OUTLINE OF MALAYSIA TORAY SCIENCE FOUNDATION

Foundation Establishment

The Malaysia Toray Science Foundation was established in 1993 through a RM4 million endowment by Toray Industries, Inc., Japan. The Foundation is registered with and recognized by the Malaysian authorities as a charitable organization formed to promote and advance science and technology in Malaysia.

Foundation Objective

To contribute to the progress and advancement of "Science and Technology" in Malaysia.

Foundation Activities

To achieve its Objective, the Foundation undertakes the following programmes :

- Presentation of the Science and Technology Awards, in recognition of outstanding achievements in the field.
- Presentation of the Science and Technology Research Grants, to provide financial assistance for basic research.
- Presentation of the Science Education Awards, in recognition of creative and innovative contributions to effective science education in secondary schools and pre-university colleges.

"Science and Technology" is limited to the fields of natural sciences, including the environment but excluding clinical medicine and mathematics.

Scale of Foundation Operations

The annual scale of operations is approximately RM600,000 financed by operating income from the MTSF endowment fund and contributions from Toray Science Foundation, Japan and Toray subsidiaries in Malaysia, Toray Malaysia Group of Companies.

Awards and Grants

- *MTSF Science and Technology Awards (STA)*
- *MTSF Science and Technology Research Grants (STRG)*
  (inclusive of grants funded by Toray Science Foundation, Japan)
- *MTSF Science Education Awards (SEA)*

MESSAGE FROM CHAIRMAN OF MTSF

This book is a compilation of the winning entries of the MTSF Science & Technology Award and the completed research projects under the MTSF Science and Technology Research Grant from Year 1994 – 2008. It is another venture in line with MTSF's continuous effort to promote science and technology in all levels of education. MTSF believes that a comprehensive strategy to advance science and technology starts with fostering a culture of innovation. Our hope is that this book will inspire young scientists and researchers to pursue excellence and venture into untapped fields of science and technology and contribute to the advancement of knowledge for the humanity.

On behalf of MTSF, I would like to thank the members of the Selection Committee for their efforts in assessing and selecting the winning entries and MTSF Secretariat for arduous tasks of compiling this book for free distribution to all universities, institutions, awardees and grantees.

MTSF will continue its objective of contributing to the overall progress of Malaysia through science and technology by rendering financial assistance to selected young researchers and awarding accomplished scientists.

Tan Sri Dato' Seri Law Hieng Ding

MESSAGE FROM CHAIRMAN OF SELECTION COMMITTEE

Since MTSF's inception, the Selection Committee has had the most rewarding time in assessing, interviewing and awarding worthy scientists and researchers for the Science & Technology Award and Science & Technology Research Grant programmes. For the past 15 years, the submissions were of the high standards and qualities that made our task very difficult indeed.

The publication highlights the winning entries of the MTSF Science & Technology Award and the completed research projects of the recipients of the Science & Technology Research Grants. It will undoubtedly serve as a good reference and resource book for everyone who are interested in basic fundamental scientific research.

Taking this opportunity, I wish to thank all the winners and grantees for their contributions, as well as the Selection Committee Members and MTSF Secretariat for their commitment and untiring effort leading to the successful compilation of this first book of records.

Academician Professor Emeritus
Tan Sri Dr. Augustine Ong

BOARD AND COMMITTEE MEMBERS

HONORARY CHAIRMAN

YBhg. Tan Sri Dato' (Dr.) Katsunosuke Maeda

(Honorary Chairman of Toray Industries, Inc., Japan)

BOARD MEMBERS

Chairman

YBhg. Tan Sri Dato' Seri Law Hieng Ding

Managing Director

Mr. Munehiro Se (Representative of Toray Japan in Malaysia)

Directors

YBhg. Academician Tan Sri Dr. Omar Abdul Rahman

YBhg. Academician Professor Emeritus Tan Sri Dr. Augustine Ong Soon Hock

YBhg. Professor Emeritus Tan Sri Dr. Syed Jalaludin Syed Salim

YBhg. Academician Datuk Dr. M Jegathesan

Mr. Yasuhiko Sasada

Secretary

YBhg. Dato' Lee Ow Kim

SELECTION COMMITTEE MEMBERS

(Science & Technology Award and Science & Technology Research Grant)

