8
New Strategies for the Selective Isolation of Industrially Important Bacteria

M. GOODFELLOW AND E. WILLIAMS

Department of Microbiology, The Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK

Introduction

The isolation and subsequent screening of bacteria from diverse habitats has led to the discovery of many novel and useful secondary metabolites. Surprisingly, the approach to the search for potentially valuable bacteria has been largely empirical and restricted to sampling a tiny fraction of the microbial community found in natural habitats. In essence, many representatives of a few well-established bacterial taxa are isolated and then screened in the hope that something useful turns up. However, it is now essential to develop new and more objective procedures for the selective isolation of uncommon and novel (that is, previously undiscovered) microorganisms in order to improve the biological quality of the material screened. Taxonomical expertise is needed to apply rapid and reliable methods to differentiate between microbial taxa in order to reduce the rediscovery of known bioactive compounds to an acceptable minimum. The value of increasing the proportion of novel actinomycetes in screening programmes is illustrated by the spectacular increase from six to over 95 in the antibiotics discovered from actinoplanetes between 1974 and 1984 (Bérdy, 1974, 1984), by the clinically significant leads provided by the gentamycin C complex from micromonosporae (Parenti and Coronelli, 1979) and by the rifampicin group of mycolateless nocardiae (Alderson *et al.*, 1981). New bioactive compounds can also be expected from new, rare or neglected bacteria other than actinomycetes.

Some kinds of bacteria are a richer source of secondary metabolites than others and this influences isolation procedures. Among the bacteria, the actinomycetes and aerobic endospore-forming bacteria have proved to be a particularly rich source of antibiotics, enzymes, enzyme inhibitors and vitamins. With respect to the actinomycetes, initial attention was focused on

Biotechnology and Genetic Engineering Reviews—Vol. 4, September 1986

the genus *Streptomyces*, given the important discovery of the antibiotics actinomycin, neomycin, streptomycin and streptothricin in the laboratory of Selman Waksman. The capacity of streptomycetes to produce commercially significant compounds, especially antibiotics, remains unsurpassed, possibly because of the extra-large DNA complement of the bacteria (Hopwood and Chater, 1980; Gładek and Zakrzewska, 1984), their capacity for genetic exchange and the presence of plasmids (Chater and Hopwood, 1984). It is now becoming clear that actinomycetes other than streptomycetes are an important source of new antibiotics (*Table 1*).

Table 1. Number of antibiotics produced by selected actinomycete taxa*

Genus	1974	1980	1984
Streptomyces	1934	2784	3477
Micromonospora	41	129	269
Nocardia	45	74	107
Actinoplanes	6	40	95
Streptoverticillium	19	41	64
Actinomadura	0	16	51
Saccharopolyspora	—	4	33
Streptosporangium	7	20	26
Actinosynnema	—	0	25
Dactylosporangium	0	4	19
Actinomyces	0	14	17
Streptoalloteichus	—	3	14
Pseudonocardia	0	3	8
Micropolyspora	2	4	7
Microbispora	4	6	6
Thermomonospora	1	3	4

*Taken from Bérdy (1974, 1984) and Nisbet (1982).

Actinomycetes were once considered to be related to both bacteria and fungi but in recent years it has been unequivocally shown that they are prokaryotic organisms. Classically, actinomycetes were seen as Gram-positive bacteria that formed branching hyphae which often developed into a mycelium, but the group is now also considered to include some coccoid and pleomorphic organisms. Reproduction is by fragmentation of the hyphae or by production of spores in special areas of the mycelium. In addition to being a rich source of antibiotics, the actinomycetes are important for the production of enzymes, such as amylases (e.g. *Thermomonospora curvata*), cellulases (e.g. *Thermomonospora* spp.), chitinases, pectinases (e.g. *Micromonospora* spp.), ligninases (e.g. *Nocardia autotrophica*), peptidases, proteases (e.g. *Nocardia* spp.), sugar isomerases (e.g. *Actinoplanes missouriensis*) and xylanases (e.g. *Microbispora* spp.), and enzyme inhibitors including elasnin (e.g. *Streptomyces noboritoensis*), esterastin (e.g. *Streptomyces lavendulae*) and leupeptin (e.g. *Streptomyces* spp.) (*see* Cross, 1982; Williams, Lanning and Wellington, 1984). Actinomycetes are also important

for transforming aromatic, sterol and steroid compounds, and for the degradation of recalcitrant molecules (*see* Cain, 1980; Peczyńska-Czoch and Mordarski, 1984; Lechevalier and Lechevalier, 1985).

There are three general areas of industrial applications of the aerobic endospore-forming bacteria: enzymes, antibiotics and insecticides. *Bacillus* species produce an extensive range of industrially useful enzymes. Those that hydrolyse starch—α-amylase, β-amylase and amyloglucosidases—are the most important commercially and find extensive usage in the brewing and food industries. The proteases, including esterases, metalloproteases and alkaline serine proteases, are important for diagnostic and industrial purposes, and are used extensively in biological detergents (Atkinson and Mavituna, 1983; Godfrey, 1983; Reichelt, 1983).

Bacillus species produce a range of antibiotics, namely peptides, which are primarily active against Gram-positive bacteria. The three main classes are bacitracins (which inhibit cell-wall synthesis), edeines (which inhibit DNA synthesis), and the gramicidin–polymixin–tyrocidin group (which modify membrane function). The most important species are *Bacillus brevis* and *Bacillus subtilis* (Katz and Demain, 1977). Many bacilli also have insecticidal properties. *Bacillus thuringiensis* insecticide has been produced on a large scale and is active against moths and mosquito larvae. It is produced as a large proteinaceous crystal which is synthesized during sporulation (*see* Dean, 1984). Other important insect pathogens are *Bacillus lentimorbus* and *Bacillus popilliae* (Bulla and Hoch, 1985).

The realization that new bioactive compounds are produced by a wide range of actinomycetes and aerobic endospore-forming bacilli has stimulated work designed to produce selective isolation techniques. The aim of such techniques is to eliminate the growth of fungi and discourage that of the more readily isolated bacterial genera in order to uncover rare and novel taxa. An alternative, but sometimes complementary, strategy involves the development of extremely sensitive screening methods for particular types of metabolites (Nisbet, 1982; Bérdy, 1984). This approach has been applied successfully in the search for monocyclic β-lactam antibiotics from a range of Gram-negative bacteria (Imada *et al.*, 1981; Sykes *et al.*, 1981). This review concentrates on some of the more objective isolation strategies that are being developed and applied in the quest for novel and uncommon bacteria which might be expected to yield new commercially significant compounds. Pride of place is given to methods intended to isolate selectively new and uncommon actinomycetes, aerobic endospore-forming bacilli, methylotrophic and C_1-utilizing autotrophic bacteria. This emphasis is testimony to the importance of these groups in industrial microbiology, but also reflects our own interests.

The development of selective isolation procedures has been hindered by the reliance placed on classical approaches and by poor classification which has made it difficult to choose representative strains for screening programmes and genetic engineering. However, recent improvements in classification and identification are being exploited to devise selective isolation procedures for specific established (Williams, Goodfellow and Vickers, 1984)

and novel taxa (Goodfellow and Dickinson, 1985). In addition, the introduction of rapid methods for the identification of isolates growing on selective media helps to optimize specific isolation procedures and provides information on the heterogeneity and distribution of new and uncommon strains. The growing links between advances in bacterial systematics and the development of selective isolation and screening techniques are explored with particular reference to the actinomycetes. The C_1 autotrophic and methylotrophic bacteria are considered separately, as a taxonomic approach to these organisms is not yet practicable.

Application of systematics to the development of selective isolation techniques

The application of new and reliable biochemical, chemical and molecular biological techniques are revolutionizing actinomycete systematics (Goodfellow and Cross, 1984; Goodfellow, 1986). As a result, established actinomycete genera and species have been defined more precisely, new taxa have been proposed for novel centres of variation, and misclassified and poorly circumscribed species have been reduced to synonyms of well-defined taxospecies. The number of recognized actinomycete genera has risen sharply to over 60 (*see Table 3*) and one of the four volumes of the forthcoming edition of *Bergey's Manual of Systematic Bacteriology* (Williams, 1987) will be devoted to these organisms. The improvements in the classification of actinomycetes are providing an essential base for the introduction of accurate and standardized methods for the identification of unknown isolates to established genera and species (Williams, Vickers and Goodfellow, 1985), for the circumscription of novel taxa (O'Donnell, 1986) and for the development of objective procedures for the isolation of specific antibiotic-producing actinomycetes (Vickers, Williams and Ross, 1984).

The genus *Bacillus* is generally considered to include those rod-shaped bacteria able to form heat-resistant endospores under aerobic conditions. This definition encompasses a wide range of organisms endowed with markedly different biochemical and physiological properties which allow members of the taxon apparently to prosper in extreme habitats such as acid hot springs (pH 2–3; 75–80°C) and the snow fields of Antarctica (Slepecky, 1975). With the application of modern taxonomic methods, such as chemotaxonomy, numerical taxonomy and nucleic acid analyses, has come the realization that the genus, as at present defined, includes bacteria of widely different properties and genetic composition (*see* Berkeley and Goodfellow, 1981). The application of the newer taxonomic methods has also helped to clarify the status of the genera *Sporolactobacillus, Sporosarcina* and *Thermoactinomyces* which contain aerobic endospore-forming bacteria (Cross and Unsworth, 1981; Norris, 1981). Improvements in *Bacillus*10 systematics have led to tighter descriptions of species, to the recognition of novel species, and to the provision of miniaturized test systems and computer-assisted methods for the identification of unknown strains (Suzuki, 1983; Logan and Berkeley, 1984; Bryant, Capey and Berkeley, 1985). In addition, several species of

Bacillus originally omitted from the *Approved Lists of Bacterial Names* (Skerman, McGowan and Sneath, 1980) have been redescribed (Moore, Cato and Moore, 1985). These taxa include *Bacillus amyloliquefaciens* which is responsible for much of the world's production of α-amylase and protease (Priest *et al.*, 1986).

Most of the methods currently applied in bacterial systematics are of limited value in detecting relationships among distantly related genera and species. However, a number of powerful methods have recently been introduced for establishing suprageneric relationships (*Table 2*). In an impressive series of 16S ribosomal (r) RNA cataloguing studies the actinomycetes have been found to form a distinct evolutionary line that fused with a second phyletic branch which encompassed most of the remaining Gram-positive taxa (Stackebrandt and Woese, 1981). The actinomycete branch, which includes taxa once assigned to the 'coryneform group of bacteria' (Minnikin, Goodfellow and Collins, 1978), accommodates organisms containing DNA with a guanine (G) plus cytosine (C) content above *c.* 55 mole % and can thereby be distinguished from the low G plus C (<50 mole %) *Bacillus–Clostridium–Lactobacillus* branch (Stackebrandt and Woese, 1981). Interestingly, the thermophilic genus *Thermoactinomyces*, long regarded as a bona fide actinomycete given its capacity to form branched hyphae which carry lateral spores on both substrate and aerial hyphae, is phylogenetically close to the family *Bacillaceae* (Goodfellow and Cross, 1984).

Table 2. Methods for constructing bacterial phylogenies

Macromolecules	Method	Ranks covered
DNA	DNA–DNA pairing	Closely related species
RNA	DNA 16S/23S rRNA pairing	Species→family
	16S rRNA oligonucleotide sequencing	Species→kingdom
	Cataloguing of 5S rRNA	Species→kingdom
Proteins	Specific proteins	
	Amino acid sequencing	Species→order
	Immunological analysis	Species→family
	Groups of proteins	
	Electrophoretic patterns	Closely related species
	Enzyme patterns and activities	Species→genera

VALUE OF PHYLOGENETIC CLASSIFICATIONS

The ribosomal RNA oligonucleotide cataloguing method provides a most exacting way of elucidating phylogenetic relationships among bacteria (Stackebrandt and Woese, 1981; Fowler, Ludwig and Stackebrandt, 1985). In this method, purified RNA is digested by T1 ribonuclease and the oligonucleotides separated by two-dimensional electrophoresis are sequenced by a combination of endonuclease digestion procedures which give a catalogue of sequences of the strain under consideration. The oligonucleotides of any

two test strains are compared one with another and oligonucleotides of six residues or larger, common to any two catalogues, are scored to produce an 'S_{AB} value' characteristic of that pair of organisms. The function S_{AB} is equivalent to twice the number of residues in sequences common to a pair of catalogues, divided by the total number of residues in all of the sequences in the two catalogues. S_{AB} values are analysed using standard clustering algorithms and the results are presented as evolutionary trees.

It is not yet possible to compile a comprehensive phylogeny of the prokaryotes by this approach but the outline of such a natural classification is emerging (Stackebrandt and Woese, 1981). Already the eubacteria, the most thoroughly characterized group with over 300 species catalogued to date, can be assigned to about 10 major groups (Woese *et al.*, 1984). As the phylogeny of the eubacteria unfolds, it becomes increasingly evident that many of the markers previously used to define suprageneric taxa have little or no predictive value. This is certainly so with respect to taxa classified in the family *Bacillaceae* and the order *Actinomycetales*. Thus, in the case of the actinomycetes, the genus *Micromonospora* has been shown to be closely related to sporangia-forming taxa such as *Actinoplanes* and *Dactylosporangium*, but sharply distinct from other monosporic genera such as *Thermomonospora* and *Thermoactinomyces* with which it was recently associated.

Actinomycetes can be assigned to 10 aggregate groups on the basis of their phylogenetic diversity (Goodfellow, 1986; *Table 3*). It is particularly interesting that many of the phenotypic properties of actinomycetes, notably morphological features traditionally weighted in the classification and identification of these organisms, are not discontinuously distributed along phylogenetically defined lines (Stackebrandt and Schleifer, 1984; Stackebrandt, 1986). Nevertheless, the cohesiveness of aggregate groups such as the Actinoplanetes, Maduromycetes, Nocardioforms and Streptomycetes is apparent (*see* Goodfellow and Cross, 1984). In contrast, it seems likely that aggregate taxa such as the Actinobacteria and Thermomonosporas are markedly heterogeneous and in need of further study. Indeed, a number of possible evolutionary lines can be recognized among the Actinobacteria (*Table 3*). The aerobic, endospore-forming bacteria also form a recognizable phyletic branch together with the genera *Planococcus* and *Staphylococcus* (Stackebrandt and Woese, 1981).

A whole panoply of modern taxonomic methods can be applied to unravelling the fine structure of major evolutionary lines detected in rRNA sequencing studies. Data derived from comparative studies on the same group of organisms can readily be examined to see which, if any, properties make good phylogenetic markers. Among the actinomycetes some chemical markers have been shown to be useful indicators of evolutionary relationships (Stackebrandt and Schleifer, 1984; Stackebrandt, 1986). Chemotaxonomy or chemical systematics is a rapidly expanding discipline in which information derived from chemical analyses of whole organisms or cell fractions is used for classification and identification. A wide array of chemical techniques have been recommended, including ones for determining DNA base, whole-organism, lipid, wall sugar and amino-acid composition of bacteria (*see*

Goodfellow and Minnikin, 1985). Chemical features have been invaluable in clarifying the taxonomic affinities of the genus *Thermoactinomyces* and the suprageneric affinities of actinomycete taxa (Goodfellow and Cross, 1984; *Table 3*). Nocardioform actinomycetes, for example, have a wall chemotype IV *sensu* Lechevalier and Lechevalier (1970), that is, they have *meso*-diaminopimelic acid as the diamino acid of the wall peptidoglycan, and arabinose and galactose as major wall sugars, an AI_γ peptidoglycan (Schleifer and Kandler, 1972), and major amounts of straight-chain saturated and monounsaturated fatty acids, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides as major phospholipids and characteristic 2-alkyl-branched 3-hydroxy acids, the mycolic acids.

For the first time ever it is possible to classify bacteria into a hierarchic system on the basis of their natural or evolutionary relationships. Now that several major lines of descent leading to recent actinomycetes have been detected (Fischer, Kroppenstedt and Stackebrandt, 1983; Stackebrandt *et al.*, 1983; Stackebrandt and Schleifer, 1984; Fowler, Ludwig and Stackebrandt, 1985), representative strains are the subject of extensive study using a variety of methods designed to elucidate the fine structure of the higher taxa. The product of such comparative studies will be a stable classification of the actinomycetes. The benefits of sound classification are wide and varied but include the possibility of relating the distribution of particular bioactive compounds to defined taxonomic groups. Clearly, such information will have a dramatic impact on the development of isolation strategies.

NUMERICAL CLASSIFICATION

The application of high-speed electronic computers to taxonomic data has led to major advances in the fields of bacterial classification and identification in recent years. The method is usually called numerical taxonomy but is also referred to as computer-assisted taxonomy. Conventional numerical taxonomy has been applied to the reclassification of many bacterial taxa but has been shown to be very effective in determining relationships among actinomycetes and aerobic, endospore-forming bacteria, albeit at the subgeneric level. The theoretical basis of the subject is well documented (Sneath and Sokal, 1973; Goodfellow, Jones and Priest, 1985) and its impact on the classification of bacilli and actinomycete taxa has been considered in detail elsewhere (Logan and Berkeley, 1981; Priest, Goodfellow and Todd, 1981; Goodfellow and Cross, 1984).

