryopreservation of diatoms. *Diatom Research* **4**, 301-318.

Morris, G.J. (1976a). The cryopreservation of *Chlorella* 2. Effect of growth temperature on freezing tolerance. *Arch. Microbiol* **107**, 309-312.

Morris, G.J. (1976b). Effect of growth temperature on the cryopreservation of *Prototheca. Journal of General Microbiology* **94**, 395-399.

Morris, G.J. (1977). Preservation of algae by the method of two step cooling. *Cryobiology* **14**, 691-692.

Morris, G.J. (1978). Cryopreservation of 250 strains of Chlorococcales by the method of two step cooling. *British Phycology Journal* **13**, 15-24.

Morris, G. J. & Canning, C. E. (1978) The cryopreservation of *Euglena gracilis*. *Journal of General Microbiology* **108**, 27-31

Morris, G. J., Coulson, G. E. & Engels, M. (1986). A cryomicroscopic study of *Cylindrocystis brebissonii* and two species of *Micrasterias conjugatophyceae* chlorophyta during freezing and thawing. *Journal of Experimental Botany* **37**, 842-856

Morris, G.J., Clarke, A. & Fuller, B.J. (1980). Methanol as a cryoprotective additive for *Chlorella. Cryo-Letters* **1**, 121-128.

Mortain-Bertrand, A., Etchart, F. & de Boucayd, M-T. (1996) A method for the cryoconservation of *Dunaliella salina* (Chlorophyceae): Effect of glycerol and cold adaptation. *Journal of Phycology* **32**, 346-352.

Mutetwa, S.M. & James E.R. (1984). Cryopreservation of *Plasmodium chabaudi*. 1. Protection by glycerol and dimethylsulphoxide during cooling and by glucose following thawing. *Cryobiology* **21**, 329-339.

Mutetwa, S.M. & James E.R. (1985). Low-temperature preservation of *Plasmodium* spp. *Parasitology* **90**, 589-603.

Norton, C.C., Pout, D.D. & Joyner, L.P. (1968). Freeze preservation of Eimeria acervulina Tyzzer, 1929. *Folia Parasitology* **15**, 203-211.

Nugent, M.J., O'Leary, S.A. & Burnell, A.M. (1994). Optimised procedures for the cryopreservation of different species of *Heterorhabditis*. Eclair Grant 151. Brussels: EU Commission.

O'Brien, M.J. & Webb, R.E. (1958). Preservation of conidia of *Albugo occidentalis* and *Peronospora effusa*, obligate parasites of spinach. *Plant Disease Reporter* **42**, 1312-1315.

Osbourne, J.A. & Lee, D. (1975). Studies on the conditions required to optimum recovery to *Tetrahymena pyriformis* strain S (Phenoset A) after freezing to and thawing from –196^{o}C. *Journal of Protozoology* **22**, 233-237.

Phillips, R.E., Boreham, P.F.L. & Shepherd, R.W. (1984). Cryopreservation of viable *Giardia intestinalis* trophozoites. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **78**, 604-606.

Popiel, I. & Vasquez, E.M. (1991). Cryopreservation of *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*. *Journal of Nematology* **23**, 432-437.

Raether, W. & Uphoff, M. (1976). Survival of monoxenically (*Crithidia* sp.)and axenically grown *Entamoeba histolytica* cultures after storage in liquid nitrogen. *International Journal of Parasitology* **6**, 121-125.

Ribeiro dos Santos, R. von Gal Furtado, C.C., Martins, J.B. & Martins A.C.P. (1978). *Rev. Bras. Pesequisas Med. Biol.* **11**, 99-104.

Ritter, E. & Ulrich, U. (1986). Langzeitkonservierung von Mykoplasmen in flűssigem Stickstoff. *Z.* ges. Hyg. **32**, 618-620.

Romo, S. & Becares, E. (1992). Preservation of filamentous cyanobacteria cultures. *Journal of Microbioogical Methods* **16**, 85-89.