- YBhg. Academician Professor Emeritus Tan Sri Dr. Augustine Ong Soon Hock
- YBhg. Academician Tan Sri Dr. Omar Abdul Rahman
- YBhg. Academician Datuk Dr. M Jegathesan
- Professor Dr. Chia Swee Ping
- Academician Professor Emeritus Dr. Yong Hoi Sen
- YBhg. Professor Dato' Goh Sing Yau

EXAMINATION COMMITTEE MEMBERS

(Science Education Award)

- YBhg. Professor Emeritus Tan Sri Dr. Syed Jalaludin Syed Salim
- YBhg. Dato' Dr. R Ratnalingam
- Dr. Chuah Chong Cheng
- YBhg. Ir. Professor Dato' Dr. Chuah Hean Teik
- Dr. Azian Binti Tengku Syed Abdullah
- Professor Dr. Siow Heng Loke

TABLE OF CONTENTS

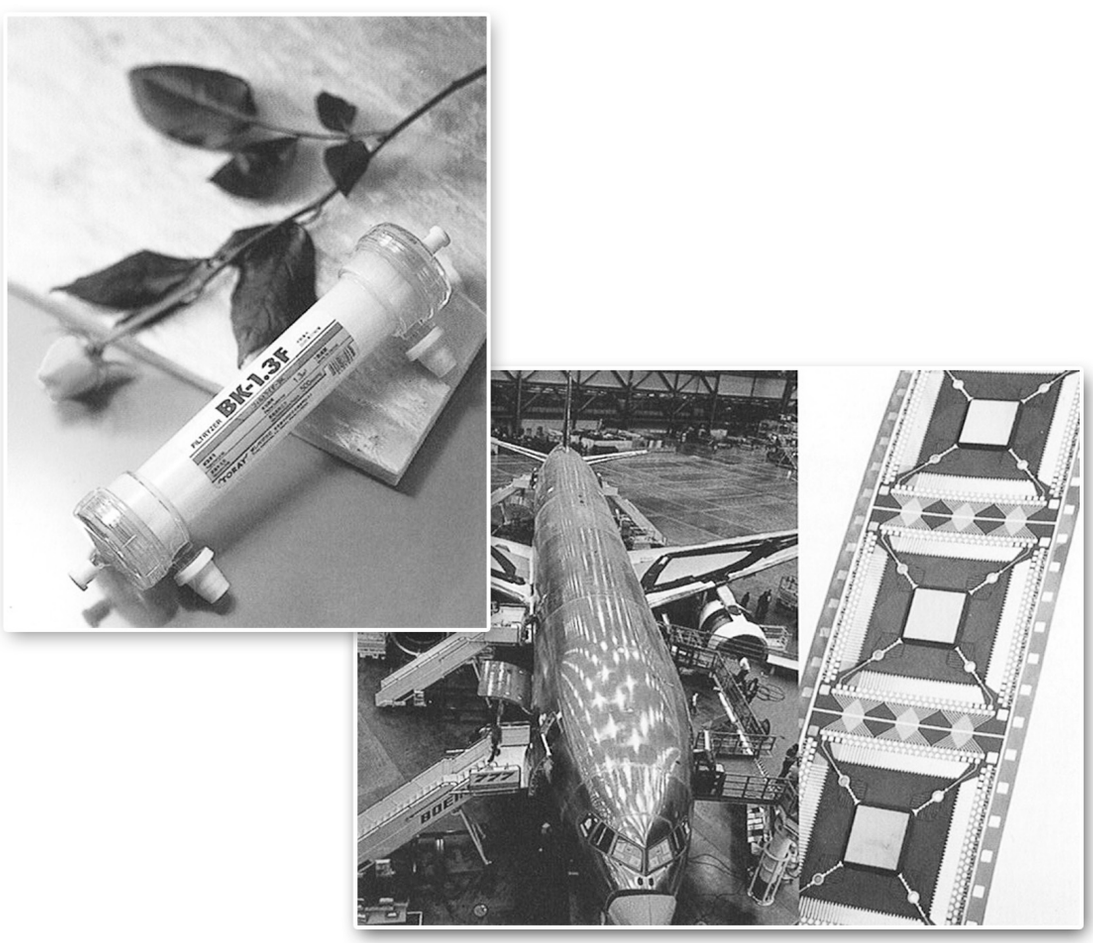
TORAY INDUSTRIES, INC., JAPAN

Toray Industries, Inc., Japan was established in 1926. It now operates on a global scale to meet the needs and expectations of its customers, shareholders and employees.

Leveraging on its core technologies of organic synthetic chemistry, polymer chemistry and biochemistry, Toray positions fibres & textiles and plastics & chemicals as foundation businesses while expanding IT-related products, carbon firbre composite materials, environmental products and life sciences as strategic growth businesses.

Apart from manufacturing, Toray is becoming increasingly active in areas such as fashion, trade and information.

Toray has thus positioned itself to enjoy sustainable corporate growth while fulfilling its corporate social responsibilities to the local communities.



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Chuo-ku, Tokyo 103-8666 Japan
Tel : (03) 3245-5111, Fax : (03) 3245-5555
Website : www.toray.co.jp

Establishment

The Toray Science Foundation was established on June 23, 1960, through a ¥1 billion endowment by Toray Industries, Inc., Japan. Subsequent donations have increased the Foundation's basic fund to ¥1.8 billion. The Foundation is recognized by the Japanese Ministry of Education and the Science and Technology Agency.

Prospectus