In essence, numerical taxonomy involves the comparison of many phenetic (i.e. observable) characters of one organism with the same phenetic features of other organisms. The degree of similarity between the test strains is then computed and the organisms are assigned to groups on this basis. Bacteria which share many features in common, that is, have a high percentage similarity (%S), will cluster together and on the basis of this clustering a classification can be constructed. Initially, all characters are given equal weight but, once a numerical taxonomy has been obtained, cluster-specific properties can be abstracted from the data base and weighted for identification. This approach is in sharp contrast to traditional practice in *Bacillus*

Table 3. Suprageneric groups of actinomycetes and some of their chemical properties

Group/Genus	Wall chemotype*	Peptidoglycan type**	Fatty acid pattern†	Phospholipid type‡	Mole % G + C§
ACTINOBACTERIA					
A. *Arthrobacter*	VI	A3α	2c	1	59–66
Cellulomonas	VIII	A4β	2c	5	71–77
Dermatophilus	III	A1γ	1a	1	57–59
Micrococcus	VI	A3α	2c	1	66–75
Oerskovia	VI	A4α	2c	5	70–75
Promicromonospora	VI	A4α	ND	5	73–75
Renibacterium	VI	A3α	2c	1	53–54
Rothia	VI	A3α	2c	1	54–57
Stomatococcus	VI	A3α	ND	ND	56–60
B. *Agromyces*	VII	B2γ	2c	1	71–76
Aureobacterium	VIII	B2β	2c	1	65–75
Clavibacter¶	VI	B2γ	2c	1	65–75
Curtobacterium	VIII	B2β	2c	1	69–75
Microbacterium	VI	B1α	2c	1	69–70
C. *Actinomyces*	V, VI	A4α,A4β A5α,A5β	1a,1c	2	60–73
Arcanobacterium	VI	A5α	1a	ND	50–52
D. *Arachnia*	I	A3γ	2c	1	63–65
Pimelobacter	I	A3γ	3a	1	69–74
E. *Brevibacterium*	III	A1γ, A4β	2c	1	60–64
ACTINOPLANETES					
Actinoplanes			2d	2†	72–73
Amorphosporangium			2d	2†	71
Ampullariella	II	A1γ	2d	2	72–73
Dactylosporangium			2d	2†	71–73
Pilimelia			2d	2†	ND
Glycomyces	II	ND	2c†	1	71–73
Micromonospora	II	A1γ	3b	2	71–73
KITASATOSPORIA					
Kitasatosporia	I,III	ND	ND	ND	66–73
MADUROMYCETES					
Actinomadura A			3c	4†	66–69
Microbispora			3c	4	70–74
Microtetraspora A			3c	4	ND
Planobispora	III	A1γ	3c	4	70–72
Planomonospora			3a	4	72
Streptosporangium			3c	4	71–73
Spirillospora	III	A1γ	3a	1/2	69–71
MICROPOLYSPORAS					
Actinopolyspora			2d	3	64
Amycolata//			3b†	3	68–72
Amycolatopsis//	IV	ND	2c	2	66–69
Kibdelosporangium#			2c†	2	66

Table 3. (contd)

Group/Genus	Wall chemotype*	Peptidoglycan type**	Fatty acid pattern†	Phospholipid type‡	Mole % G + C§
Micropolyspora			2d	3	ND
Pseudonocardia	IV	A3γ	2b	3	79
Saccharomonospora			2a	2	74–75
Saccharopolyspora			2d	3	77
MULTILOCULAR SPORANGIA					
Frankia	III	ND	ND	1	68–72
Geodermatophilus	III	A1γ	2b	2	73–75
NOCARDIOFORMS					
Caseobacter			1b	ND	60–67
Corynebacterium			1a	1	51–59
Mycobacterium	IV	A1γ	1b	2	62–70
Nocardia			1b	2	64–69
Rhodococcus			1b	2	59–69
'aurantiaca' taxon			1b	2	ND
NOCARDIOIDES					
Nocardioides	I	A3γ	3a	1	ND
STREPTOMYCETES					
Intrasporangium			2b	4	ND
Sporichthya			3c	ND	ND
Streptomyces	I	A3γ	2c	2	69–78
Streptoverticillium			2c	2	69–73
Kineosporia			ND	3	ND
THERMOMONOSPORAS					
Actinomadura B		A1γ	3a	1	65–70
Actinosynnema			2a†	2	ND
Microtetraspora B			3a†	1	ND
Nocardiopsis	III	ND	3d	3	65–76
Saccharothrix			2c†	2	73
Streptoalloteichus			ND	ND	ND
Thermomonospora			3c	2,4†	ND

For explanation of characteristics, *see* the following references:
* Lechevalier and Lechevalier (1970, 1980); Goodfellow and Cross (1984)
**Schleifer and Kandler (1972); Kusser and Fiedler (1983); Goodfellow and Cross (1984)
† Goodfellow and Cross (1984); Kroppenstedt (1985); R. M. Kroppenstedt, unpublished data
‡ Lechevalier, De Biévre and Lechevalier (1977); Lechevalier and Lechevalier (1980); Lechevalier, Stern and Lechevalier (1981)
§ Goodfellow and Cross (1984)
ND not determined

For information on new taxa, *see* the following references:
¶ Davis *et al.* (1984)
// Lechevalier *et al.* (1986)
Shearer *et al.* (1986)

and actinomycete taxonomy, as species are defined not by a small number of subjectively chosen morphological and staining properties but by overall similarities based on many equally weighted features. The clusters defined in numerical phenetic surveys are polythetic, i.e. no single character is either indispensable or sufficient to entitle an organism to group membership.

A lot of work has been devoted to constructing numerical taxonomies of both bacilli and actinomycetes (*Table 4*). In particular, numerical phenetic surveys have provided sound taxonomic frameworks for the genera *Mycobacterium* (Goodfellow and Wayne, 1982), *Nocardia* (Goodfellow and Minnikin, 1981) and *Streptoverticillium* (Locci *et al.*, 1981), have led to the reintroduction of the genus *Rhodococcus* (Goodfellow and Cross, 1984), and have underscored proposals for the recognition of the genera *Actinomadura* (Goodfellow, Alderson and Lacey, 1979), *Nocardiopsis* (Athalye *et al.*, 1985), *Oerskovia* (Goodfellow, 1971) and *Rothia* (Holmberg and Hallander, 1973). However, it is the reclassification of the genus *Streptomyces* which is especially interesting as the definition of species within this taxon has provided taxonomists with a major problem for many years. Hundreds of streptomycete species have been described (Pridham and Tresner, 1974) and included in the *Approved Lists of Bacterial Names* (Skerman, McGowan and Sneath, 1980). Still more have been cited in the patent literature (Trejo, 1970). However, in an extensive numerical phenetic survey (Williams *et al.*, 1983a) the type strains of over 300 species of *Streptomyces* were assigned to 23 clusters or subclusters (6–38 strains), 37 minor clusters (<5 strains) and 13 single-member clusters. The minor and single-member clusters were considered to form species and the major clusters were equated with species groups. The genera *Actinopycnidium, Actinosporangium, Chainia, Elytrosporangium, Kitasatoa* and *Microellobosporia*, all defined on the basis of single morphological properties, were found to be closely related to streptomycetes. They were subsequently reduced to synonyms of the genus *Streptomyces* (Goodfellow, Williams and Alderson, 1986a,b,c,d).

Numerical taxonomy with its emphasis on many strains and tests (*Table 4*) remains the method of choice for circumscribing species. It is, however, imperative to evaluate numerical taxonomies using other powerful taxonomic methods, as similarities between strains can be distorted by factors such as test and sampling errors, the statistics used, and failure to allow for differences in growth rates and metabolic activities (Jones and Sackin, 1980). Indeed, the congruence found between classifications obtained by the application of several independent taxonomic methods can be taken as an index of the reliance that can be placed in a classification. Ideally, following numerical analyses, strains should be chosen to represent the whole range of variation within defined clusters and examined using the more analytical techniques that cannot readily be applied to large numbers of strains. Chemical, nucleic-acid pairing, serological and phage host-range studies have all been successfully used to evaluate numerical taxonomies of actinomycetes (Goodfellow and Cross, 1984). This multifaceted or polyphasic approach to classification has been most rigorously applied to the genera *Mycobacterium*

Table 4. Examples of recent numerical phenetic surveys of actinomycetes and aerobic endospore-forming bacteria

Taxa	Data matrix		Test error (%)	References
	Strains	Tests		
Aerobic endospore-forming bacteria				
Bacillus	47	108	ND	Pichinoty, Garcia and Mandel (1980)
Bacillus	600	139	1·3	Logan and Berkeley (1981)
Bacillus	89	107	ND	Sheard and Priest (1981)
Bacillus	338	118	ND	Priest, Goodfellow and Todd (1981)
Bacillus coagulans and *B. stearothermophilus*	74	116	ND	Garcia *et al.* (1982)
Bacillus sphaericus	35	160		de Barjac, Véron and Dumanoir (1980)
Actinomycetes				
Actinomadura	156	90	4·5	Goodfellow, Alderson and Lacey (1979)
Actinomadura and *Nocardiopsis*	170	120	4·9	Athalye *et al.* (1985)
Actinomyces	222	124	1·6	Schofield and Schaal (1981)
Mycobacterium	89	118	ND	Meissner *et al.* (1974)
Mycobacterium	66	140	ND	Wayne *et al.* (1971)
Mycobacterium	54	81	ND	Wayne *et al.* (1978)
Mycobacterium agri	165	104	ND	Tsukamura (1981)
Mycobacterium farcinogenes and *M. senegalense*	82	96	2·5	Ridell and Goodfellow (1983)
Nocardia	197	137	7·1	Orchard and Goodfellow (1980)
Nocardia	396	107	4·0	Hookey (1983)
Nocardia amarae	123	92	1·5	Goodfellow *et al.* (1982)
Renibacterium salmoninarum	56	86	0·4	Goodfellow, Embley and Austin (1985)
Rhodococcus	177	92	3·7	Goodfellow and Alderson (1977)
Rhodococcus coprophilus	36	78	3·8	Rowbotham and Cross (1977a)
Rhodococcus equi	205	160	2·9	Goodfellow, Beckham and Barton (1982)
Rhodococcus globerulus	130	92	2·5	Goodfellow, Weaver and Minnikin (1982)
Streptomyces	475	139	3·4	Williams *et al.* (1983a)
Streptoverticillium	111	185	3·2	Locci *et al.* (1981)
Thermomonospora	113	101	1·1	McCarthy and Cross (1984)

ND: not determined

(Goodfellow and Wayne, 1982), *Nocardia* and *Rhodococcus* (Goodfellow and Minnikin, 1981). Statistical procedures are now being developed for comparing quantitative data obtained by applying different taxonomic methods to common strains (Sneath, 1983). In an interesting preliminary study (Mordarski *et al.*, 1986), partial congruence was found between numerical phenetic and DNA homology values obtained with a common set of streptomycete strains.

COMPUTER-ASSISTED IDENTIFICATION

The lack of objective criteria for the differentiation of microbial taxa makes it difficult to choose balanced sets of strains for industrial screening programmes and genetic engineering. The problem is partly a historical one as the need to identify unknown bacteria has rarely been seen as a distinctive and major task for industrial microbiologists. Consequently, current practice mainly consists of a tangled web of more or less useful diagnostic techniques with little evidence of any rational design. This situation contrasts markedly with that faced by the diagnostic medical or veterinary bacteriologist.

Identification of actinomycetes can be seen as a twofold procedure. Reliable criteria are needed to assign organisms to the genus level and appropriate diagnostic tests are required for identification to individual species. A combination of chemical and morphological properties has been recommended for assigning unknown strains to certain actinomycete genera (Minnikin, Goodfellow and Collins, 1978; Lechevalier and Lechevalier, 1980, 1981; Minnikin and Goodfellow, 1980; Schaal, 1985) but few well-tested schemes are available for the differentiation of species. There is also considerable room for improvement in the identification of aerobic endospore-forming rods. Classical test systems using few characters do not usually allow identification of atypical and intermediate strains, in spite of the excellent work of Gordon and her colleagues (Smith, Gordon and Clark, 1952; Gordon, Haynes and Pang, 1973).

It is not always appreciated that sound classifications with high information contents are needed for the construction of accurate identification schemes. One of the advantages of conventional numerical taxonomy is that it provides a pool of quantitative data on the test reactions of the strains within each of the defined clusters. This is usually expressed as the percentage of strains within each cluster which show a positive state for each character used to construct the classification. When classification is complete, data bases can be trawled and presumptive diagnostic characters abstracted. Presumptive diagnostic tests shown to be reproducible can then be used (a posteriori weighting) to construct dichotomous keys, diagnostic tables and computerized identification matrices.

Computer-assisted probabilistic identification schemes have many advantages over the widely used monothetic sequential keys and diagnostic tables which are very susceptible to test error (Sneath, 1974). Numerical taxonomies are now being used to construct identification matrices (*see* Goodfellow, Jones and Priest, 1985), which contain the minimum number of characters

needed to discriminate between taxa. Once formed, the matrices can be used for the probabilistic identification of unknown strains. Surprisingly few numerical classifications of bacteria have been supported by probabilistic identification schemes, possibly because of the extensive work associated with reproducibility studies (Wayne *et al.*, 1976). However, theoretically sound, workable computer-assisted procedures based on numerical classifications are available for the identification of slow-growing mycobacteria (Wayne *et al.*, 1980), streptomycetes (Williams *et al.*, 1983b), streptoverticillia (Williams *et al.*, 1985) and vibrios (Dawson and Sneath, 1985), and for bacteria isolated from Alaskan outer continental shelf regions (Davis, Atlas and Krichevsky, 1983). Probabilistic identification schemes have also been constructed using data less comprehensive than those provided by numerical classifications. They include systems for the identification of *Bacillus* (Willemse-Collinet, Tromp and Huizinga, 1980) and *Micrococcus* species (Feltham and Sneath, 1982). Improved identification systems based on numerical taxonomies are available for actinobacteria (Seiler, 1983) and nearly all of the recognized species of *Bacillus* (Logan and Berkeley, 1984).

The theoretical basis of numerical identification and the use of this method to identify streptomycetes have been reviewed in detail elsewhere (Hill, 1974; Willcox, Lapage and Holmes, 1980; Williams *et al.*, 1984; Williams, Vickers and Goodfellow, 1985). The streptomycete probabilistic matrix was based on 23 clusters which included all of the major clusters and *Streptomyces fradiae*, a well-known source of antibiotics. Forty-one characters found to be most diagnostic for these taxa (*Table 5*) were abstracted from the data base containing the 139 tests used to construct the numerical classification (Williams *et al.*, 1983b). The rather high number of tests required reflected the variation within the clusters and the necessity of having at least as many tests as taxa in the matrix (Sneath and Chater, 1978). Identification of strains against the matrix was achieved using three coefficients from the MATIDEN program (Sneath, 1979). The three coefficients were Willcox probability (Willcox *et al.*, 1973), taxonomic distance and the standard error of taxonomic distance:

1. *Willcox probability*. This is the likelihood of unknown character-state values against a particular group divided by the sum of the likelihoods against all groups; the closer the score is to 1, the better is the fit;
2. *The taxonomic distance*. This gives the distance of an unknown from the centroid of the group with which it is being compared: a low score indicates relatedness to the group, and ideally it is less than about 0·15;
3. *Standard error of taxonomic distance*. This is based on the assumption that groups are in hyperspherical normal clusters. An acceptable score is less than about 2·0–3·0, and about half the members of a taxon will have negative scores, that is, they are closer to the centroid than average.

The criteria adopted for a successful identification were:

1. A Willcox probability greater than 0·850 with low scores for taxonomic distance and its standard error;

Table 5. Characters selected for construction of the *Streptomyces* identification matrix

Type of test	*Tests and unit characters
Antibiosis	Inhibition of: *Aspergillus niger*, soil isolate; *Bacillus subtilis* NCIB 3610; *Streptomyces murinus* ATCC 19788
Antibiotic inhibition	Neomycin sulphate Rifampicin
Degradation	Allantoin Arbutin Hydrogen sulphide production Lecithinase production Nitrate reduction Pectin Xanthine
Morphological	Aerial spore mass colour—green, grey or red Fragmentation of substrate mycelium Spore chain shape—biverticillate, rectus-flexibilis, retinaculum apertum or spiral Substrate pigment colour—red/orange or yellow/brown Melanin production
Nutritional	Utilization of sole carbon sources—adonitol, cellobiose, fructose, inositol, inulin, mannitol, raffinose, rhamnose and xylose Utilization of sole nitrogen sources—α-aminobutyric acid, L-histidine and L-hydroxyproline
Physiological	Growth in presence of: phenol (0·1%,w/v), sodium azide (0·01%, w/v) and sodium chloride (7%, w/v) Growth at 45°C

*See Williams *et al.* (1983b) for details of tests

2. A first group score significantly better than those against the next best two alternatives;
3. A small number of characters of the unknown listed as being atypical of those of the group in which it is placed.

An example of the print-out provided by the MATIDEN program is given in *Table 6*. Similarly, examples to illustrate the range of scores obtained for identified and non-identified isolates are shown in *Table 7*.

The probability matrix and identification program have been successfully used to identify unknown streptomycetes from soil (Williams *et al.*, 1983b; Williams, Goodfellow and Vickers, 1984), fresh water (Stanton, 1984) and marine habitats (Goodfellow and Haynes, 1984). To date, over 70% of isolates tested have been identified to one of the major clusters in the matrix (Williams, Vickers and Goodfellow, 1985). The computer-assisted identification procedure, like its counterparts for slow-growing mycobacteria and streptoverticillia, provides a practical and reliable way of identifying unknown isolates.