Rossi, P., Pozio, E. & Besse, M.G. (1990). *Transactions of the Royal Society of Tropical Medicine and Hygiene* **84**, 68.

Sanfilippo, A. & Lewin, R.A. (1970). Preservation of viable flexibacteria at low temperatures. *Canadian Journal of Microbiology* **16**, 441-444.

Simione, F.P., Daggett, P.-M. (1976). Freeze preservation of pathogenic and nonpathogenic *Naegleria* species. *Journal of Parasitology* **62**, 49.

Simione, F.P. & Daggett, P-M. (1977). Recovery of a marine dinoflagellate following controlled and uncontrolled freezing. *Cryobiology* **14**, 362-366.

Simon, E.M. (1972). Freezing and storage in liquid nitrogen of axenically and monoxenically cultivated *Tetrahymena pyriformis. Cryobiology* **9**, 75-81.

Simon, E.M. & Schneller, M.V. (1973). The preservation of ciliated protozoa at low temparature. *Cryobiology* **10**, 421-426.

Smentek, P. & Windisch, S. (1982). Zur Frage des Überlebens von Hefestämmen unter flussigem Stickstoff. *Zbl. Bakt. I. Orig. C.* **3**, 432-439.

Smith, B.S., Hodgson-Smith, A., Popiel, I., Minter, D.M. & James, E.R. (1990). Cryopreservation of the entomogenous nematode parasite *Steinernema feltiae* (*=Neoaplectana carpocapsae*). *Cryobiology* **27**, 319.

Smith, D. (1982). Liquid nitrogen storage of fungi. *Transactions of the British Mycological Society* **79**, 415-421.

Taylor, R. & Fletcher, R.L. (1998). Cryopreservation of eukaryotic algae - a review of methodologies. *Journal of Applied Phycology***10**, 481-501.

Tetsuka, Y. & Katsuya, K. (1983). Storage of sporangia of hop and vine downy mildews in liquid nitrogen. *Annals of the Phytopathological Society of Japan* **49**, 731-735.

Thompson, J.P. (1987). Cryopreservation of Azotobacteriaceae in liquid nitrogen. *MIRCEN Journal – Journal of Applied Microbiology and Biotechnology* **3**, 323-336.

Tooley, P.W. (1988). Use of uncontrolled freezing for liquid nitrogen storage of *Phytophthora* spp. *Plant Diseases* **72**, 680-682.

Tsuru, S. (1973) Preservation of marine and freshwater algae by means of freezing and freeze-drying. *Cryobiology* **10**, 445-452.

Vega, C.A., Buening, G.M., Rodriguez, S.D., Carson, C.A. & McLaughlin, K. (1985). Cryopreservation of *Babesia bigemina* for *in vitro* cultivation. *American Journal of Veterinary Research* **46**, 421-423.

Wang, G. –T, Lin, C.T. & Hua, J. (1990). Long-term preservation of *Ganoderma* mycelia. *Journal of Chinese Agriculture and Chemistry Society* **28**, 86-93.

Watanabe, M.M. & Sawaguchi, T. (1995). Cryopreservation of a water bloom forming cyanobacterium *Microcystis aeruginosa. Japanese Journal of Phycol*ogy **43**, 111-118.

Watanabe, M.M., Shimizu, A. & Satake, K. (1992). NIES-Microbial Culture Collection at the National Institute of Environmental Studies: Cryopreservation and database of culture strains of microalgae. In: Watanabe M.M. (ed.), Proceedings of Symposium on Culture Collection of Algae. NIES, Tsukuba, Japan, 33-41.

Yarlett, N.C., Yarlett, N., Orpin, C.G. & Lloyd, D. (1986). Cryopreservation of the anaerobic rumen fungus *Neocallimastix patriciarum*. *Letters on Aplied Microbiology* **3**, 1-3.