Building a firm foundation of scientific and technological progress is essential in the current age to contribute to the future prosperity of our country. To achieve this objective, we believe it is of greatest importance to create propitious circumstances under which scientific and technological development can proceed. The prominence of the scientific and technological innovations made in the United States and European countries gives impetus to the need for Japan to upgrade and advance scientific and technological standards to enable the country to successfully compete in the international arena.

Toray Science Foundation aims to contribute to achieving these objectives through offering support for national scientific and technological advancement as well as providing opportunities for public enlightenment in these areas. The Foundation assists scientists and researchers from academic areas through grants and access to reference materials and awards prizes to persons who have made outstanding achievements to contribute to scientific and technological progress. It is the Founder's hope that through their activities made possible through endowments from Toray Industries, Inc., Japan that our purpose will be fulfilled and we will contribute to the betterment of our society and further national growth.

Activities

The major activities of the Foundation include the following :

1. **Toray Science and Technology Prize :**
   An award honoring outstanding achievements in the field of science and technology.
2. **Toray Science and Technology Grant :**
   Financial assistance to creative young researchers for basic research in science and technology.
3. **Toray Science Education Prize :**
   Recognizes creative and innovative contributions to effective scientific education by junior and senior high school teachers, and the publishing of works that have received the Toray Science Education Prize by
   - Publishing and distributing a series of books about the award-winning works;
   - Producing and lending videotapes featuring award-winning works.
4. Sponsoring of lectures on scientific topics for the general audience.
5. Toray Science Foundation International Research Grant, which subsidizes basic research projects in the field of natural science at overseas universities and research institutes.
6. Sponsoring of other events to promote the Foundation's objectives.