Table 6. Example of the output provided by the MATIDEN program to identify an unknown strain against the identification matrix

INPUT THE CHARACTER VALUES OF THE UNKNOWN
REFERENCE NUMBER OF UNKNOWN IS S149
ISOLATE S149 BEST IDENTIFICATION IS S. ALBIDOFLAVUS
SCORES FOR COEFFICIENT: 1 (Willcox probability), 2 (Taxonomic distance),
3 (Standard error of taxonomic distance)

	1	2	3
S. ALBIDOFLAVUS	0·998	0·382	1·233
S. EXFOLIATUS	$0{\cdot}215 \times 10^{-4}$	0·438	2·589
S. ATROOLIVACEUS	$0{\cdot}627 \times 10^{-5}$	0·439	3·657

ADDITIONAL CHARACTERS THAT ASSIST IN SEPARATING S. ALBIDOFLAVUS FROM:

S. EXFOLIATUS: NONE
S. ATROOLIVACEUS: NONE

Table 7. Examples of identification scores for unknown strains isolated from marine habitats

Isolate number	Cluster identification	Identification scores		
		Willcox probability	Taxonomic distance	Standard error of taxonomic distance
25	*Streptomyces albidoflavus*	0·998	0·37	0·75
148	*Streptomyces albidoflavus*	0·998	0·39	1·23
64	*Streptomyces griseoflavus*	0·997	0·38	0·38
52	*Streptomyces chromofuscus*	0·996	0·36	0·78
65	*Streptomyces rochei*	0·987	0·34	0·11
41	*Streptomyces diastaticus*	0·984	0·34	−0·58
78	*Streptomyces albidoflavus*	0·976	0·37	0·85
146	*Streptomyces atroolivaceus*	0·943	0·36	1·41
239	Not identified	0·792	0·46	4·15
246	Not identified	0·584	0·48	3·16
57	Not identified	0·470	0·46	5·12

FORMULATION AND EVALUATION OF SELECTIVE MEDIA

Most selective isolation media have been developed on an empirical basis without regard either to the nutritional and growth requirements or to the tolerances of the organisms under investigation (Williams, Goodfellow and Vickers, 1984). Semi-defined media containing supplements such as peptone and yeast extract for soil bacteria (Goodfellow, Hill and Gray, 1968) or beef extract and peptone for aerobic endospore-forming bacilli (Norris *et al.*, 1981) are widely used to provide growth factors, but innumerable more ill-defined and bizarre supplements have also been recommended. These include canned tomato juice for lactobacilli (Yoshizumi, 1975), hay extract for facultatively anaerobic spirochaetes (Canale-Parola, 1981), paraffin wax

for nocardiae (Gordon and Hagan, 1936) and sheep dung extract for thermophilic streptomycetes (Tendler and Burkholder, 1961).

The rationale behind the formulation of more defined media such as colloidal chitin (Lingappa and Lockwood, 1962) and starch–casein agars (Küster and Williams, 1964) is not always clear. The selectivity of the widely used colloidal chitin was considered to rest on the virtual universal ability of streptomycetes to hydrolyse this compound (Hsu and Lockwood, 1975), yet only 25% of the strains included in the numerical phenetic survey on *Streptomyces* were strongly chitinolytic (Williams *et al.*, 1983a). Similarly, starch–casein agar supplemented with sodium chloride (4·6%, w/v) to reduce the growth of spreading Gram-negative bacteria (Mackay, 1977) will not support the growth of streptomycetes unable to tolerate salt at this concentration (Williams *et al.*, 1983a). However, given recent improvements in classification and identification of some bacterial groups, notably streptomycetes, it is now possible to prepare selective isolation media on sound objective criteria and to identify accurately the strains thus isolated.

The multiplicity of approaches to the selective isolation of actinomycetes (Cross, 1982; Williams and Wellington, 1982a,b; Wellington and Cross, 1983) clearly show that differences in the nutritional, physiological and antibiotic-sensitivity profiles of different groups can be exploited for the isolation of particular taxa from natural habitats. Physical characteristics of actinomycetes such as resistance to desiccation, heat and salt also provide grist to the selective isolation mill. Numerical taxonomic data bases which contain extensive information on the biochemical, nutritional and physiological requirements of taxa provide an ideal resource for the formulation of new selective isolation media. Indeed, information abstracted from data matrices has already been successfully used in the isolation of particular actinomycete taxa (*Table 8*), the most comprehensive studies having been carried out on streptomycetes. The emphasis on streptomycetes is not surprising for until recently the inability to identify these organisms below the genus level made it impossible to choose representative strains for screening programmes.

Information in the streptomycete identification matrix (Williams *et al.*, 1983b) has been used to design and evaluate media intended for the isolation of members of the streptomycete community other than those, such as *Streptomyces albidoflavus*, which predominate on colloidal chitin and starch–casein agars. The formulation of the new media was assisted by the application of the DIACHAR program (Sneath, 1980), which selects the most diagnostic characters for individual clusters within the matrix. Not surprisingly, the highest diagnostic scores were given by those characters which were either consistently positive or negative for species of one major cluster when compared with those of all of the other numerically defined taxa studied. As the identification tests included ones for nutritional and tolerance characters, the program highlighted those constituents which could be used in a medium to isolate selectively a particular group of streptomycetes. Initial experiments were designed to select against members of the *Streptomyces albidoflavus* group in the expectation that less-commonly isolated streptomycete species would develop more readily. The data matrix

Table 8. Selective isolation media based on information taken from numerical taxonomic data bases

Taxon	Selective agent	Reference
Actinomadura spp.	Rifampicin	Athalye, Lacey and Goodfellow (1981); Athalye *et al.* (1985)
Faenia rectivirgula	Hippurate	D. Rose, J. Lacey and M. Goodfellow (unpublished data)
Nocardia asteroides	Chlortetracycline; demethylchlortetracycline; methacycline	Goodfellow and Orchard (1974); Orchard and Goodfellow (1974)
Streptomyces diastaticus	Rifampicin	Williams, Goodfellow and Vickers (1984)
Streptomyces chromofuscus, *S. cyaneus*, *S. rochei*	Raffinose, histidine	Vickers, Williams and Ross (1984)
Thermomonospora chromogena	Kanamycin	McCarthy and Cross (1981)

Table 9. Examples of scores included in the percentage positive probability matrix for streptomycete clusters (from Williams *et al.*, 1983b)

	Characters*				
	Utilization of:			Resistance to:	
Clusters*	Raffinose	Xylose	Histidine	Neomycin (50 μg/ml)	Rifampicin (50 μg/ml)
S. albidoflavus	0·17	0·93	0·65	0·01	0·54
S. chromofuscus	0·22	0·99	0·78	0·01	0·33
S. cyaneus	0·99	0·90	0·85	0·01	0·46
S. diastaticus	0·84	0·90	0·68	0·01	0·68
S. fulvissimus	0·89	0·99	0·99	0·89	0·99
S. griseoruber	0·99	0·89	0·99	0·01	0·78
S. griseoviridis	0·51	0·83	0·83	0·01	0·83
S. lavendulae	0·08	0·33	0·08	0·50	0·33
S. platensis	0·82	0·27	0·36	0·18	0·09
S. rochei	0·69	0·96	0·77	0·08	0·89

*Only ten of the 23 species groups and five of the 41 characters included in the full matrix are listed in this Table.

(*Table 9*) showed that a medium based on raffinose and histidine as major carbon and nitrogen sources, respectively, might support reduced numbers of *Streptomyces albidoflavus*, while allowing growth of a variety of other streptomycete species. The isolates obtained on control and test isolation media were identified using the computer probability matrix and identification programs described earlier.

The use of objectively formulated media, and the subsequent identification of isolates, allowed clear qualitative differences to be detected in strep-

Table 10. Computer-assisted identification of streptomycetes isolated from a sample of sand-dune soil using three different selective media (from Williams, Goodfellow and Vickers, 1984)

Species-groups	Percentage of total isolates on: Starch–casein medium	Starch–casein medium + rifampicin (50 μg/ml)	Raffinose–histidine medium
Streptomyces albidoflavus	6·5	13·3	0
S. chromofuscus	2·2	0	0
S. cyaneus	28·3	0	63·6
S. diastaticus	0	80·0	2·3
S. platensis	37·0	0	4·5
S. rochei	8·7	6·6	13·6
Unidentified isolates	17·4	0	15·9

tomycetes isolated from the same soil sample (*Table 10*). Comparison of these results with information in the data matrix (*Table 9*) made it possible to determine whether an observed increase or decrease in numbers of a major cluster on the new media, compared with that on the control, was mainly due to selection by media constituents or to the effects of competition. Thus, from the data matrix, it was evident that all (or nearly all) of the *Streptomyces cyaneus* group were able to use raffinose and histidine, whereas the *Streptomyces albidoflavus* group was only 17% and 65% positive for raffinose and histidine respectively. Competition on the selective isolation plates may account for the reduction in the number of strains of other groups, notably *Streptomyces platensis*, on the raffinose–histidine medium. Conversely, the isolation of large numbers of *Streptomyces diastaticus* strains on the starch–casein medium supplemented with rifampicin suggested that competition had previously excluded this group from both the control and raffinose–histidine media. The results of these and other experiments (Vickers, Williams and Ross, 1984), demonstrate that the concept of a non-selective medium for streptomycetes is not plausible and that several selective media must be used if representatives of the indigenous streptomycete flora are to be isolated from natural habitats.

The ability to isolate and characterize large numbers of unusual streptomycetes has clear implications for industrial screening programmes, especially where it has been shown that particular antimicrobial activities are associated with certain species-groups defined by overall phenetic similarity (*Table 11*). Good examples of this phenomenon are the high activity of members of the *Streptomyces lavendulae* cluster against Gram-negative bacteria and fungi, and the activity of *Streptomyces fulvissimus, Streptomyces griseoviridis* and *Streptomyces platensis* strains against Gram-positive bacteria. Similarly, *Streptomyces cyaneus* and *Streptomyces diastaticus* are notable for their ability to produce β-lactamase, and *Streptomyces chro-*

Table 11. Antimicrobial activity (percentage positive reactions) within some streptomycete species-groups defined by numerical classification (from Williams *et al.*, 1983a)

	S. albidoflavus	*S. chromofuscus*	*S. cyaneus*	*S. diastaticus*	*S. fulvissimus*	*S. griseoruber*	*S. griseoviridis*	*S. lavendulae*	*S. platensis*	*S. rochei*
Inhibition of:										
Aspergillus niger	32	0	10	11	22	0	33	75	100	27
Candida albicans	34	0	3	0	11	0	33	67	27	19
Saccharomyces cerevisiae	32	0	5	0	22	0	5	67	27	15
Escherichia coli	9	0	3	0	0	0	0	83	0	8
Pseudomonas fluorescens	4	0	0	0	0	0	0	50	0	8
Bacillus subtilis	28	11	44	21	78	0	100	92	73	35
Micrococcus luteus	39	11	33	21	89	0	100	83	100	35
Streptomyces murinus	39	22	62	5	100	22	80	100	100	39
Production of:										
β-Lactamase	69	25	87	90	40	22	33	92	40	36
β-Lactamase inhibitor	3	38	0	0	20	11	0	0	0	8

mofuscus for a capacity to provide β-lactamase inhibitors. It is, however, well known that the ability to produce a particular type of antibiotic is not necessarily confined to a single species or genus. It has, for instance, been shown on the basis of recent sensitive screening methods that diverse micro-organisms are able to produce β-lactam antibiotics.

The development and application of new selective media is altering our understanding of the numbers, types and distribution of actinomycetes in natural habitats (Cross, 1981; Orchard, 1981; Goodfellow and Williams, 1983; Goodfellow and Haynes, 1984). Thus, it has been found that nocardiae are common in soil (*Table 12*; Orchard and Goodfellow, 1974; Orchard, Goodfellow and Williams, 1977) and that media containing tetracyclines are useful for studying their distribution and numbers. It is also interesting that many of the nocardiae growing on selective isolation plates cannot be accommodated into established taxa but form distinct numerically defined clusters (Orchard and Goodfellow, 1980; Hookey, 1983). Similarly, large numbers of actinoplanetes (Makkar and Cross, 1982), actinomadurae (Athalye, Lacey and Goodfellow, 1981), and rhodococci (Rowbotham and Cross, 1977b) have been isolated from a variety of natural habitats. An increased ability to relate the distribution of specific antibiotic-producing taxa with particular habitats or microsites should help in the development of more objective approaches to the search for novel antibiotics. At present, very little is known about the geographical or ecological distribution of antibiotic-producing actinomycetes although this is partly because workers rarely provide precise descriptions of the habitats from which they have isolated commercially significant strains (Cross, 1982; Williams, Goodfellow and Vickers, 1984).

Table 12. Dilution plate counts of nocardiae (no./g dry weight × 10^3) on Diagnostic Sensitivity Test Agar supplemented with various antibiotics. Data from Orchard and Goodfellow (1974), Orchard, Goodfellow and Williams (1977) and Hookey (1983)

		Antibiotic combinations*			
Soil	pH	Demethylchlortetracycline (5 μg/ml)	Methacycline (45 μg/ml)	Demethylchlortetracycline (5 μg/ml) + chlortetracycline (45 μg/ml)	Methacycline (5 μg/ml) + chlortetracycline (45 μg/ml)
Sand dune, England	7·5	0·2	0	0	0·1
Arable, England	6·3	13·0	14·0	11·0	2·7
Sandy, Ghana	7·8	2·0	0·5	1·0	0·4
Garden, Mexico	7·2	4·6	0·9	3·6	1·8
Pasture, Mexico	8·0	5·5	1·2	2·7	1·2
Garden, Thailand	6·0	1·2	4·0	3·2	3·2
Garden, Thailand	7·2	2·1	3·2	2·2	1·3
Garden, Venezuela	7·7	73·0	26·0	4·4	0·3

*All of the media contained the antifungal antibiotics actidione (50 μg/ml) and nystatin (50 μg/ml)

DETECTION AND CHARACTERIZATION OF NOVEL TAXA

It is not always realized by industrial microbiologists that a sound classification is needed to prove novelty in its broadest sense. Indeed, novelty is still often perceived as a product of the aims, interests and judgement of the investigator (Williams, Goodfellow and Vickers, 1984). As these vary considerably, so do the criteria invoked to justify novelty. Thus, the isolation of *Thermosulfobacterium commune* (Zeikus *et al.*, 1983) during ecological studies of decomposing algal–bacterial mats associated with hot aquatic habitats in Yellowstone National Park led to the discovery of an organism with a highly unusual combination of biochemical, chemical, physiological and structural properties. Other very unusual bacteria isolated from 'exotic' habitats include *Acetoanaerobium* (Sleat, Mah and Robinson, 1985), *Acidophilus* (Harrison, 1981), *Dictyoglomus* (Saiki *et al.*, 1985), *Erythrobacter* (Shiba and Simidu, 1982), *Methanothermus* (Stetter *et al.*, 1981), *Stella* (Vasilyeva, 1985), *Thermococcus* (Zillig *et al.*, 1983) and *Thermoproteus* (Zillig *et al.*, 1981). At the other extreme, many newly isolated actinomycete species, such as *Dactylosporangium roseum* (Shomura *et al.*, 1985), *Microbispora viridis* (Miyadoh *et al.*, 1985) and *Streptomyces sulfonofaciens* (Miyadoh *et al.*, 1983), differ from established species by little other than their ability to produce a novel secondary metabolite.

An idea of some of the previous and present disparities in the number of species assigned to bacterial genera can be obtained from *Table 13*. The number of species in a genus is partly a measure of its natural diversity, and reflects how comprehensively it has been studied, but is also influenced by the criteria used to define the species and by the objectives of the investigator. Thus, improved classification has led to fewer but better-described species being recognized in the genera *Corynebacterium, Nocardia, Rhizobium* and *Streptomyces*, and extensive taxonomic surveys have resulted in the circumscription of new species of *Actinomyces, Actinoplanes, Azotobacter, Bacillus, Micrococcus, Mycobacterium, Thermomonospora* and *Vibrio*. Similarly, comprehensive taxonomic studies have led to the establishment or redescription of genera such as *Actinomadura, Oerskovia* and *Rhodococcus* (Goodfellow and Cross, 1984).

The introduction of the *Approved Lists of Bacterial Names* (Skerman, McGowan and Sneath, 1980) caused the demise of many bacterial taxa that had been defined by a few subjectively weighted characters, notably staining and morphological features. Recommendation 30b of the *International Code of Nomenclature of Bacteria* (Lapage *et al.*, 1975), which called for the establishment of recommended minimal standards for the definition of new species, was intended to prevent any further proliferation of poorly described species. Although these measures are to be welcomed, the criteria for recognition of species and higher taxa can still be expected to vary between different taxonomists and microbial groups. The philosophy of taxonomists is still reflected in the subdivisions they create ('lumpers' or 'splitters') and the group on which they work (Cowan, 1965).

Table 13. Number of species in selected bacterial genera. Compiled from *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974) and the forthcoming edition of *Bergey's Manual of Systematic Bacteriology*

Genus	1974	1986
Streptomyces	463	142
Clostridium	61	83
Mycobacterium	29	55
Lactobacillus	26	50
Bacillus	48	34
Actinomadura	—	28
Streptococcus	21	25
Vibrio	5	20
Staphylococcus	3	19
Corynebacterium	39	16
Arthrobacter	7	15
Actinoplanes	4	14
Rhodococcus	—	14
Streptoverticillium	11	13
Actinomyces	5	10
Micrococcus	3	9
Nocardia	31	9
Cellulomonas	1	7
Thermoactinomyces	2	7
Azotobacter	4	6
Thermomonospora	2	5
Acetobacter	3	4
Agrobacterium	4	4
Leuconostoc	6	4
Rhizobium	6	3
Oerskovia	—	2
Frankia	10	1
Sporolactobacillus	1	1

Procedures used to distinguish new from established taxa must be based on sound taxonomic principles. Numerical phenetic surveys of restricted groups of bacteria have resulted in the discovery of many new centres of variation (*see* Goodfellow and Dickinson, 1985). This approach has, for instance, led to the discovery of new actinoplanetes (Stanton, 1984), mycobacteria (*see* Goodfellow and Wayne, 1982), nocardiae (Orchard and Goodfellow, 1980; Hookey, 1983), rhodococci (Rowbotham and Cross, 1977a), thermomonosporas (McCarthy and Cross, 1984) and wall chemotype IV taxa lacking mycolic acids (Goodfellow, Alderson and Lacey, 1979).

Numerical taxonomy has also revealed that acidophilic streptomycetes, which grow between pH 3·5 and 6·5, but not at pH 7·0, are a diverse group. Thus, in a study based on the simple matching coefficient and average linkage clustering, over 200 representative strains were assigned to 11 major (64% of the strains), 20 minor (26% of the strains) and 10 single-member clusters (Lonsdale, 1985). The low test error, high cophenetic correlation values and good congruence between classifications based on different resemblance coefficients indicated that confidence could be placed in the numerical

classification. Information from the data base has been used to devise and evaluate media designed to isolate specific fractions of the acidophilic streptomycete community from natural habitats. These organisms are common in natural and man-made acidic soils (Williams *et al.*, 1971; Khan and Williams, 1975). Acidophilic streptomycetes are probably active in decomposition processes in such soils and their exo-enzymes, chitinases and diastases, are adapted to function at a lower pH than those from neutrophilic streptomycetes (Williams and Flowers, 1978; Williams and Robinson, 1981).