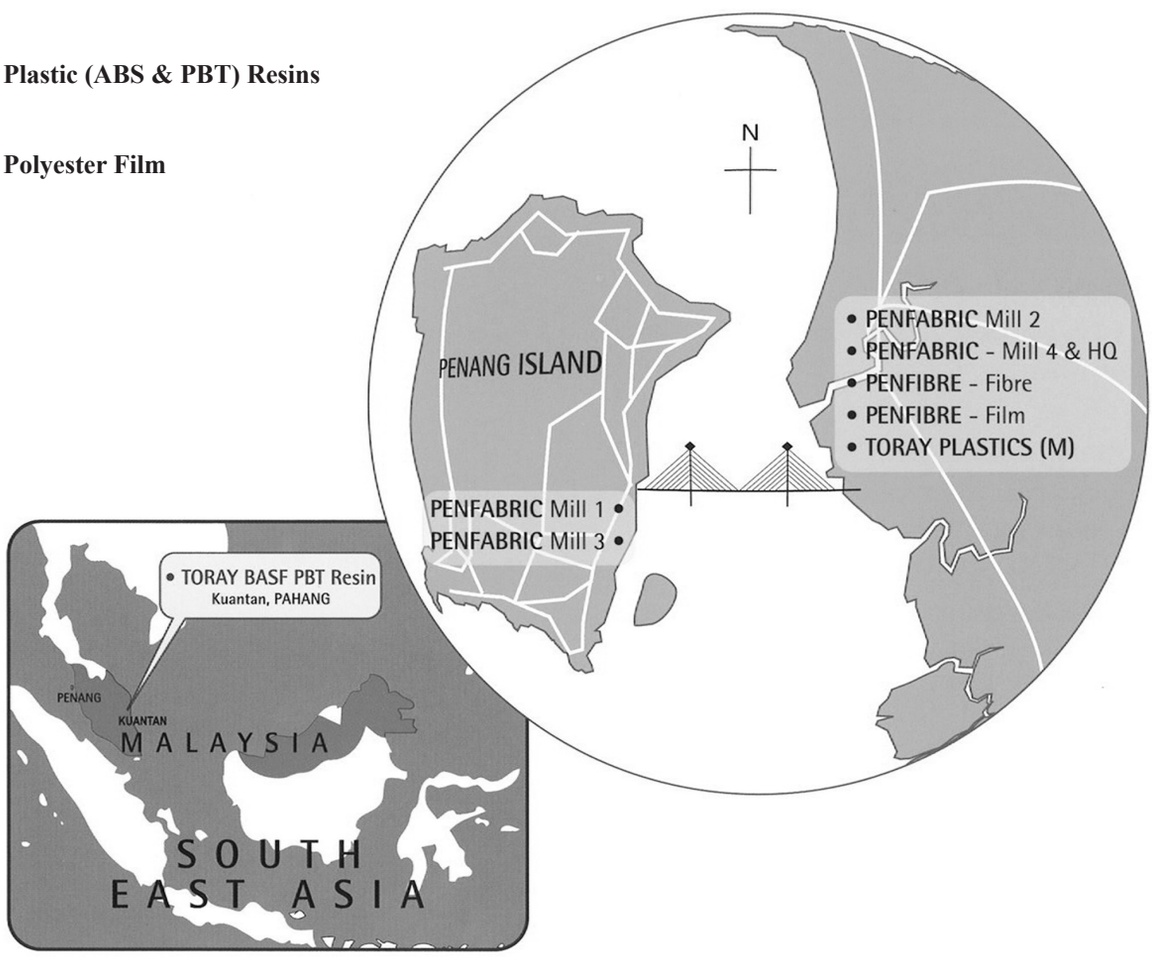
TORAY MALAYSIA GROUP

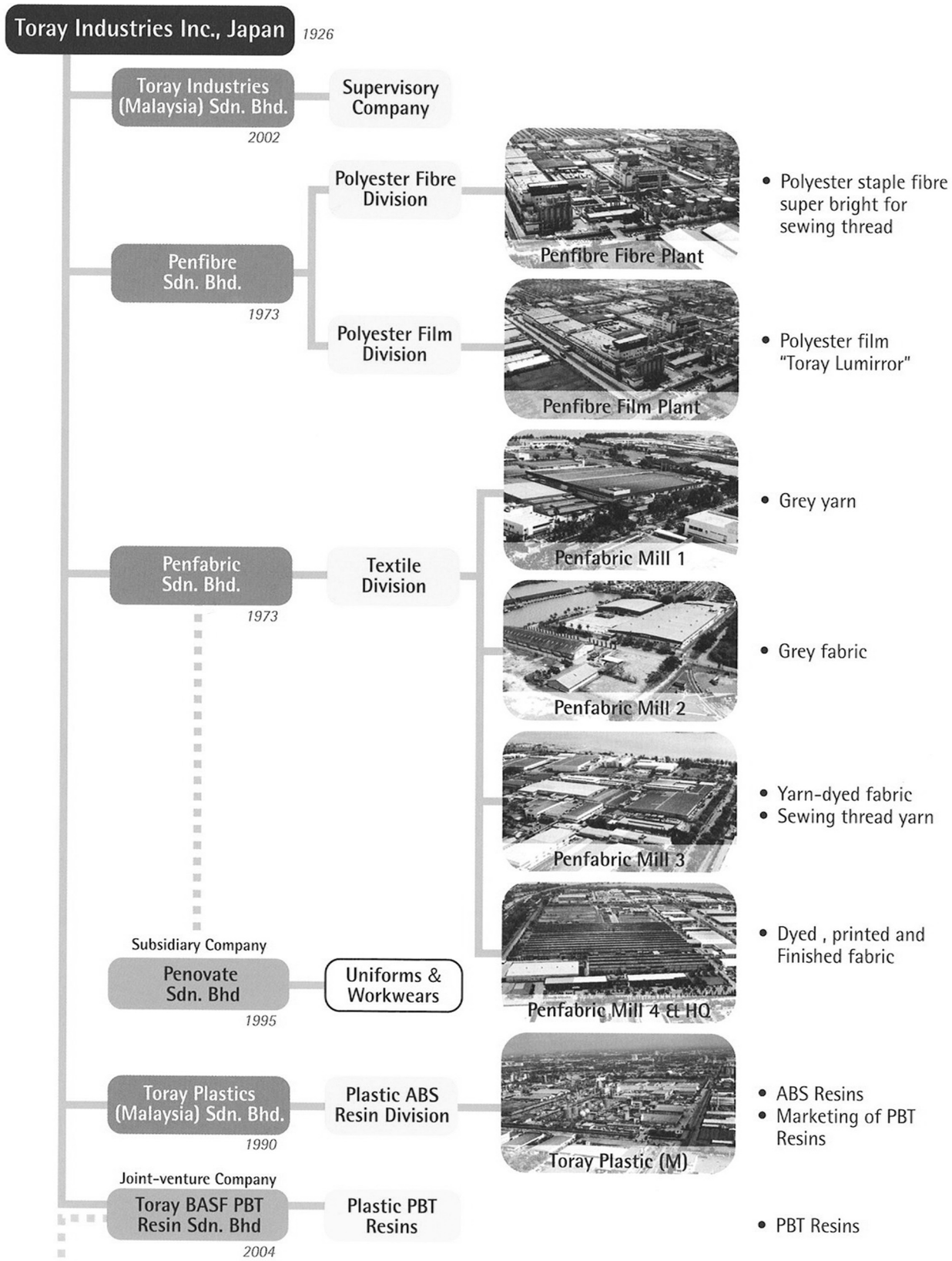
Toray Industries Inc., Japan is a chemical conglomerate boasting of top-notch technologies and products. Owing to its globalization policy, Toray has evolved into a strong multinational organization with diversified businesses worldwide, a true pioneer in promoting Malaysia's industrial growth since the 1970s.

TORAY MALAYSIA GROUP of Companies, which operates 7 manufacturing plants in Penang's strategic industrial parks and 1 joint-venture project in Kuantan, Pahang represents one of Toray's major investments outside Japan.

Under the supervision of **Toray Industries (Malaysia),** today Toray's multi-million Ringgit investments in **Penfibre, Penfabric, Toray Plastics (Malaysia)** and T**oray BASF PBT Resin** encompass the following key business areas :

- **Polyester Fibre**
- **Textiles**
- **Plastic (ABS & PBT) Resins**
- **Polyester Film**




Toray Industries Inc., Japan
1926
Toray Industries (Malaysia) Sdn. Bhd.
2002
Supervisory Company
Penfibre Sdn. Bhd.
1973
Polyester Fibre Division
Penfibre Fibre Plant
• Polyester staple fibre super bright for sewing thread
Polyester Film Division
Penfibre Film Plant
• Polyester film "Toray Lumirror"
Penfabric Sdn. Bhd.
1973
Textile Division
Penfabric Mill 1
• Grey yarn
Penfabric Mill 2
• Grey fabric
Penfabric Mill 3
• Yarn-dyed fabric
• Sewing thread yarn
Penfabric Mill 4 & HQ
• Dyed , printed and Finished fabric
Subsidiary Company
Penovate Sdn. Bhd
1995
Uniforms & Workwears
Toray Plastics (Malaysia) Sdn. Bhd.
1990
Plastic ABS Resin Division
Toray Plastic (M)
• ABS Resins
• Marketing of PBT Resins
Joint-venture Company
Toray BASF PBT Resin Sdn. Bhd
2004
Plastic PBT Resins
• PBT Resins

MTSF SCIENCE & TECHNOLOGY AWARD

LIST OF AWARDEES FROM 1994 TO 2008

SUMMARY OF ACHIEVEMENTS

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY AWARD

LIST OF AWARDEES (1994 - 2008)

Year Awarded	Name of Recipient	Name of University/ Institution	Field of Specialization/ Research
1994	Academician Professor Emeritus Dr. Yong Hoi Sen	Universiti Malaya	Genetics, Systematics, Evolutionary Biology and Biodiversity
	Dr. Hoi Why Kong	Forest Research Institute Malaysia	Fuel Technology - Conversion of Biomass Residues into Solid, Liquid and Gaseous Fuels
1995	Dr. Lim Boo Liat	Wildlife and National Parks	Vertebrate Ecology (small mammals, reptiles and amphibians) Parasitology of small mammals in relation to zoonotic diseases, Control of Vector-borne diseases and relationship between diseases and the Environment
	Dr. Abdul Latiff Mohamad	Universiti Kebangsaan Malaysia	Plant Taxonomy, Ethnobotany and Plant Diversity
1996	Professor Fon Wai Chu	Universiti Malaya	Theory of Atomic collison processes Atomic & Molecular Data for Fusion
	Professor Dr Koh Chong Lek	Universiti Malaya	Molecular Genetics and Molecular Biology (including recombinant DNA Technology or Genetic Engineering)
1997	Dr. Ho Chee Cheong	Universiti Malaya	Colloid and Surface Chemistry of Dispersed Systems, in particular (1) Natural Rubber Latex, (2) Palm Oil Emulsions, (3) Tin Tailings Slurry (ponds), (4) Waste-water Discharge from Factories and Mills, and (5) Solid-phase Immunoassay
	Professor Dr. Abdul Salam Abdullah and Group Members	Universiti Putra Malaysia	Anti-Nurtritional Factors in local feedstuffs
1998	Professor Yap Han Heng	Universiti Sains Malaysia	Medical Entomology especially Biology and Control of Mosquitoes and other Household Insect Pests (Cockroaches and Ants)
	Professor Dr. Mak Chai @ Mak Lian Fong	Universiti Malaya	Plant Breeding and Biotechnology
1999	Professor Dato' Dr. Khairul Anuar B Abdullah	Universiti Malaya	Parasitology, Immunology and Molecular Biology especially in the field of Toxoplasma and Filaria
	Ir Professor Dato' Dr. Chuah Hean Teik	Multimedia University	Microwave Remote Sensing : Theoretical Modelling, Sensor Development and Applications