Rapid and reliable chemical techniques are widely employed in bacterial systematics (*see* Goodfellow and Minnikin, 1985). This is particularly true of the actinomycetes, for which the most useful chemical information has been derived from analyses of wall amino-acid and sugar composition and whole-organism lipid patterns (*Table 3*). Indeed, for many actinomycete genera, details of their wall diamino acid and lipid composition is an integral part of the generic description (Goodfellow and Cross, 1984). Most chemosystematic studies have been restricted to a visual comparison of quantitative data generated by analytical techniques such as gas chromatography and high performance liquid chromatography. To date, chemical markers have proved to be most useful in the classification of actinomycetes at generic and supra-generic levels but analysis of quantitative data, by appropriate cluster analysis techniques, has given valuable information for the characterization of species and subspecies (O'Donnell, 1985). It is essential in such quantitative studies to know that the chemical data are suitable for multivariate analysis (Saddler *et al.*, 1986). Preliminary studies suggest that the examination of fatty acid profiles using a microcomputer-based package called SIMCA may provide a quick and accurate way of detecting novel streptomycetes (O'Donnell, 1986).

Statistically, the characterization and detection of novel isolates is primarily a matter of pattern recognition in which the unknown fatty acid profile is compared with a library of known profiles and its similarity or dissimilarity determined. SIMCA (Soft Independent Modelling of Class Analogy), a commercially available package (SEPANOVA AB, Enskede, Sweden), uses the well-established statistical technique of principal components analysis (Gower, 1966) to identify unknown isolates. The basis of the SIMCA method of identification is to describe each taxon or group of strains by a separate principal components model. An advantage of the SIMCA approach over some discriminant analyses, such as canonical variates, is that it does not assume equal interspecies homogeneity and thereby accommodates the different degrees of homogeneity found within microbial taxa. The allocation of unknown profiles to each class model is determined by linear multiple regression. Using this procedure, successful identification depends on the residual standard deviation, obtained when the unknown is tested against a given principal components model, being within the known standard deviation of the test class or taxon. Samples which are not assigned to any of the class models used to describe a particular taxon may belong to hitherto undiscovered classes and therefore constitute novel isolates. This strategy could be incorporated into screening programmes designed to select novel isolates (*see* O'Donnell, 1986).

OTHER TAXONOMIC CRITERIA

Most substrates require some form of treatment before their incorporation into selective isolation media. Actinomycete spores are relatively resistant to desiccation so that simply air-drying soil samples will significantly enhance the chances of recovering spore-forming taxa. Resistance to desiccation is often accompanied by some degree of resistance to heat and dry soils can be heated to over 100°C to reduce the numbers of unwanted bacteria. Less severe heat treatments have been successfully used to isolate a variety of actinomycete genera (*Table 14*) although the basis of this practice is not clearly understood. It appears, however, that many actinomycete propagules, both spores (e.g. streptomycetes) and hyphal fragments (e.g. rhodococci), are more resistant to heat than vegetative cells of Gram-negative bacteria. It may be possible to design taxon-specific heat pretreatment regimes as some actinomycetes vary with respect to their heat-sensitivity profiles (Haynes, 1982; Goodfellow and Simpson, 1986). The endospores of *Bacillus* species survive mild heat treatments but the subsequent use of isolation media lacking peptone or the amino acids necessary to trigger germination can help to restrict the number of their colonies on isolation plates (Cross, 1982). Pasteurization at 80°C for 10 minutes, or some similar temperature and time, is a standard initial step in the selection of aerobic endospore-forming bacilli.

Selective isolation of actinomycetes: ground rules

CHOICE OF SUBSTRATE

Soil is the primary reservoir of most actinomycetes (Williams, Lanning and Wellington, 1984). Streptomycetes tend to predominate, but actinomadurae, arthrobacters, micromonosporas, nocardiae and rhodococci are common in most soils. Actinomycetes can also be isolated readily from freshwater (Cross, 1981) and marine environments (Goodfellow and Haynes, 1984) and from more defined habitats such as root nodules of *Comptonia* (Callaham, Tredici and Torrey, 1978) and the gut microflora of *Bibio marci* larvae (Szabo *et al.*, 1967). Samples may be taken at random or from habitats where the microbial community is adapted to relatively extreme environmental factors. In order to obtain new strains likely to produce novel metabolites it is advisable to examine samples from diverse habitats and to use media and incubation conditions which will facilitate the isolation of acidophilic, alkalophilic, neutrophilic, mesophilic, psychrophilic, thermophilic and osmophilic strains. To date, the screening of anaerobic actinomycetes has received little attention (Bull, Ellwood and Ratledge, 1979).

PRETREATMENT OF MATERIAL

In order to isolate actinomycetes from natural habitats it is necessary to eliminate or reduce fungal and bacterial growth on selective isolation media

Table 14. Examples of heat pretreatment of material for isolation of actinomycetes

Treatment	Material	Media	Target organism(s)	Reference
120°C for 1 h	Air-dried soil	AV agar	*Microbispora* and *Streptosporangium* spp.	Nonomura and Ohara (1969)
100°C for 1 h	Air-dried soil	C2 and MGA-SE agars	*Microbispora* and *Microtetraspora*	Nonomura and Ohara (1971)
40°C for 2–16 h	Soil and roots	Starch–casein agar	*Streptomyces* spp.	Williams *et al.* (1972)
55°C for 6 min	Water, soil and dung	M3 agar	*Rhodococcus coprophilus*	Rowbotham and Cross (1977b)
55°C for 6 min	Soil	Diagnostic Sensitivity Test Agar (Oxoid) + methacycline	*Nocardia asteroides*	Orchard (1978)
100°C for 15 min	Air-dried soil	Glucose yeast extract agar + rifampicin	*Actinomadura* spp.	Athalye, Lacey and Goodfellow (1981)
60°C for 40 min	Soil	Cellulose asparagine seawater agar + novobiocin	*Micromonospora*	Goodfellow and Haynes (1984)

without adversely affecting the target organisms. This is usually achieved by applying appropriate selective pressures at various stages of the dilution plate procedure (Williams and Wellington, 1982a). Fungal contaminants can usually be eliminated by supplementing isolation media with antifungal antibiotics such as actidione, nystatin and pimaricin (Williams and Davies, 1965).

Selectivity may be enhanced by chemical or physical pretreatment of material or propagules in suspensions prior to plating on to selective isolation media (*Table 15*). Membrane filtration and centrifugation have been used to concentrate actinomycete propagules in soil, water and sediment samples (Trolldenier, 1966; Goodfellow and Haynes, 1984) and nutrient enrichment of material *en masse* has been employed to increase the numbers of streptomycetes prior to isolation (Williams and Mayfield, 1971). A further departure from the dilution plate technique has been to isolate thermophilic actinomycetes from dry, self-heating plant material. The latter is shaken in a wind tunnel (Gregory and Lacey, 1963; Lacey, 1971; Lacey and Dutkiewicz, 1976a) or sedimentation chamber (Lacey and Dutkiewicz, 1976b) and the spore cloud impacted on to plates of surface-dried medium in an Andersen (1958) sampler. As this latter procedure has rarely been used in selective isolation work it is described in detail here.

The Andersen sampler consists of six aluminium stages that are held together by three strong springs which clamp the sampler together and seal with O-ring gaskets. Each of the stages has a section containing 400 holes for the intake of air. The holes become progressively smaller at each stage: they range from 0·118 cm at the top to 0·0254 cm at the bottom (*Figure 1*). Each stage holds a Petri dish to collect the spores. Unimpacted particles flow around the Petri dish and into the next stage.

Table 15. Examples of physical and chemical pretreatments for isolation of streptomycetes (from Goodfellow and Simpson, 1986)

Treatment	Material	Predominant isolates
Physical:		
Agitation in sedimentation chamber	Mouldy hay	Thermophilic streptomycetes
Centrifugation	Seawater and mud	*Streptomyces* spp.
Membrane filtration	Soil	*Streptomyces* spp.
	Water	*Micromonospora* and *Streptomyces* spp.
Chemical:		
Ammonia and sodium hypochlorite	Water	*Streptomyces* spp.
Chloramine	Water	*Micromonospora* and *Streptomyces* spp.
Calcium carbonate to increase pH with incubation	Soil	*Streptomyces* spp.
Nutrient enrichment with chitin and incubation	Soil	*Streptomyces* spp.
Phenol	Soil and water	*Streptomyces* spp.

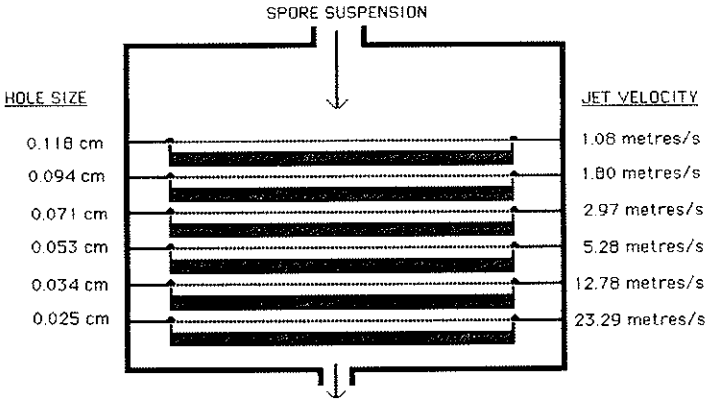

Figure 1. Andersen sampler.

Soil or compost samples are dried overnight and placed in a muslin bag suspended in a sedimentation chamber (*Figure 2*). The bag is agitated vigorously to produce an aerial suspension of spores and small particles from the sample. Overnight drying of the samples improves the development of the aerial suspension and decreases the counts of non-sporing organisms. The aerial suspension of spores is mixed thoroughly with an electric fan, and is then allowed to sediment slowly to separate the spore types. The air from the sedimentation chamber is then withdrawn and passed through the Andersen sampler at the rate of one cubic foot per minute. Typical sampling times are 10–30 seconds. As air is drawn through the sampler, a jet from each of the 400 holes is directed on to the surface of the agar medium. The size of the holes, and hence the jet velocity, is constant for each stage but as the holes become smaller at each stage, the jet velocity increases. A spore will leave the air stream and become impinged on the surface of the agar

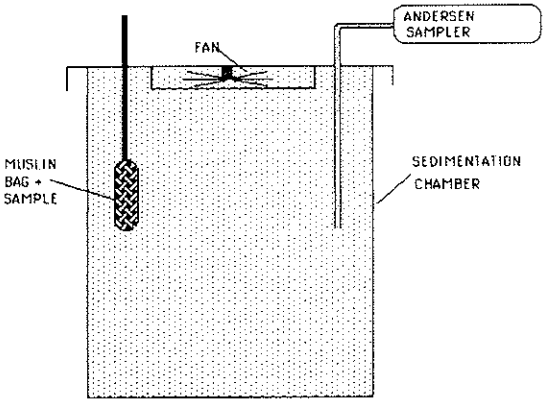

Figure 2. Sedimentation chamber of Andersen sampler containing a spore suspension.

medium when the velocity is sufficiently great to allow it to overcome the aerodynamic drag; otherwise, it remains in the air stream and proceeds to the next stage. All particles of 1 μm or more may be collected by the sampler. The plates are removed, inverted in their lids and incubated. Colonies may be counted by direct observation or by using a microscope. Alternatively, on plates containing many colonies the 'positive hole' method may be used. This is a count of the jets which delivered viable spores to the plates. It is based on the assumption that, as the number of viable spores increases, the probability of a spore passing through an unused hole decreases. The viable count is determined from a 'positive hole' probability table, based on the formula:

$$P^r = N\left[\frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + \ldots \frac{1}{N-r+1}\right]$$

where P_r is the expected number of viable spores to produce r positive holes and N is the total number of holes per stage.

If several hours are allowed for sedimentation, and the sampling times are short (10–20 seconds), fairly clean and easily counted plates are usually obtained. Alternatively, using the 'positive hole' method and counting micro-colonies before they merge, counts as high as 4×10^4 can be made per sample (Andersen, 1958). Although equating counts obtained from the Andersen sampler to actual numbers of spores in soils or composts is problematical, the Andersen sampler is a good method for the collection and separation of spores from these environments and for the isolation of novel types of spore-forming bacteria.

GROWTH ON LABORATORY MEDIA

Most actinomycetes are outgrown by other inhabitants of the microbial community when soil dilutions are plated out on to the surface of standard isolation media such as peptone–yeast extract agar. However, many isolation media are available which will allow the growth of actinomycetes but will discourage that of other bacteria. It has already been demonstrated that the sensitivity of an isolation medium can be influenced by its nutrient composition, its pH and by the addition of selective inhibitors. Incorporation of antifungal antibiotics such as actidione and nystatin into isolation media is essential when dealing with most soils, and such antibiotics are not active against actinomycetes. It is advisable to dry isolation plates, as free moisture on the surface of agar encourages the growth and spread of Gram-negative bacteria which can significantly reduce the germination and subsequent outgrowth of actinomycete spores. It is also good practice to dry the agar surface further when the spread-plate technique is used. It should also be remembered that absolute selectivity is rarely achieved.

INCUBATION

Actinomycete isolation plates are usually incubated at 25–30°C under aerobic conditions. Thermophilic actinomycetes are isolated at 45–55°C and psychrophilic strains at 4–10°C. Little attention has been paid to the selective isolation of psychrophilic and anaerobic taxa. The major variable is the length of the incubation period before colony selection. Commonly isolated mesophilic taxa, such as *Micromonospora* and *Nocardia*, can be selected at 7–14 days; common thermophilic strains, such as *Faenia rectivirgula*, require only 1 to 2 days. There is some evidence that novel or unusual isolates may be overlooked unless longer periods of incubation are used. Thus, Nonomura and Ohara (1971) discovered several new taxa by incubating plates at 30° and 40°C for up to one month. Goodfellow and Haynes (1984) isolated actinomycetes from marine habitats after incubation at 18°C for up to 10 weeks.

COLONY SELECTION

The final, but frequently the most critical, stage in the isolation of an actinomycete is its recognition and transfer to axenic culture. When selective isolation media are used the target organism(s) can often be identified tentatively by microscopic examination of the isolation plate using a high-power objective with a long working distance. However, it is rarely possible to distinguish between different species of the same genus on isolation plates. In such instances the consequent selection of large numbers of colonies can sometimes lead to considerable duplication and wasted effort in screening programmes. This can be overcome by overlaying isolation plates with appropriate sensitive strains or by the use of replica-plating techniques which allow colonies to be studied against a battery of sensitive test organisms. Such strategies have their limitations and there is still a need for more efficient methods for choosing colonies which produce interesting metabolites.

Isolation of methylotrophs and C_1-utilizing autotrophs

Methylotrophs are defined by Anthony (1982) as 'those micro-organisms able to grow at the expense of reduced carbon compounds containing one or more carbon atoms but containing no carbon–carbon bonds'. Reduced C_1 compounds are widespread in nature and are produced by a variety of mechanisms, including biological, photochemical and anthropogenic (*Table 16*). Two groups are recognized: obligate methylotrophs, which grow only on reduced C_1 compounds, and facultative methylotrophs, which are able to grow on reduced C_1 compounds and also on a variety of multicarbon compounds. The definition of an autotroph has changed with time and under pressure from these organisms which are able to grow autotrophically, heterotrophically or methylotrophically according to the carbon substrate present in the growth medium. One definition is 'an organism able to grow with

Table 16. Distribution of reduced C_1 compounds in nature (from Anthony, 1982; Kim and Hegeman, 1983; Large, 1983)

C_1 compound	Chemical formula	Occurrence
Methane	CH_4	Produced by methanogenic bacteria in anaerobic environments, e.g. lakes, paddy fields
Methanol	CH_3OH	Decomposition of lignin, hemicellulose and pectin; photooxidation of methane
Formaldehyde	HCHO	Oxidation of methanol; waste from chemical processing
Formate	HCOOH	Oxidation of formaldehyde; mixed acid fermentation; tanning and rubber processing
Formamide	$HCONH_2$	Industrial waste
Cyanide	CN	Produced by plants, bacteria and fungi; industrial waste
Carbon monoxide	CO	Produced biologically by animals, bacteria, algae and fungi; incomplete combustion processes; photochemical oxidation of organic matter
Methylated amines	CH_3NH_2; $(CH3)_2NH$; $(CH_3)_3N$; $(CH_3)_4N^+$	Decomposition of fish; industrial waste
Trimethylamine *N*-oxide	$(CH_3)_3NO$	Decomposition of fish and invertebrates
Dimethyl sulphide; sulphoxide; sulphone	$(CH_3)_2S$; $(CH_3)_2SO$; $(CH_3)_2SO_3$	Plants and algae
Dimethyl ether	$(CH_3)_2O$	Photochemical oxidation of methane

CO_2 as its major carbon source, using light or the oxidation of reduced inorganic compounds as energy source'. However, the distinctions between autotrophs and methylotrophs are not always clear. For example, some organisms such as *Methylococcus capsulatus* (Taylor, Dalton and Dow, 1981) may assimilate carbon as formaldehyde or CO_2 (via the ribulosebisphosphate carboxylase cycle), and some autotrophs such as *Rhodopseudomonas* spp. (Hirsch, 1968), *Rhodopseudomonas gelatinosa* (Uffen, 1975, 1976) and '*Rhodopseudomonas rubrum*' (Uffen, 1981) may assimilate reduced C_1 compounds.

Methylotrophs and autotrophs have often been isolated with a specific biotechnological application in mind. The impetus for much of the work in this area came from the commercial desire to produce a cheap and competitive source of single-cell protein, taking advantage of the ubiquitous nature and relative cheapness of methane, methanol and carbon monoxide. These applications have been extensively reviewed (Hamer and Harrison, 1980; Meyer, 1980, 1981; Smith, 1981; Vasey and Powell, 1984). The main characteristics desired in organisms for single-cell protein production are:

1. A high yield coefficient on the chosen substrate (mass of dry microbial cells or biomass produced per unit mass of substrate utilized);

2. A high growth rate;
3. A high optimum growth temperature (over 40°C for fermentation);
4. A high affinity for the substrate;
5. Ability to grow at high cell densities;
6. No expensive growth factor requirement;
7. Stable growth in continuous culture;
8. High nutritional value.

These requirements have influenced the methods of isolation of methylotrophs and particularly the criteria for selecting organisms from isolation media. The most obvious of these is the selection for high growth rates and the rejection of slow-growing organisms. This has been particularly important as many methylotrophs have been isolated by liquid enrichment culture, a technique that favours the fastest-growing organisms from the sample of natural material used (Whittenbury, Phillips and Wilkinson, 1970).

Other biotechnological applications of methylotrophs include the oxidation of hydrocarbons and their derivatives (Dalton, 1980), electroenzymology and biofuel cells (Higgins *et al.*, 1980), and the overproduction of metabolites such as vitamins, carboxylic acids and amino acids (Tani and Yamada, 1980; Tani, 1985). The main reason for the choice of C_1 compound for these applications is again availability and price. However most C_1 compounds are toxic or inhibitory to those micro-organisms unable to oxidize or assimilate them. They may therefore be used to select large numbers of novel micro-organisms that may be exploited for the production of unusual primary or secondary metabolites.

The taxonomic status of C_1 autotrophic and methylotrophic bacteria has often to be treated with caution as the organisms have rarely been the subject of detailed taxonomic studies. In this review, binomials in inverted commas are not on the *Approved Lists of Bacterial Names* (Skerman, McGowan and Sneath, 1980) and have not been validly published since 1 January 1980. The application of taxonomy to selective isolation of C_1-utilizing micro-organisms is therefore not possible at present. Methylotrophs are a physiologically related group of micro-organisms with special properties. They have mostly been isolated on C_1 substrates and do not easily fit into previously characterized groups. The obligate methylotrophs have very few properties that may be used in conventional numerical taxonomic studies (Byrom, 1981; Green and Bousfield, 1981). In addition, there is a wide variety of micro-organisms able to utilize C_1 compounds, and a great biological and biochemical diversity amongst these microbes (*Tables 17* and *18*). Some of these methylotrophs belong to genera of importance in biotechnology. Methods for the isolation of methylotrophs on various C_1 compounds are described below.

METHANE

Methanotrophs are a specialized group of micro-organisms able to grow on methane. Some methanotrophs are able to grow on methanol or dimethyl

Table 17. Bacteria and yeasts able to grow on methane (methanotrophic)

Organisms		Growth on methanol
Obligate Gram-negative bacteria		
Genus: *Methylococcus*	G + C content 62–64%	
Methylococcus capsulatus		–
'Methylococcus minimus'		–
Methylococcus mobilis		–
Methylococcus thermophilus		–
Genus *Methylomonas*	G + C content 50–54%	
Methylomonas methanica		+
'Methylomonas albus'		+
'Methylomonas streptobacterium'		–
'Methylomonas agile'		+
'Methylomonas rubrum'		+
'Methylomonas rosaceus'		–
Genus: *'Methylobacter'*	G + C content 50–54%	
'Methylobacter bovis'		–
'Methylobacter capsulatus'		–
'Methylobacter chroococcum'		–
'Methylobacter vinelandii'		–
Genus: *'Methylosinus'*	G + C content 62·5%	
'Methanomonas methanooxidans'		–
'Methylosinus sporium'		–
'Methylosinus trichosporium'		+
'Pseudomonas methanica'		+
Genus: *'Methylocystis'*	G + C content 62·5%	
'Methylocystis parvus'		–
'Methylovibrio sohngenii'		–
Facultative Gram-negative bacteria		
Genus: *Methylobacterium*	G + C content 58–66%	
'Methylobacterium ethanolicum'		–
'Methylobacterium hypolimneticum'		–
Methylobacterium organophilum		+
Methylobacterium strain R6		+
Gram-positive bacteria		
Mycobacterium sp.		ND
Nocardia sp.		ND
Yeasts		
Rhodotorula glutinis		–
Rhodotorula rubra		–
Sporobolomyces gracilis		–
Sporobolomyces roseus		–

ND: not determined

Table 18. Gram-positive facultative methylotrophs unable to grow on methane

Organism	Growth on methanol	Growth on methylamine
'Arthrobacter rufescens'	+	+
Arthrobacter globiformis SK-200	−	+
Arthrobacter globiformis B-175, B-126, B-53	−	+
Arthrobacter strains 1A1, 1A2, 2B2	+	+
Arthrobacter strain P1	−	+
Bacillus cereus M-33-1	+	−
Bacillus strain PM6	−	+
Bacillus strain S2A1	−	+
'Brevibacterium fuscum' 24	−	+
Mycobacterium vaccae	+	+
Streptomyces strain 239	+	ND

ND: not determined

ether. Unlike most of the C_1 compounds, methane is non-toxic and therefore exerts no selective pressure. Organisms unable to use methane can often outgrow methanotrophs on agar medium containing no added carbon source. Direct isolation on plates is therefore difficult and liquid enrichment procedures have mainly been used.

Before 1970 it was considered extremely difficult to isolate methanotrophs and only four strains had been described: '*Bacillus methanicus*' (Söhngen, 1906), reisolated and renamed as '*Pseudomonas methanica*' by Dworkin and Foster (1956); '*Methanomonas methanooxidans*' (Brown, Strawinski and McCleskey, 1964); '*Pseudomonas methanitrificans*' (Davis, Coty and Stanley, 1964), and *Methylococcus capsulatus* (Foster and Davis, 1966). In 1970, Whittenbury, Phillips and Wilkinson isolated over 100 strains of obligate methanotrophs from samples of mud and water, taken from ponds, rivers, streams and ditches, and soil samples from various habitats. Approximately 1 g of the material was added to 25 ml of mineral salts minimum medium (pH 6·8) containing either ammonia or nitrate as nitrogen source in a 250 ml bottle, sealed with a Suba-seal top. Methane (20 ml) was injected with a hypodermic syringe and the liquid enrichments were incubated statically at 30, 45 and 55°C. After 3–4 days a surface pellicle formed and the flasks became turbid. Other workers had previously described the successful development of enrichment cultures, but had experienced great difficulty in isolating pure cultures from the primary enrichment flasks (Vary and Johnson, 1967). Turbid cultures were serially diluted in sterile tap water and spread on to mineral salts agar plates, then incubated in methane:air mixtures (approximately 30:70 v/v) in vacuum desiccators or 'Tupperware' polythene containers. Methanotrophs appeared after 5–7 days and continued to increase in size over 2–3 weeks. Colonies unable to utilize methane reached their maximum size in 3 days. The counts of colonies unable to use methane, and

presumably using dissolved organic materials in the agar, were often 10–100-fold more than counts of methanotrophs. Isolates were transferred to mineral salts plates at the small-colony stage (0·2 mm diameter) using a straight wire and a plate microscope. The ability to use methane was determined by analysing gas samples at intervals.

The organisms were classified into five genera (*Methylobacter, Methylococcus, 'Methylocystis', Methylomonas* and *'Methylosinus'*) on the basis of morphology, fine structure, the type of resting stage formed (either exospore or cyst), and subgrouped on other properties, such as growth temperature, growth on methanol, motility, and enhancement of growth on methane by the addition of yeast extract, malate, acetate, or succinate. All of the isolates are Gram-negative rods or cocci, catalase- and oxidase-positive, strictly aerobic, obligate methanotrophs, utilizing only methane and, in some cases, methanol for growth. The main isolates are listed in *Table 17*. *'Pseudomonas methanica', 'Methanomonas methanooxidans', 'Pseudomonas methanitrificans'* and *Methylococcus capsulatus* have characteristics in common with the obligate methanotrophs of Whittenbury, Phillips and Wilkinson (1970), and are included in *Table 17* in the most appropriate group.

The isolation of facultative methanotrophs has been described, mostly from freshwater lakes and from depths where the oxygen concentration is low (Patt *et al.*, 1974; Patel, Hou and Felix, 1978; Lynch, Wopat and O'Conner, 1980). They are all Gram-negative rods and are able to grow on multicarbon compounds as well as methane (*Table 17*). The ability to grow on methane has often been reported to be an unstable character and a recent report questions the existence of these organisms, as *'Methylobacterium ethanolicum'* H414 was demonstrated to be a mixture of an obligate methanotroph, similar to *'Methylocystis'* sp. and a *Xanthobacter* sp. which grows on methanol and multicarbon compounds (Lidstrom-O'Connor, Fulton and Wopat, 1983).

Whittenbury, Phillips and Wilkinson (1970), commented on their failure to isolate any Gram-positive bacterium or yeast able to utilize methane, although their methods were not specifically designed to isolate these organisms. Seto, Sakayanagi and Lizuka (1975) described a number of Gram-positive bacteria (*Mycobacterium* sp. and *Nocardia* sp.) able to grow on methane, but the descriptions were incomplete and little information has been reported.

Five strains of methane-utilizing yeasts belonging to four species (*Rhodotorula glutinis, Rhodotorula rubra, Sporobolomyces gracilis* and *Sporobolomyces roseus*) have been described in detail (Wolf and Hanson, 1979; Wolf, 1981). They grow very slowly on methane, with a generation time of more than two days, do not grow on methanol, but utilize methylamine, higher alkanes and complex organic compounds such as dicarboxylic acids, alcohols and saccharides. Although the first strain was isolated from an enrichment for methane-utilizing bacteria, an enrichment procedure to select specifically for methanotrophic yeasts was subsequently developed (Wolf, 1981). This consisted of inoculating a rich medium at pH 3·5 with lake samples and incubating at 25°C until turbidity developed. Dilution series

were then performed on filters or plates of ammonia mineral salts medium at pH 6·0, incubated with 70% methane: 10% carbon dioxide: 20% air. Single colonies containing budding cells were transferred and grown under methane to establish pure cultures. Classification of the strains was based on the type of asexual reproduction, the formation of spores and the ability to use various carbon and nitrogen sources (*Table 17*).

Methane is produced anaerobically by methanogenic bacteria and diffuses upwards to the more aerobic zones. Approximately 10^{15} g are produced annually, mainly in paddy fields, swamps and marshes, ruminants, and river and lake muds. Anthropogenic sources are coal mining, lignite mining, automobile exhaust and natural gas wells (Ehhalt, 1976). Although much of the methane produced by methanogenic bacteria is oxidized anaerobically by sulphate-reducing bacteria (Zehnder and Brock, 1979), some reaches the aerobic zones and is oxidized by methanotrophic bacteria. In a freshwater lake these organisms are found on or near the thermocline and may be the main reason for the sudden fall in oxygen concentration at the thermocline (Patt *et al.*, 1974; Large, 1983). Many methanotrophs fix atmospheric nitrogen under reduced oxygen tensions, but this property has not been exploited in isolation procedures.

METHANOL AND METHYLATED AMINES

Growth on methanol is a difficult property to establish and many methanotrophs will grow on methanol only if it is supplied in the vapour phase. However, methanol is produced by the oxidation of methane, and by the hydrolysis of structural components of plants, particularly pectin and lignin, and is therefore widespread in nature. Trimethylamine is produced by the anaerobic degradation of carnitine and lecithin. It is also produced from trimethylamine *N*-oxide during the decomposition of fish. Methylamine is found in some plant material and is produced by the oxidation of dimethylamine, which in turn is produced by the oxidation of trimethylamine (Hanson, 1980).

Methanol and methylated amine-utilizing micro-organisms can be isolated from most samples of soil, water or sewage. More bacteria are able to grow on methylated amines than on methanol. Methylotrophs isolated on methanol can usually grow on methylated amines, but many of those isolated on methylated amines are unable to use methanol (Large, 1981). Liquid enrichment in mineral salts medium containing methanol is the usual method of isolation, followed by plating on to the same medium solidified with agar. However, a selective isolation method for *Hyphomicrobium* species is to incubate the sample with methanol or methylated amines and nitrate under anaerobic conditions, the nitrate acting as terminal electron acceptor (Colby, Dalton and Whittenbury, 1979; Large, 1981; Anthony, 1982).

Methylotrophic bacteria isolated on methanol or methylated amines are usually placed into several artificial groups. The obligate bacteria are all Gram-negative, oxidase- and catalase-positive, aerobic rods, motile by a single polar flagellum. G + C contents vary between 52% and 56%. They

include organisms named as various *Pseudomonas* species, '*Methylomonas methylovora*', '*Methylophilus methylotrophus*' (the organism used by ICI for single-cell protein 'Pruteen' production; Vasey and Powell, 1984), '*Methylomonas aminofaciens*', '*Methylomonas clara*' and '*Methylomonas methanolica*'. Anthony (1982) suggests that they could all be included in a single genus, '*Methylophilus*'.

The facultative bacteria are divided by Anthony (1982) into seven groups. Group 1 consists of Gram-negative, catalase- and oxidase-positive, motile rods with a G + C ratio of between 60 and 70 mol %. They all produce carotenoid pigments and colonies appear pink–red. Although they are usually described as *Pseudomonas* species, it has been suggested that they might be included in a single genus, *Methylobacterium* (Green and Bousfield, 1981). Group 2 consists of bacteria which resemble Group 1 organisms, except that they are non-pigmented. Methylotrophs of this type have predominantly been isolated on methylated amines, not methanol, and many strains are unable to grow on methanol. They have again been designated as *Pseudomonas* species. Group 3 are a diverse collection of Gram-negative, non-motile rods and coccoid-rods, usually assigned to the genera *Alcaligenes, Achromobacter, Acinetobacter* or *Klebsiella*. Group 4 are the Gram-positive methylotrophs. The Gram-variable *Arthrobacter* spp. described in Group 3 are included with the Gram-positive methylotrophs in *Table 18*. Group 5 represents a diverse range of autotrophs and phototrophs that are able to grow on methanol or formate. It includes *Alcaligenes eutrophus*, '*Blastobacter viscosus*', *Microcyclus aquaticus*, '*Microcyclus ebrunous*', '*Nitrobacter agilis*', *Paracoccus denitrificans*, '*Pseudomonas gazotropha*' (also grows on carbon monoxide), '*Pseudomonas oxalaticus*', *Rhodopseudomonas acidophila, Rhodopseudomonas palustris* and *Thiobacillus novellus*. Group 6 are Gram-negative stalked motile bacteria that reproduce by budding: the hyphomicrobia. Group 7 are Gram-negative non-sporing motile rods isolated from marine environments.

Three species of mycelial fungi have been shown to grow on methanol, formaldehyde and formate: *Gliocladium deliquescens, Paecilomyces varioti* and *Trichoderma lignorum* (Hanson, 1980). Methanol utilization by yeasts is more widespread and occurs in some members of the genera *Candida, Hansenula, Pichia* and *Torulopsis*, but is not as common as bacterial methanol utilization. Yeasts can be isolated by enrichment with 0·1–0·5% methanol at pH 4·5 in a mineral salts medium containing vitamins and antibacterial antibiotics. Most strains grow best at temperatures below 30°C. The most successful sources of material are samples rich in organic material (Tani, Kato and Yamada, 1978; Van Dijken, Harder and Quayle, 1981; Anthony, 1982; Large, 1983; Goldberg, 1985).

Although this short review of the methylotrophs has indicated the enormous diversity of micro-organisms capable of utilizing one-carbon compounds, the methods used for their isolation have been conservative. For example, heat treatment of samples has been successfully used to isolate *Bacillus* species, but this method has only rarely been used. A more imaginative approach to selective isolation might be expected to extend considerably the diversity of methylotrophs and C_1-utilizing autotrophs.

Conclusions

The selective isolation and characterization of novel and rare micro-organisms from natural habitats is an integral part of industrial screening programmes designed to discover and evaluate new metabolites. The continued detection of novel isolates will remain a matter of chance to some extent, but methods are now available which allow the preferential isolation and rapid characterization of uncommon and new microbes. The new methods involve the use of selective isolation media and pretreatment of the substrate to reduce the number of unwanted bacteria. They have mainly been applied to the isolation of actinomycetes but are equally applicable to other industrially important groups of bacteria, given the necessary high-quality taxonomic data bases. Extensive numerical taxonomic data bases are available for many bacterial taxa, including the aerobic endospore-forming bacteria (Logan and Berkeley, 1984; Priest *et al.*, 1986) and some Gram-negative facultatively methylotrophic organisms (Green and Bousfield, 1982).

The concept of the general isolation medium is no longer tenable. Organisms exhibit a bewildering variety of nutritional profiles and a battery of selective media must be used if a representative sample of bacteria are to be isolated from the complex microbial communities found in nature. The pioneering studies on the actinomycetes underline the limitations of conventional isolation procedures and reinforce the view that even in intensively studied habitats many novel microbes await discovery (Williams, Goodfellow and Vickers, 1984). In the final analysis the microbes should be seen as friends and partners who may well take care of our future if we seek them out and treat them sensitively and imaginatively.

Acknowledgement

M.G. gratefully acknowledges the support of the Science and Engineering Research Council (Grant GR/C/18830) for some of the work reported here.

References

ALDERSON, G., GOODFELLOW, M., WELLINGTON, E.M.H., WILLIAMS, S.T., MINNIKIN, S.M. AND MINNIKIN, D.E. (1981). Chemical and numerical taxonomy of *Nocardia mediterranei*. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Abteilung 1, Supplement 11*, 39–46.

ANDERSEN, A.A. (1958). New sampler for the collection, sizing and enumeration of viable airborne particles. *Journal of Bacteriology* **76**, 471–484.

ANTHONY, C. (1982). *The Biochemistry of Methylotrophs*. Academic Press, London.

ATHALYE, M., LACEY, J. AND GOODFELLOW, M. (1981). Selective isolation and enumeration of actinomycetes using rifampicin. *Journal of Applied Bacteriology* **51**, 289–297.

ATHALYE, M., GOODFELLOW, M., LACEY, J. AND WHITE, R.P. (1985). Numerical classification of *Actinomadura* and *Nocardiopsis*. *International Journal of Systematic Bacteriology* **35**, 86–98.