2000	Dr. Chan Ying Kwok and Group Members	Malaysian Agricultural Research & Development Institute	Breeding and selection for improvement of Papaya and Pineapple varieties
	Dr. Ng Seik Weng	Universiti Malaya	Chemical crystallagraphy of Organotin Chemicals
2001	Dato' Dr. Choo Yuen May	Malaysian Palm Oil Board	Development of Efficient and Green processes for the Palm-based Industry
	Dr. Tee E Siong	Institute for Medical Research	Human Nutrition
2002	Professor Tou Teck Yong and Group Members	Multimedia University	Laser Systems and Applications
	Professor Dr. Asma Binti Ismail and Group Members	Universiti Sains Malaysia	Medical Microbiology, Medical Biotechnology, Rapid Diagnosis of Infectious Diseases especially on Typhoid and Paratyphoid fevers and Development of new technology platforms in especially for Rapid Diagnosis of Diseases
2003	Dr. Lee Han Lim	Institute for Medical Research	Research and Development of Innovative approaches for the control of Insect Vectors of public health importance
	Associate Professor Dr. Zarita Bt Zainuddin	Universiti Sains Malaysia	Neural Networks
2004	Professosr Dr. Kurunathan a/l Ratnavelu	Universiti Malaya	Theoretical Atomic Collision Physics
	Professor Dr. Yaakob B Che Man	Universiti Putra Malaysia	Instrumental methods for Fats and Oil Analysis with special emphasis on Palm Oil products
2005	Professor Dr. Wong Chiow San	Universiti Malaya	Plasma Technology, Pulse Power & Gas discharge Physics
	Professor Dr. Soh Aik Chin	Applied Agricultural Resources	Plant Breeding & Genetics
2006	Professor Dr. Chua Kaw Bing	National Public Health Laboratory, Ministry of Health	Clinical and Molecular Virology
	Professor Dr. Roslan Abd Shukor	Universiti Kebangsaan Malaysia	Condensed Matter Physics - High Temperature Superconductivity
2007	Professor Dr. Harith Bin Ahmad	Universiti Malaya	Photonics and Laser Technologies
	Professor Dr. Thong Kwai Lin	Universiti Malaya	Molecular Microbiology of Foodborne and Nosocomial Bacterial Pathogens
2008	Professor Dr. G. Suresh Kumar	Universiti Malaya	Medical Parasitology
	Professor Dr. Abdul Latif Ahmad	Universiti Sains Malaysia	Membrane Separation Technology, Wastewater Treatment, Separation Technology

ACADEMICIAN PROFESSOR EMERITUS DR. YONG HOI SEN

Universiti Malaya

"Genetics, Systematics, Evolutionary Biology and Biodiversity "
Year 1994 MTSF Science & Technology Award Recipient

Academician Professor Emeritus Dr Yong Hoi Sen is an outstanding biologist/scientist who has since the 1960s contributed greatly to the knowledge of science, particularly the life sciences. He was among the pioneers to adopt a holistic and innovative approach to solving problems in biosystematics, employing both classical and modern methods. He has published some 300 articles on various aspects of biology and four books on natural history, and edited many academic works. Some of his research findings have direct applications in clinical and agricultural practices. In addition to his significant and pioneering research achievements and scientific contributions, Dr Yong has been actively involved in the advancement and dissemination of knowledge and awareness of science and technology. He founded and edited the fully illustrated quarterly NATURE MALAYSIANA (the only publication of this kind in this part of the world) for 20 years. Dr Yong's expertise has been sought after both locally and overseas. His achievements and contributions have been duly and widely recognized locally and internationally. He is a Foundation and Senior Fellow of the Academy of Sciences Malaysia, and Professor Emeritus in Genetics and Zoology at University of Malaya.

Contribution and Achievement

Academician Professor Emeritus Dr Yong Hoi Sen is an outstanding biologist/scientist in Malaysia and the surrounding region. He was the first Malaysian to be appointed as Professor for the Chair of Zoology at University of Malaya. It's noteworthy that he received his entire tertiary education at the University of Malaya, at a time when facilities and expertise were still at the infancy stage. Despite having to work under very difficult conditions and not having the benefits/guidance from established researchers/scientists, Dr Yong has gained fairly wide experience in both classical and modern biology, basic and applied biology, and laboratory (experimental) and field biology. This is already evidenced in his PhD thesis, which incorporated both classical (anatomy, morphology) and modern (behaviour, ecology, cytogenetics, molecular biology, serology) approaches in systematics.

Since the 1960s, Dr Yong has contributed greatly to the knowledge of genetics, systematics, evolutionary biology, biological diversity, and basic biology of the Malaysian natural heritage. Dr Yong was among the pioneers to adopt a holistic and innovative approach to solving problems in biological systematics, employing both classical and modern methods. He has published over 300 articles and four books on the fauna and flora of Malaysia. In addition, he has edited several journals, magazines, monographs, encyclopedia, proceedings and books. The Citation Databases of the Institute for Scientific Information (ISI) listed at least 109 of his papers in the 1990s.