ATKINSON, B. AND MAVITUNA, F. (1983). *Biochemical Engineering and Biotechnology Handbook*. Macmillan, London.

BARJAC, H.DE., VÉRON, M. AND DUMANOIR, V.C. (1980). Caractérisation biochimique

et sérologique de souches de *Bacillus sphaericus* pathogènes ou non pour les moustiques. *Annales de Microbiologie de l'Institut Pasteur* **131**, 191–202.

BÉRDY, J. (1974). Recent developments of antibiotic research and classification of antibiotics according to chemical structure. *Advances in Applied Microbiology* **18**, 309–406.

BÉRDY, J. (1984). New ways to obtain new antibiotics. *Chinese Journal of Antibiotics* **7**, 272–290.

BERKELEY, R.C.W. AND GOODFELLOW, M., EDS (1981). *The Aerobic Endospore-forming Bacteria: Classification and Identification*. Academic Press, London.

BROWN, L.R., STRAWINSKI, R.J. AND MCCLESKEY, C.S. (1964). The isolation and characterisation of *Methanomonas methanooxidans* Brown and Strawinski. *Canadian Journal of Microbiology* **10**, 791–800.

BRYANT, T.N., CAPEY, A.G. AND BERKELEY, R.C.W. (1985). Microcomputer-assisted identification of *Bacillus* species. *Computer Applications in the Biosciences* **1**, 23–27.

BUCHANAN, R.E. AND GIBBONS, N.E. (1974). *Bergey's Manual of Determinative Bacteriology, 8th edn.* The Williams and Wilkins Company, Baltimore.

BULL, A.T., ELLWOOD, D.C. AND RATLEDGE, C. (1979). The changing scene in microbial technology. In *Microbial Technology: Current State, Future Prospects* (A.T. Bull, D.C. Ellwood and C. Ratledge, Eds), pp. 1–28. Cambridge University Press, Cambridge.

BULLA, L.A. AND HOCH, T.A. (1985). Biology of the bacilli. In *Biology of Industrial Microorganisms* (A.L. Demain and N.A. Solomon, Eds), pp. 57–78. Benjamin/Cummings Publishing Company, Inc., California, USA.

BYROM, D. (1981). Taxonomy of methylotrophs: a reappraisal. In *Microbial Growth on C1 Compounds. Proceedings of the Third International Symposium at the University of Sheffield, 12–16 August, 1980* (H. Dalton, Ed.), pp. 278–285. Heyden, London.

CAIN, R.B. (1980). Transformation of aromatic hydrocarbons. In *Hydrocarbons in Biotechnology. Proceedings of a Meeting Organised by the Institute of Petroleum, University of Kent, 25–26 September 1979* (D.E.F. Harrison, I.J. Higgins and R. Watkinson, Eds), pp. 99–132. Heyden, London.

CALLAHAM, D., TREDICI, P.D. AND TORREY, J.G. (1978). Isolation and cultivation of the actinomycete causing root nodulation in *Comptonia*. *Science* **199**, 899–902.

CANALE-PAROLA, E. (1981). Free-living anaerobic and facultatively anaerobic spirochaetes: The genus *Spirochaeta*. In *The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria*, (M.P. Starr, H. Stolp, H.G. Trüper, A. Balows and H.G. Schlegel, Eds), volume 1, pp. 538–547. Springer-Verlag, Berlin.

CHATER, K.F. AND HOPWOOD, D.A. (1984). *Streptomyces* genetics. In *The Biology of the Actinomycetes* (M. Goodfellow, M. Mordarski and S.T. Williams, Eds), pp. 229–286. Academic Press, London.

COLBY, J., DALTON, H. AND WHITTENBURY, R. (1979). Biological and biochemical aspects of microbial growth on C1 compounds. *Annual Review of Microbiology* **33**, 481–517.

COWAN, S.T. (1965). Principles and practice of bacterial taxonomy: a forward look. *Journal of General Microbiology* **39**, 143–153.

CROSS, T. (1981). Aquatic actinomycetes: a critical survey of the occurrence, growth and role of actinomycetes in aquatic habitats. *Journal of Applied Bacteriology* **50**, 397–424.

CROSS, T. (1982). Actinomycetes: a continuing source of new metabolites. *Developments in Industrial Microbiology* **23**, 1–18.

CROSS, T. AND UNSWORTH, B.A. (1981). The taxonomy of the endospore-forming actinomycetes. In *The Aerobic Endospore-forming Bacteria: Classification and Identification* (R.C.W. Berkeley and M. Goodfellow, Eds), pp. 17–32. Academic Press, London.

DALTON, H. (1980). Transformations by methane monooxygenase. In *Hydrocarbons in Biotechnology. Proceedings of a Meeting Organised by the Institute of Petroleum, University of Kent, 25–26 September 1979* (D.E.F. Harrison, I.J. Higgins and R. Watkinson, Eds), pp. 85–97. Heyden, London.

DAVIS, A.W., ATLAS, R.M. AND KRICHEVSKY, M.I. (1983). Development of probability matrices for identification of Alaskan marine bacteria. *International Journal of Systematic Bacteriology* **33**, 803–810.

DAVIS, J.B., COTY, V.F. AND STANLEY, J.P. (1964). Atmospheric nitrogen fixation by methane-oxidising bacteria. *Journal of Bacteriology* **88**, 468–472.

DAVIS, M.J., GILLASPIE, A.G. JR., VIDAVER, A.K. AND HARRIS, R.W. (1984). *Clavibacter*: a new genus containing some phytopathogenic coryneform bacteria, including *Clavibacter xyli* subsp. *xyli* sp. nov., subsp. nov. and *Clavibacter xyli* subsp. *cynodontis* subsp. nov., pathogens that cause ratoon stunting disease of sugarcane and bermudagrass stunting disease. *International Journal of Systematic Bacteriology* **34**, 107–117.

DAWSON, C.A. AND SNEATH, P.H.A. (1985). A probability matrix for the identification of vibrios. *Journal of Applied Bacteriology* **58**, 407–423.

DEAN, D.H. (1984). Biochemical genetics of the bacterial insect-control agent *Bacillus thuringiensis*: basic principles and prospects for genetic engineering. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed.), volume 2, pp. 341–363. Intercept, Ponteland, Newcastle upon Tyne.

DWORKIN, M. AND FOSTER, J.W. (1956). Studies on *Pseudomonas methanica* (Söhngen) *nov. comb. Journal of Bacteriology* **72**, 646–659.

EHHALT, D.H. (1976). The atmospheric cycle of methane. In *Symposium on Microbial Production and Utilisation of Gases (H_2,CH_4,CO)* (H.G. Schlegel, G. Gottshalk and N. Pfennig, Eds), pp. 13–22. E. Goltze KG, Gottingen.

FELTHAM, R.K.A. AND SNEATH, P.H.A. (1982). Construction of matrices for computer-assisted identification of aerobic Gram-positive cocci. *Journal of General Microbiology* **128**, 713–720.

FISCHER, A., KROPPENSTEDT, R.M. AND STACKEBRANDT, E. (1983). Molecular-genetic and chemotaxonomic studies on *Actinomadura*. *Journal of General Microbiology* **129**, 3433–3446.

FOSTER, J.W. AND DAVIS, R.H. (1966). A methane-dependent coccus, with notes on classification and nomenclature of obligate methane-utilising bacteria. *Journal of Bacteriology* **91**, 1924–1931.

FOWLER, V.J., LUDWIG, W. AND STACKEBRANDT, E. (1985). Ribosomal ribonucleic acid cataloguing in bacterial systematics: the phylogeny of *Actinomadura*. In *Chemical Methods in Bacterial Systematics* (M. Goodfellow and D.E. Minnikin, Eds), pp. 17–40. Academic Press, London.

GARCIA, J.L., ROUSSOS, S., BENSOUSSAN, M., BIANCHI, A. AND MANDEL, M. (1982). Taxonomie numérique de *Bacillus thermophiles* isolés de sols de rizière de l'afrique de l'ouest. *Annales de Microbiologie de l'Institut Pasteur (Paris)* **133A**, 471–488.

GŁADEK, A. AND ZAKRZEWSKA, J. (1984). Genome size of *Streptomyces*. *FEMS Microbiology Letters* **24**, 73–76.

GODFREY, T. (1983). Brewing. In *Industrial Enzymology. The Application of Enzymes in Industry* (T. Godfrey and J.R. Reichelt, Eds), pp. 221–259. Macmillan, London.

GOLDBERG, I. (1985). Biology of methylotrophs. In *Biology of Industrial Microorganisms* (A.L. Demain and N.A. Solomon, Eds), pp. 223–260. Benjamin/Cummings Publishing Company, Inc., California.

GOODFELLOW, M. (1971). Numerical taxonomy of some nocardioform bacteria. *Journal of General Microbiology* **69**, 33–80.

GOODFELLOW, M. (1986). Actinomycete systematics: present state and future prospects. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes* (G. Szabo, S. Biro and M. Goodfellow, Eds). Kiado Press, Budapest, in press.

GOODFELLOW, M. AND ALDERSON, G. (1977). The actinomycete genus *Rhodococcus*: a home for the '*rhodochrous*' complex. *Journal of General Microbiology* **100**, 99–122.

GOODFELLOW, M. AND CROSS, T. (1984). Classification. In *The Biology of the Actinomycetes* (M. Goodfellow, M. Mordarski and S.T. Williams, Eds), pp. 7–164. Academic Press, London.

GOODFELLOW, M. AND DICKINSON, C.H. (1985). Delineation and description of microbial populations using numerical methods. In *Computer-assisted Bacterial Systematics* (M. Goodfellow, D. Jones and F.G. Priest, Eds), pp. 165–225. Academic Press, London.

GOODFELLOW, M. AND HAYNES, J.A. (1984). Actinomycetes in marine sediments. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes* (L. Ortiz-Ortiz, L.F. Bojalil and V. Yakoleff, Eds), pp. 453–472. Academic Press, Orlando.

GOODFELLOW, M. AND MINNIKIN, D.E. (1981). The genera *Nocardia* and *Rhodococcus*. In *The Prokaryotes: A Handbook of Habitats, Isolation and Identification of Bacteria,* (M.P. Starr, H. Stolp, H.G. Trüper, A. Balows and H.G. Schlegel, Eds), volume 2, pp. 2016–2027. Springer-Verlag, Berlin.

GOODFELLOW, M. AND MINNIKIN, D.E. (EDS) (1985). *Chemical Methods in Bacterial Systematics*. Academic Press, London.

GOODFELLOW, M. AND ORCHARD, V.A. (1974). Antibiotic sensitivity of some nocardioform bacteria and its value as a criterion for taxonomy. *Journal of General Microbiology* **83**, 375–387.

GOODFELLOW, M. AND SIMPSON, K.E. (1986). Ecology of streptomycetes. *Frontiers of Applied Microbiology* **2**, in press.

GOODFELLOW, M. AND WAYNE, L.G. (1982). Taxonomy and nomenclature. In *The Biology of the Mycobacteria, Volume 1. Physiology, Identification and Classification* (C. Ratledge and J.L. Stanford, Eds), pp. 472–521. Academic Press, London.

GOODFELLOW, M. AND WILLIAMS, S.T. (1983). Ecology of actinomycetes. *Annual Review of Microbiology* **37**, 189–216.

GOODFELLOW, M., ALDERSON, G. AND LACEY, J. (1979). Numerical taxonomy of *Actinomadura* and related actinomycetes. *Journal of General Microbiology* **12**, 95–111.

GOODFELLOW, M., BECKHAM, A.R. AND BARTON, M.D. (1982). Numerical classification of *Rhodococcus equi* and related actinomycetes. *Journal of Applied Bacteriology* **53**, 199–207.

GOODFELLOW, M., EMBLEY, T.M. AND AUSTIN, B. (1985). Numerical taxonomy and emended description of *Renibacterium salmoninarum*. *Journal of General Microbiology* **131**, 2739–2752.

GOODFELLOW, M., HILL, I.R. AND GRAY, T.R.G. (1968). Bacteria in a pine forest soil. In *The Ecology of Soil Bacteria* (T.R.G. Gray and D. Parkinson, Eds), pp. 500–515. University Press, Liverpool.

GOODFELLOW, M., JONES, D. AND PRIEST, F.G. (EDS) (1985). *Computer-assisted Bacterial Systematics*. Academic Press, London.

GOODFELLOW, M., WEAVER, C.R. AND MINNIKIN, D.E. (1982). Numerical classification of rhodococci, corynebacteria and related organisms. *Journal of General Microbiology* **128**, 731–745.

GOODFELLOW, M., WILLIAMS, S.T. AND ALDERSON, G. (1986a). Transfer of *Elytrosporangium brasiliense* Falcão de Morais *et al.*, *Elytrosporangium carpinense* Falcão de Morais *et al.*, *Elytrosporangium spirale* Falcão de Morais, *Microellobosporia cinerea* Cross *et al.*, *Microellobosporia flavea* Cross *et al.*, *Microellobosporia grisea* (Konev *et al.*) Pridham and *Microellobosporia violacea* (Tsyganov *et al.*) Pridham to the genus *Streptomyces*, with emended descriptions of the species. *Systematic and Applied Microbiology* **8**, 48–54.

GOODFELLOW, M., WILLIAMS, S.T. AND ALDERSON, G. (1986b). Transfer of *Chainia*

species to the genus *Streptomyces* with emended description of species. *Systematic and Applied Microbiology* **8**, 55–60.

GOODFELLOW, M., WILLIAMS, S.T. AND ALDERSON, G. (1986c). Transfer of *Actinosporangium violaceum* Krasil'nikov and Yuan, *Actinosporangium vitaminophilum* Shomura *et al.* and *Actinopycnidium caeruleum* Krasil'nikov to the genus *Streptomyces*, with amended descriptions of the species. *Systematic and Applied Microbiology* **8**, 61–64.

GOODFELLOW, M., WILLIAMS, S.T. AND ALDERSON, G. (1986d). Transfer of *Kitasatoa purpurea* Matsumae and Hata to the genus *Streptomyces* as *Streptomyces purpureus comb. nov. Systematic and Applied Microbiology* **8**, 65–66.

GOODFELLOW, M., MINNIKIN, D.E., TODD, C., ALDERSON, G., MINNIKIN, S.M. AND COLLINS, M.D. (1982). Numerical and chemical classification of *Nocardia amarae*. *Journal of General Microbiology* **128**, 1283–1297.

GORDON, R.E. AND HAGAN, W.A. (1936). A study of some acid-fast actinomycetes from soil with special reference to pathogenicity to animals. *Journal of Infectious Diseases* **59**, 200–206.

GORDON, R.E., HAYNES, W.C. AND PANG, C.H.-N. (1973). *The Genus* Bacillus. United States Department of Agriculture Publication, Washington, DC.

GOWER, J.C. (1966). Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* **53**, 325–338.

GREEN, P.N. AND BOUSFIELD, I.J. (1981). The taxonomy of the pink-pigmented facultatively methyltrophic bacteria. In *Microbial Growth on C1 Compounds. Proceedings of the Third International Symposium at the University of Sheffield, 12–16 August, 1980* (H. Dalton, Ed.), pp. 284–294. Heyden, London.

GREEN, P.N. AND BOUSFIELD, I.J. (1982). A taxonomic study of some Gram-negative facultatively methylotrophic bacteria. *Journal of General Microbiology* **128**, 623–638.

GREGORY, P.H. AND LACEY, M.E. (1963). Mycological examination of dust from mouldy hay associated with farmer's lung disease. *Journal of General Microbiology* **30**, 75–88.

HAMER, G. AND HARRISON, D.E.F. (1980). Single cell protein: The technology, economics and future potential. In *Hydrocarbons in Biotechnology. Proceedings of a Meeting Organised by the Institute of Petroleum, University of Kent, 25–26 September 1979* (D.E.F. Harrison, J. Higgins and R. Watkinson, Eds), pp. 59–73. Heyden, London.

HANSON, R.S. (1980). Ecology and diversity of methylotrophic organisms. *Advances in Applied Microbiology* **26**, 3–39.

HARRISON, A.P. (1981). *Acidophilum cryptum* gen. nov., sp. nov., heterotrophic bacterium from acidic mineral environments. *International Journal of Systematic Bacteriology* **31**, 327–332.

HAYNES, J.A. (1982). *Actinomycetes Associated with Marine Habitats*. PhD thesis, University of Newcastle upon Tyne.

HIGGINS, I.J., HAMMOND, R.C., PLOTKIN, E., HILL, H.A.O., UOSAKI, H., EDDOWES, M.J. AND CASS, A.E.G. (1980). Electroenzymology and biofuel cells. In *Hydrocarbons in Biotechnology. Proceedings of a Meeting Organised by the Institute of Petroleum, University of Kent, 25–26 September 1979* (D.E.F. Harrison, I.J. Higgins and R. Watkinson, Eds), pp. 181–193. Heyden, London.

HILL, L.R. (1974). Theoretical aspects of numerical identification. *International Journal of Systematic Bacteriology* **24**, 494–499.

HIRSCH, P. (1968). Photosynthetic bacteria growing under carbon monoxide. *Nature* **217**, 555–556.

HOLMBERG, K. AND HALLANDER, H.O. (1973). Numerical taxonomy and laboratory identification of *Bacterionema matruchotii, Rothia dentocariosa, Actinomyces naeslundii, Actinomyces viscosus*, and some related bacteria. *Journal of General Microbiology* **76**, 43–63.

HOOKEY, J.V. (1983). *Selective Isolation, Classification and Ecology of Nocardiae*

from Soil, Water and Biodeteriorating Rubber. PhD thesis, University of Newcastle upon Tyne.

HOPWOOD, D.A. AND CHATER, K.F. (1980). Fresh approaches to antibiotic production. *Philosophical Transactions of the Royal Society, Series B,* **290**, 313–328.

HSU, S.C. AND LOCKWOOD, J.L. (1975). Powdered chitin as a selective medium for enumeration of actinomycetes in water and soil. *Applied Microbiology* **29**, 422–426.

IMADA, A., KITANO, K., KINTAKA, K., MUROI, M. AND ASAI, M. (1981). Sulfazecin and isosulfazecin, novel β-lactam antibiotics of bacterial origin. *Nature* **289**, 590–591.

JONES, D. AND SACKIN, M.J. (1980). Numerical methods in the classification and identification of bacteria with especial reference to the *Enterobacteriaceae*. In *Microbiological Classification and Identification* (M. Goodfellow and R.G. Board, Eds), pp. 73–106. Academic Press, London.

KATZ, E. AND DEMAIN, A.L. (1977). The peptide antibiotics of *Bacillus*: chemistry, biogenesis and possible functions. *Bacteriological Reviews* **41**, 449–474.

KHAN, M.R. AND WILLIAMS, S.T. (1975). Studies on the ecology of actinomycetes in soil. VIII. Distribution and characteristics of acidophilic actinomycetes. *Soil Biology and Biochemistry* **7**, 345–348.

KIM, Y.M. AND HEGEMAN, G.D. (1983). Oxidation of carbon monoxide by bacteria. *International Review of Cytology* **81**, 1–32.

KROPPENSTEDT, R.M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics* (M. Goodfellow and D.E. Minnikin, Eds), pp. 173–199. Academic Press, London.

KUSSER, W. AND FIEDLER, F. (1983). Murein type and polysaccharide composition of cell walls from *Renibacterium salmoninarum. FEMS Microbiology Letters* **20**, 391–394.

KÜSTER, E. AND WILLIAMS, S.T. (1964). Selection of media for isolation of streptomycetes. *Nature* **202**, 928–929.

LACEY, J. (1971). The microbiology of moist barley storage in unsealed silos. *Annals of Applied Biology* **69**, 187–212.

LACEY, J. AND DUTKIEWICZ, J. (1976a). Methods for examining the microflora of mouldy hay. *Journal of Applied Bacteriology* **41**, 13–27.

LACEY, J. AND DUTKIEWICZ, J. (1976b). Isolation of actinomycetes and fungi from mouldy hay using a sedimentation chamber. *Journal of Applied Bacteriology* **41**, 315–319.

LAPAGE, S.P., SNEATH, P.H.A., LESSEL, E.F., SKERMAN, V.B.D., SEELIGER, H.R.P. AND CLARK, W.A. (1975). *International Code of Nomenclature of Bacteria.* American Society for Microbiology, Washington.

LARGE, P.J. (1981). Microbial growth on methylated amines. In *Microbial Growth on C1 Compounds. Proceedings of the Third International Symposium at the University of Sheffield, 12–16 August, 1980* (H. Dalton, Ed.), pp. 55–69. Heyden, London.

LARGE, P.J. (1983). *Methylotrophy and Methanogenesis. Aspects of Microbiology 8.* Van Nostrand Reinhold (UK) Co., Wokingham, Berkshire.

LECHEVALIER, H.A. AND LECHEVALIER, M.P. (1981). Introduction to the order *Actinomycetales*. In *The Prokaryotes: A Handbook of Habitats, Isolation and Identification of Bacteria* (H.P. Starr, H. Stolp, H.G. Trüper, A. Balows and H.G. Schlegel, Eds), volume 2, pp. 1915–1922. Springer-Verlag, Berlin.

LECHEVALIER, M.P. AND LECHEVALIER, H.A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *International Journal of Systematic Bacteriology* **20**, 435–443.

LECHEVALIER, M.P. AND LECHEVALIER, H.A. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy: Society for Industrial Microbiology Special Publication Number 6* (A. Dietz and D.W. Thayer, Eds), pp. 227–291. Society for Industrial Microbiology, Arlington, Virginia.

LECHEVALIER, M.P. AND LECHEVALIER, H.A. (1985). Biology of actinomycetes not

belonging to the genus *Streptomyces*. In *Biology of Industrial Microorganisms* (A.L. Demain and N.A. Solomon, Eds), pp. 315–358. Benjamin/Cummings Publishing Company, Inc., California.

LECHEVALIER, M.P., BIÉVRE, C. DE AND LECHEVALIER, H.A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochemical Systematics and Ecology* **5**, 249–260.

LECHEVALIER, M.P., STERN, A.E. AND LECHEVALIER, H.A. (1981). Phospholipids in the taxonomy of actinomycetes. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene. Abteilung 1, Supplement 11*, 111–115.

LECHEVALIER, M.P., PRAUSER, H., LABEDA, D.P. AND RUAN, J-S. (1986). Two new genera of nocardioform actinomycetes: *Amycolata* gen. nov. and *Amycolatopsis* gen. nov. *International Journal of Systematic Bacteriology* **36**, 29–37.

LIDSTROM-O'CONNOR, M.E., FULTON, G.L. AND WOPAT, A.E. (1983). *'Methylobacterium ethanolicum':* a syntrophic association of two methylotrophic bacteria. *Journal of General Microbiology* **129**, 3139–3148.

LINGAPPA, Y. AND LOCKWOOD, J.L. (1962). Chitin media for selective isolation and culture of actinomycetes. *Phytopathology* **52**, 317–323.

LOCCI, R., ROGERS, J., SARDI, P. AND SCHOFIELD, G.M. (1981). A preliminary numerical study on named species of the genus *Streptoverticillium*. *Annali di Microbiologia* **31**, 115–121.

LOGAN, N.A. AND BERKELEY, R.C.W. (1981). Classification and identification of members of the genus *Bacillus* using API tests. In *The Aerobic Endospore-forming Bacteria* (R.C.W. Berkeley and M. Goodfellow, Eds), pp. 105–140. Academic Press, London.

LOGAN, N.A. AND BERKELEY, R.C.W. (1984). Identification of *Bacillus* strains using the API system. *Journal of General Microbiology* **130**, 1871–1882.

LONSDALE, J.T. (1985). *Aspects of the Biology of Acidophilic Actinomycetes*. PhD thesis, University of Newcastle upon Tyne.

LYNCH, M.J., WOPAT, A.E. AND O'CONNOR, M.L. (1980). Characterisation of two new facultative methanotrophs. *Applied and Environmental Microbiology* **40**, 400–407.

MCCARTHY, A.J. AND CROSS, T. (1981). A note on a selective medium for the thermophilic actinomycete *Thermomonospora chromogena*. *Journal of Applied Bacteriology* **51**, 299–302.

MCCARTHY, A.J. AND CROSS, T. (1984). A taxonomic survey of *Thermomonospora* and other monosporic actinomycetes. *Journal of General Microbiology* **130**, 5–25.

MACKAY, S.J. (1977). Improved enumeration of *Streptomyces* spp. on a starch–casein–salt medium. *Applied and Environmental Microbiology* **33**, 227–230.

MAKKAR, N.S. AND CROSS, T. (1982). Actinoplanetes in soil and on plant litter from freshwater habitats. *Journal of Applied Bacteriology* **52**, 209–218.

MEISSNER, G., SCHRÖDER, K.H., AMADIO, G.E., ANZ, W., CHAPARAS, S., ENGEL, H.W.B., JENKINS, P.A., KÄPPLER, W., KLEEBERG, H.H., KUBALA, E., KUBIN, M., LAUTERBACH, D., LIND, A., MAGNUSSON, M., MIKOVA, Z., PATTYN, S.R., SCHAEFER, W.B., STANFORD, J.L., TSUKAMURA, M., WAYNE, L.G., WILLERS, I. AND WOLINSKY, E. (1974). A co-operative numerical analysis of nonscoto- and nonphotochromogenic slowly growing mycobacteria. *Journal of General Microbiology* **83**, 207–235.

MEYER, O. (1980). Using carbon monoxide to produce single cell protein. *Bioscience* **30**, 405–407.

MEYER, O. (1981). Growth of carbon monoxide oxidising bacteria with industrial gas mixtures, automobile exhaust gas and other unconventional CO-containing gases. *Studies in Environmental Science* **9**, 79–86.

MINNIKIN, D.E. AND GOODFELLOW, M. (1980). Lipid composition in the classification and identification of acid-fast bacteria. In *Microbiological Classification and Identification* (M. Goodfellow and R.G. Board, Eds), pp. 189–256. Academic Press, London.

MINNIKIN, D.E., GOODFELLOW, M. AND COLLINS, M.D. (1978). Lipid composition in

the classification and identification of coryneform and related taxa. In *Coryneform Bacteria* (I.J. Bousfield and A.G. Callely, Eds), pp. 85–160. Academic Press, London.

MIYADOH, S., SHOMURA, T., ITO, T. AND NIIDA, T. (1983). *Streptomyces sulfofaciens* sp. nov. *International Journal of Systematic Bacteriology* **33**, 321–324.

MIYADOH, S., TOHYAMA, H., AMANO, S., SHOMURA, T. AND NIIDA, T. (1985). *Microbispora viridis*, a new species of *Actinomycetales*. *International Journal of Systematic Bacteriology* **35**, 281–284.

MOORE, W.E.C., CATO, E.P. AND MOORE, L.V.H. (1985). Index of bacterial and yeast nomenclatural changes published in the *International Journal of Systematic Bacteriology* since the 1980 *Approved Lists of Bacterial Names* (7 January 1980 to 1 January 1985). *International Journal of Systematic Bacteriology* **35**, 382–407.

MORDARSKI, M., GOODFELLOW, M., WILLIAMS, S.T. AND SNEATH, P.H.A. (1986). Evaluation of species groups in the genus *Streptomyces*. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes* (G. Szabo, S. Biro and M. Goodfellow, Eds), Kiado Press, Budapest, in press.

NISBET, L.J. (1982). Current strategies in the search for bioactive microbial metabolites. *Journal of Chemical Technology and Biotechnology* **32**, 251–270.

NONOMURA, H. AND OHARA, Y. (1969). Distribution of actinomycetes in soil. VI. A culture method effective for both preferential isolation and enumeration of *Microbispora* and *Streptosporangium* strains in soil. *Journal of Fermentation Technology* **47**, 463–469.

NONOMURA, H. AND OHARA, Y. (1971). Distribution of actinomycetes in soil. IX. New species of the genera *Microbispora* and *Microtetraspora* and their isolation method. *Journal of Fermentation Technology* **49**, 887–894.

NORRIS, J.R. (1981). *Sporosarcina* and *Sporolactobacillus*. In *The Aerobic, Endospore-forming Bacteria: Classification and Identification* (R.C.W. Berkeley and M. Goodfellow, Eds), pp. 337–357. Academic Press, London.

NORRIS, J.R., BERKELEY, R.C.W., LOGAN, N. AND O'DONNELL, A.G. (1981). The genera *Bacillus* and *Sporolactobacillus*. In *The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria* (M.P. Starr, H. Stolp, H.G. Trüper, A. Balows and H.G. Schlegel, Eds), volume 2, pp. 1711–1742. Springer-Verlag, Berlin.

O'DONNELL, A.G. (1985). Numerical analysis of chemotaxonomic data. In *Computer-assisted Bacterial Systematics* (M. Goodfellow, D. Jones and F.G. Priest, Eds), pp. 403–414. Academic Press, London.

O'DONNELL, A.G. (1986). Chemical and numerical methods in the classification of novel isolates. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes* (G. Szabo, S. Biro and M. Goodfellow, Eds), Kiado Press, Budapest, in press.

ORCHARD, V.A. (1978). Effect of irrigation with municipal water or sewage effluent on the biology of soil cores. III. Actinomycete flora. *New Zealand Journal of Agricultural Research* **21**, 21–28.

ORCHARD, V.A. (1981). The ecology of *Nocardia* and related taxa. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene, Supplement* 11, 167–180.

ORCHARD, V.A. AND GOODFELLOW, M. (1974). The selective isolation of *Nocardia* from soil using antibiotics. *Journal of General Microbiology* **85**, 160–162.

ORCHARD, V.A. AND GOODFELLOW, M. (1980). Numerical classification of some named strains of *Nocardia asteroides* and related isolates from soil. *Journal of General Microbiology* **118**, 295–312.

ORCHARD, V.A., GOODFELLOW, M. AND WILLIAMS, S.T. (1977). Selective isolation and occurrence of nocardiae in soil. *Soil Biology and Biochemistry* **9**, 233–238.

PARENTI, F. AND CORONELLI, C. (1979). Members of the genus *Actinoplanes* and their antibiotics. *Annual Review of Microbiology* **33**, 389–411.

PATEL, R.N., HOU, C.T. AND FELIX, A. (1978). Microbial oxidation of methane and methanol: Isolation of methane-utilising bacteria and characterisation of a

facultative methane-utilising isolate. *Journal of Bacteriology* **136**, 352–358.

PATT, T.E., COLE, G.C., BLAND, J. AND HANSON, R.S. (1974). Isolation and characterisation of bacteria that grow on methane and organic compounds as sole sources of carbon and energy. *Journal of Bacteriology* **120**, 955–964.

PECZYŃSKA-CZOCH, W. AND MORDARSKI, M. (1984). Transformation of xenobiotics. In *The Biology of the Actinomycetes* (M. Goodfellow, M. Mordarski and S.T. Williams, Eds), pp. 287–336. Academic Press, London.

PICHINOTY, F., GARCIA, J.-L. AND MANDEL, M. (1980). Taxonomie numérique de 46 souches dénitrifiantes et mesophiles de *Bacillus* isolées à partir du sol par culture elective en présence de nitrite. *Canadian Journal of Microbiology* **26**, 787–795.

PRIDHAM, T.G. AND TRESNER, H.D. (1974). Family VII *Streptomycetaceae* Waksman and Henrici 1943. In *Bergey's Manual of Determinative Bacteriology* (R.E. Buchanan and N.E. Gibbons, Eds), pp. 747–845. Williams and Wilkins, Baltimore, Maryland.

PRIEST, F.G., GOODFELLOW, M. AND TODD, C. (1981). The genus *Bacillus*: a numerical analysis. In *The Aerobic Endospore-Forming Bacteria* (R.C.W. Berkeley and M. Goodfellow, Eds), pp. 91–103. Academic Press, London.

PRIEST, F.G., GOODFELLOW, M., SHUTE, L.A. AND BERKELEY, R.C.W. (1986). *Bacillus amyloliquefaciens* sp. nov., nom. rev. *International Journal of Systematic Bacteriology*, in press.

REICHELT, J.R. (1983). Baking. In *Industrial Enzymology. The Application of Enzymes in Industry* (T. Godfrey and J.R. Reichelt, Eds), pp. 210–220. Macmillan, London.

RIDELL, M. AND GOODFELLOW, M. (1983). Numerical classification of *Mycobacterium farcinogenes, Mycobacterium senegalense* and related taxa. *Journal of General Microbiology* **129**, 599–611.

ROWBOTHAM, T.J. AND CROSS, T. (1977a). *Rhodococcus coprophilus* sp. nov.: an aerobic nocardioform actinomycete belonging to the '*rhodochrous*' complex. *Journal of General Microbiology* **100**, 123–138.

ROWBOTHAM, T.J. AND CROSS, T. (1977b). Ecology of *Rhodococcus coprophilus* and associated actinomycetes in fresh water and agricultural habitats. *Journal of General Microbiology* **100**, 231–240.

SADDLER, G.S., GOODFELLOW, M., MINNIKIN, D.E. AND O'DONNELL, A.G. (1986). Influence of the growth cycle on the fatty acid and menaquinone composition of *Streptomyces cyaneus* NCIB 9616. *Journal of Applied Bacteriology* **60**, 51–56.

SAIKI, T., KOBAYASHI, Y., KAWAGOE, K. AND BEPPU, T. (1985). *Dictyoglomus thermophilus* gen. nov., sp. nov., a chemoorganotrophic, anaerobic, thermophilic bacterium. *International Journal of Systematic Bacteriology* **35**, 253–259.

SCHAAL, K.P. (1985). Laboratory diagnosis of actinomycete diseases. In *The Biology of the Actinomycetes* (M. Goodfellow, M. Mordarski and S.T. Williams, Eds), pp. 425–456. Academic Press, London.

SCHLEIFER, K.H. AND KANDLER, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriological Reviews* **36**, 407–477.

SCHOFIELD, G.M. AND SCHAAL, K.P. (1981). A numerical taxonomic study of members of the *Actinomycetaceae* and related taxa. *Journal of General Microbiology* **127**, 237–259.

SEILER, H. (1983). Identification key for coryneform bacteria derived by numerical taxonomic studies. *Journal of General Microbiology* **129**, 1433–1471.

SETO, N., SAKAYANAGI, S. AND IIZUKA, H. (1975). Utilisation of methane by Gram-positive bacteria. In *Microbial Growth on C1 Compounds*, pp. 35–44. Society of Fermentation Technology, Tokyo, Japan.

SHEARD, M.A. AND PRIEST, F.G. (1981). Numerical classification of some psychrotrophic bacilli isolated from frozen foods. *Journal of Applied Bacteriology* **51**, xxii–xxiii.

SHEARER, M.C., COLMAN, P.M., FERRIN, R.M., NISBET, L.J. AND NASH, C.H. (1986). New genus of the *Actinomycetales: Kibdelosporangium aridum* gen. nov., sp.

nov. *International Journal of Systematic Bacteriology* **36**, 47–54.

SHIBA, T. AND SIMIDU, U. (1982). *Erythrobacter longus* gen. nov. sp. nov., anaerobic bacterium which contains bacteriochlorophyll *a*. *International Journal of Systematic Bacteriology* **32**, 211–217.

SHOMURA, T., AMANO, S., TOHYAMA, H., YOSHIDA, J., ITO, T. AND NIIDA, T. (1985). *Dactylosporangium roseum* sp. nov. *International Journal of Systematic Bacteriology* **35**, 1–4.

SKERMAN, V.B.D., MCGOWAN, V. AND SNEATH, P.H.A. (1980). Approved lists of bacterial names. *International Journal of Systematic Bacteriology* **30**, 225–420.

SLEAT, R., MAH, R.A. AND ROBINSON, R. (1985). *Acetoanaerobium noterae* gen. nov., sp. nov., an anaerobic bacterium that forms acetate from H_2 and CO_2. *International Journal of Systematic Bacteriology* **35**, 10–15.

SLEPECKY, R.A. (1975). Ecology of bacterial sporeformers. In *Spores VI* (P. Gerhardt, H.L. Sadoff and R.N. Costilow, Eds), pp. 297–313. American Society for Microbiology, Washington.

SMITH, N.R., GORDON, R.E. AND CLARK, F.E. (1952). *Aerobic Sporeforming Bacteria*. Monograph no. 16. United States Department of Agriculture, Washington, DC.

SMITH, S.R.L. (1981). Some aspects of ICI's single cell protein process. In *Microbial Growth on C1 Compounds. Proceedings of the Third International Symposium at the University of Sheffield, 12–16 August 1980* (H. Dalton, Ed.), pp. 342–349. Heyden, London.

SNEATH, P.H.A. (1974). Test reproducibility in relation to identification. *International Journal of Systematic Bacteriology* **24**, 508–523.

SNEATH, P.H.A. (1979). BASIC program for identification of an unknown with presence-absence data against an identification matrix of percent positive characters. *Computers and Geosciences* **5**, 195–213.

SNEATH, P.H.A. (1980). BASIC program for the most diagnostic properties of groups from an identification matrix of percent positive characters. *Computers and Geosciences* **6**, 21–26.

SNEATH, P.H.A. (1983). Distortion of taxonomic structure from incomplete data on a restricted set of reference strains. *Journal of General Microbiology* **129**, 1045–1073.

SNEATH, P.H.A. AND CHATER, A.O. (1978). Information content of keys for identification. In *Essays in Plant Taxonomy* (H.E. Street, Ed.), pp. 79–95. Academic Press, London.

SNEATH, P.H.A. AND SOKAL, R.R. (1973). *Numerical Taxonomy: The Principles and Practice of Numerical Classification*. W.H. Freeman, San Francisco.

SÖHNGEN, N.L. (1906). Uber Bakterien, welche Methan als Kohlenstoffnahrung und Energiequelle gebrauchen. *Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene, Abteilung 1, Originale* **C 15**, 513–517.

STACKEBRANDT, E. (1986). The significance of 'wall types' in phylogenetically based taxonomic studies on actinomycetes. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes* (G. Szabo, S. Biro and M. Goodfellow, Eds), Kiado Press, Budapest, in press.

STACKEBRANDT, E. AND SCHLEIFER, K-H. (1984). Molecular systematics of actinomycetes and related organisms. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes* (L. Ortiz-Ortiz, L.F. Bojalil and V. Yakoleff, Eds), pp. 485–504. Academic Press, Orlando.

STACKEBRANDT, E. AND WOESE, C.R. (1981). The evolution of prokaryotes. In *Molecular and Cellular Aspects of Microbial Evolution* (M.J. Carlile, J.F. Collins and B.E.B. Moseley, Eds), pp. 1–31. Cambridge University Press, Cambridge.

STACKEBRANDT, E., LUDWIG, W., SEEWALDT, E. AND SCHLEIFER, K.H. (1983). Phylogeny of sporeforming members of the order *Actinomycetales*. *International Journal of Systematic Bacteriology* **33**, 173–180.

STANTON, L.J. (1984). *Actinomycetes Associated with Freshwater Habitats*. PhD thesis, University of Newcastle upon Tyne.

STETTER, K.O., THOMM, M., WINTER, G., WILDGRUBER, G., HUBER, H., ZILLIG, W.,

JANEKOVIC, D., KONIG, H., PALM, P. AND WUNDERL, S. (1981). *Methanothermus fervidus*, sp. nov., a novel extremely thermophilic methanogen isolated from an Icelandic hot spring. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung 1, Originale* **C2**, 166–178.

SUZUKI, Y. (1983). In Y. Suzuki; T. Kishigami; K. Inoue; Y. Mizoguchi; N. Eto; M. Takagi and S. Abe. *Bacillus thermoglucosidasius* sp.nov., a new species of obligately thermophilic bacilli. *Systematic and Applied Microbiology* **4**, 487–495.

SYKES, R.B., CIMARUSTI, C.M., BONNER, D.P., BUSH, K., FLOYD, D.M., GEORGOPAPADAKOU, N.H., KOSTER, W.H., LIU, W.C., PARKER, W.L., PRINCIPE, P.A., RATHNUM, M.L., SLUSARCHYK, W.A., TREJO, W.H. AND WELLS, J.S. (1981). Monocyclic β-lactam antibiotics produced by bacteria. *Nature* **291**, 489–491.

SZABO, I., MARTON, M., FERENCZY, L. AND BUTI, I. (1967). Intestinal microflora of the larvae of St. Mark's fly. II. Computer analysis of intestinal actinomycetes from the larvae of a bibio population. *Acta microbiologia Academiae scientarum hungaricae* **14**, 239–249.

TANI, Y. (1985). Methylotrophs for biotechnology: methanol as a raw material for fermentative production. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed.), volume 3, pp. 111–135. Intercept, Ponteland, Newcastle upon Tyne.

TANI, Y. AND YAMADA, H. (1980). Microbial utilisation of C1 compounds. *Biotechnology and Bioengineering* **22**, 163–175.

TANI, Y., KATO, N. AND YAMADA, H. (1978). Utilisation of methanol by yeast. *Advances in Applied Microbiology* **24**, 165–186.

TAYLOR, S.C., DALTON, H. AND DOW, C.S. (1981). Ribulose-1,5 biphosphate carboxylase/oxygenase and carbon assimilation in *Methylococcus capsulatus* (Bath). *Journal of General Microbiology* **122**, 89–94.

TENDLER, M.D. AND BURKHOLDER, P.R. (1961). Studies on the thermophilic actinomycetes. I. Methods of cultivation. *Applied Microbiology* **9**, 394–399.

TREJO, W.G. (1970). An evaluation of some concepts and criteria used in the speciation of streptomycetes. *Transactions of the New York Academy of Sciences* **32**, 989–997.

TROLLDENIER, G. (1966). Uber die Eignung enthaltender Nährsubstrate zur Zählung und Isolierung von Bodenmikroorganismen auf Membranfiltern. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung II* **120**, 496–508.

TSUKAMURA, M. (1981). Numerical analysis of rapidly growing, nonphotochromogenic mycobacteria, including *Mycobacterium agri* (Tsukamura 1972) Tsukamura sp. nov. nom. rev. *International Journal of Systematic Bacteriology* **31**, 247–258.

UFFEN, R.L. (1975). Utilisation of CO by bacteria isolated from river and pond sediments. *Abstracts of the Annual Meeting of the American Society for Microbiology* **75**, 131.

UFFEN, R.L. (1976). Anaerobic growth of *Rhodopseudomonas* spp. in the dark with carbon monoxide as sole carbon and energy source. *Proceedings of the National Academy of Sciences of the United States of America* **73**, 3298–3302.

UFFEN, R.L. (1981). Metabolism of carbon monoxide. *Enzyme Microbiology and Technology* **3**, 197–206.

VAN DIJKEN, J.P., HARDER, W. AND QUAYLE, J.R. (1981). Energy transduction and carbon assimilation in methylotrophic yeasts. In *Microbial Growth on C1 Compounds. Proceedings of the Third International Symposium at the University of Sheffield, 12–16 August, 1980* (H. Dalton, Ed.), pp. 191–201. Heyden, London.

VARY, P.S. AND JOHNSON, M.J. (1967). Cell yield of bacteria grown on methane. *Applied Microbiology* **15**, 1473–1478.

VASEY, R.B. AND POWELL, K.A. (1984). Single-cell protein. In *Biotechnology and Genetic Engineering Reviews* (G.E. Russell, Ed.), volume 2, pp. 285–311. Intercept, Ponteland, Newcastle upon Tyne.

VASILYEVA, L.V. (1985). *Stella*, a new genus of soil prosthecobacteria, with proposals

for *Stella humosa* sp. nov. and *Stella vacuolata* sp. nov. *International Journal of Systematic Bacteriology* **35**, 518–521.

VICKERS, J.C., WILLIAMS, S.T. AND ROSS, G.W. (1984). A taxonomic approach to selective isolation of streptomycetes from soil. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes* (L. Ortiz-Ortiz, L.F. Bojalil and V. Yakoleff, Eds), pp. 553–561. Academic Press, Orlando.

WAYNE, L.G., DIETZ, T.M., GERNEZ-RIEUX, C., JENKINS, P.A., KÄPPLER, W., KUBICA, G.P., KWAPINSKI, J.B.G., MEISSNER, G., PATTYN, S.R., RUNYON, E.H., SCHRÖDER, K.H., SILCOX, V.A., TACQUET, A., TSUKAMURA, M. AND WOLINSKY, E. (1971). A cooperative numerical analysis of scotochromogenic slowly growing mycobacteria. *Journal of General Microbiology* **66**, 255–271.

WAYNE, L.G., ENGEL, H.W.B., GRASSI, C., GROSS, W., HAWKINS, J., JENKINS, P.A., KÄPPLER, W., KLEEBERG, H.H., KRASNOW, I., NEL, E.E., PATTYN, S.R., RICHARDS, P.A., SHOWALTER, S., SLOSAREK, M., SZABO, I., TÁRNOK, I., TSUKAMURA, M., VERGMANN, B. AND WOLINSKY, E. (1976). Highly reproducible techniques for use in systematic bacteriology in the genus *Mycobacterium*: Tests for niacin and catalase and for resistance to isoniazid, thiophene 2-carboxylic hydrazide, hydroxylamine and *p*-nitrobenzoate. *International Journal of Systematic Bacteriology* **26**, 311–318.

WAYNE, L.G., ANDRADE, L., FROMAN, S., KÄPPLER, W., KUBALA, E., MEISSNER, G. AND TSUKAMURA, M. (1978). A cooperative numerical analysis of *Mycobacterium gastri, Mycobacterium kansasii* and *Mycobacterium marinum*. *Journal of General Microbiology* **109**, 319–327.

WAYNE, L.G., KRICHEVSKY, E.J., LOVE, L.L., JOHNSON, R. AND KRICHEVSKY, M.I. (1980). Taxonomic probability matrix for use with slowly growing mycobacteria. *International Journal of Systematic Bacteriology* **30**, 528–538.

WELLINGTON, E.M.H. AND CROSS, T. (1983). Taxonomy of antibiotic-producing actinomycetes and new approaches to their selective isolation. In *Progress in Industrial Microbiology* **17** (M.E. Bushell, Ed.), pp. 7–36. Elsevier, Amsterdam.

WHITTENBURY, R., PHILLIPS, K.C. AND WILKINSON, J.F. (1970). Enrichment, isolation and some properties of methane-utilising bacteria. *Journal of General Microbiology* **61**, 205–218.

WILLCOX, W.B., LAPAGE, S.P., BASCOMB, S. AND CURTIS, M.A. (1973). Identification of bacteria by computer: theory and programming. *Journal of General Microbiology* **77**, 317–330.

WILLCOX, W.B., LAPAGE, S.P. AND HOLMES, B. (1980). A review of numerical methods in bacterial identification. *Antonie van Leeuwenhoek* **46**, 233–299.

WILLEMSE-COLLINET, M.E., TROMP, T.F.J. AND HUIZINGA, T. (1980). A simple and rapid computer-assisted technique for the identification of some selected *Bacillus* species using biochemical tests. *Journal of Applied Bacteriology* **49**, 385–394.

WILLIAMS, S.T. (ED.) (1987). *Bergey's Manual of Systematic Bacteriology, Volume 4*. Williams and Wilkins, Baltimore, in press.

WILLIAMS, S.T. AND DAVIES, F.L. (1965). Use of antibiotics for selective isolation and enumeration of actinomycetes in soil. *Journal of General Microbiology* **38**, 251–261.

WILLIAMS, S.T. AND FLOWERS, T.H. (1978). The influence of pH on starch hydrolysis of neutrophilic and acidophilic streptomycetes. *Microbios* **20**, 99–106.

WILLIAMS, S.T. AND MAYFIELD, C.I. (1971). Studies on the ecology of actinomycetes in soil. III. The behaviour of streptomycetes in acid soil. *Soil Biology and Biochemistry* **3**, 197–208.

WILLIAMS, S.T. AND ROBINSON, C.S. (1981). The role of streptomycetes in decomposition of chitin in acidic soils. *Journal of General Microbiology* **127**, 55–63.

WILLIAMS, S.T. AND WELLINGTON, E.M.H. (1982a). Principles and problems of selective isolation of microbes. In *Bioactive Products: Search and Discovery* (J.D. Bu'Lock, L.J. Nisbet and D.J. Winstanley, Eds), pp. 9–26. Academic Press, London.

WILLIAMS, S.T. AND WELLINGTON, E.M.H. (1982b). Actinomycetes. In *Methods of Soil Analysis, part 2, Chemical and Microbiological Properties*, 2nd edn. (A.L. Page, R.H. Miller and D.R. Keeney, Eds), pp. 969–987. American Society of Agronomy/Soil Science Society of America, Madison.

WILLIAMS, S.T., GOODFELLOW, M. AND VICKERS, J.C. (1984). New microbes from old habitats? In *The Microbe 1984, II: Prokaryotes and Eukaryotes* (D.P. Kelly and N.G. Carr, Eds), pp. 219–256. Cambridge University Press, Cambridge.

WILLIAMS, S.T., LANNING, S. AND WELLINGTON, E.M.H. (1984). Ecology of actinomycetes. In *The Biology of the Actinomycetes* (M. Goodfellow, M. Mordarski and S.T. Williams, Eds), pp. 481–528. Academic Press, London.

WILLIAMS, S.T., VICKERS, J.C. AND GOODFELLOW, M. (1985). Application of new theoretical concepts to the identification of streptomycetes. In *Computer-assisted Bacterial Systematics* (M. Goodfellow, D. Jones and F.G. Priest, Eds), pp. 289–306. Academic Press, London.

WILLIAMS, S.T., DAVIES, F.L., MAYFIELD, C.I. AND KHAN, M.R. (1971). Studies on the ecology of actinomycetes in soil. II. The pH requirements of streptomycetes from two acid soils. *Soil Biology and Biochemistry* **3**, 187–195.

WILLIAMS, S.T., SHAMEEMULLAH, M., WATSON, E.T. AND MAYFIELD, C.I. (1972). Studies on the ecology of actinomycetes in soil. VI. The influence of moisture tension on growth and survival. *Soil Biology and Biochemistry* **4**, 215–225.

WILLIAMS, S.T., GOODFELLOW, M., ALDERSON, G., WELLINGTON, E.M.H., SNEATH, P.H.A. AND SACKIN, M.J. (1983a). Numerical classification of *Streptomyces* and related genera. *Journal of General Microbiology* **129**, 1743–1813.

WILLIAMS, S.T., GOODFELLOW, M., WELLINGTON, E.M.H., VICKERS, J.C., ALDERSON, G., SNEATH, P.H.A., SACKIN, M.J. AND MORTIMER, A.M. (1983b). A probability matrix for identification of some streptomycetes. *Journal of General Microbiology* **129**, 1815–1830.

WILLIAMS, S.T., VICKERS, J.C., GOODFELLOW, M., ALDERSON, G., WELLINGTON, E.M.H., SNEATH, P.H.A., SACKIN, M.J. AND MORTIMER, A.M. (1984). Numerical classification and identification of streptomycetes. In *Biological, Biochemical and Biomedical Aspects of Actinomycetes* (L. Ortiz-Ortiz, L.F. Bojalil and V. Yakoleff, Eds), pp. 537–551. Academic Press, Orlando.

WILLIAMS, S.T., LOCCI, R., VICKERS, J., SCHOFIELD, G.M., SNEATH, P.H.A. AND MORTIMER, A.M. (1985). Probabilistic identification of *Streptoverticillium* species. *Journal of General Microbiology* **131**, 1681–1689.

WOESE, C.R., STACKEBRANDT, E., WEISBURG, N.G., PASTER, B.J., MADIGAN, M.T., FOWLER, V.J., HAHN, C.M., BLANZ, P., GUPTA, R., NEALSON, K.H. AND FOX, G.E. (1984). The phylogeny of purple bacteria: the alpha subdivision. *Systematic and Applied Microbiology* **5**, 315–326.

WOLF, H.J. (1981). Biochemical characterisation of methane oxidising yeasts. In *Microbial Growth on C1 Compounds. Proceedings of the Third International Symposium at the University of Sheffield, 12–16 August, 1980* (H. Dalton, Ed.), pp. 202–210. Heyden, London.

WOLF, H.J. AND HANSON, R.S. (1979). Isolation and characterisation of methane-utilising yeasts. *Journal of General Microbiology* **114**, 187–194.

YOSHIZUMI, H. (1975). A malo-lactic bacterium and its growth factor. In *Lactic Acid Bacteria in Beverages and Food* (J.G. Carr, C.V. Cutting and G.C. Whiting, Eds), pp. 87–102. Academic Press, New York and London.

ZEHNDER, A.J.B. AND BROCK, T.D. (1979). Methane formation and methane oxidation by methanogenic bacteria. *Journal of Bacteriology* **137**, 420–432.

ZEIKUS, J.D., DAWSON, M.A., THOMPSON, T.E., INGVORSEN, K. AND HATCHIKIAN, E.C. (1983). Microbial ecology of volcanic sulphidogenesis: isolation and characterisation of *Thermosulfobacterium commune* gen. nov. and sp. nov. *Journal of General Microbiology* **129**, 1159–1169.

ZILLIG, W., HOLZ, I., JANEKOVIC, D., SCHÄFER, W. AND REITER, W.D. (1983). The archaebacterium *Thermococcus celer* represents a novel genus within the

thermophilic branch of the archaebacteria. *Systematic and Applied Microbiology* **4**, 88–94.

ZILLIG, W., STETTER, K.O., SCHÄFER, W., JANEKOVIC, D., WUNDERL, S., HOLZ, I. AND PALM, P. (1981). *Thermoproteales:* a novel type of extremely thermoacidophilic anaerobic archaebacteria isolated from Icelandic sulfataras. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung I, Originale C*, 205–227.