Among Dr Yong's outstanding research achievements was the significant discovery for the first time in the world of X-monosomy (presence of a single X-chromosome instead of the normal two in female individuals) in the house rat *(Rattus rattus*) in 1971 (published in the premier journal NATURE) and in wild Norway rat (*Rattus norvegicus*) in 1989. Unlike XO humans (with a single X-chromosome, known as Turner's syndrome) and larger mammals that are infertile, XO female rats are fertile (due to short maturation period). After Dr Yong's discovery, some 10 other cases have been reported throughout the world.

Dr Yong was also the first to report: (1) centric fusion (apparent reduction of chromosome number due to fusion without loss of genetic material; 1971) and accessory (B-) chromosomes (presence of extra chromosomes; 1972) in the house rat; (2) Robertsonian translocations (gain or reduction of chromosome number without gain/loss of genetic material) in the house shrew *Suncus murinus* (1971, 9172, 1973); and (3) the chromosomes of a variety of Malaysian animals. Together with M. Volleth (Germany), they reported the first known Old-World bat with X-autosome translocation in 1987. More recently, he reported female heterogamety (producing two kinds of gametes; in most animals the male produces two kinds of gametes) in the frog *Rana paramacrodon* and the lowest chromosome number (2n=20) for frogs of the genus *Rana* in the world (1994).

In addition to the finding of unique/unusual phenomena, Dr Yong's studies on chromosomes have been gainfully employed in taxonomy/systematics and clinical diagnosis. For example, Dr Yong demonstrated without doubt that two sibling species (that is, morphologically very similar) of rat are genetically distinct and are valid species, with one possessing 36 chromosomes and the other 52 chromosomes – prior to that (1972) these two species had been also regarded as colour phases of one species.

For many years Dr Yong provided free cytogenetic services to the University Hospital and other hospitals and clinics for the diagnosis/confirmation of specific diseases/disorders due to chromosomal abnormalities. In this respect he provided letters of confirmation, particularly to expatriates returning home so that their children could be admitted to special institutions.

In 1972, Dr Yong confirmed that subline differentiation was a continuing process in inbred lines of mice. This phenomenon has an important bearing on the practice of including a control group in conducting scientific experiments involving animals and other organisms.

Although extensively and intensively studied elsewhere in the world, particularly U.S.A., Dr Yong first reported the phenomenon of isocitrate dehydrogenase gene duplication (presence of two copies of the gene for this enzyme) in soya bean (1981). This marker can be used for cultivar and seed identification.

During the 1980s, Dr Yong initiated the genetic studies on vectors, parasites, pests and their hosts. This endeavour has increased our knowledge (both basic and applied) concerning mosquito vectors, parasites of public health importance, and insects of agriculture importance. For example, Dr Yong had confirmed that: (1) the Malaysian schistosome parasite was a new species, now named as *Schistosoma malayensis;* (2) the filarial worm subperiodic *Brugia malayi* was genetically heterogeneous; (3) the filarial worms *Brugia malayi* and *Brugia pahangi* were genetically distinct; (4) the Cocoa Pod Borers (a moth) from various host plants were conspecific (of the same species); (5) host specificity in tephritid fruit flies was directly related to genetic diversity; and (6) many plants contain specific attractants for male tephritid fruit flies – these attractants could be used for monitoring and control purposes. A molecular marker discovered by Praphathip Eamsobhana (a PhD student from Thailand under SEAMEO-TROPMED Scholarship) for the diagnosis or confirmation of human angiostrongyliasis (a disease due to lung worm) has been gainfully employed in Thailand.

In 1988, Dr Yong confirmed the first case of direct development in the frog *Philautus*. Unlike other frogs and toads whose tadpoles are free swimming, development of this *Philautus* takes place completely within the egg (as in birds and reptiles). This provides an example for the evolutionary process of conquering the land.

Over the years, Dr Yong has confirmed the occurrence of a number of insect pests in Malaysia and found many new species of organisms. Noteworthy among the new species is *Trypanosoma raksasa* (1994; discovered with Akira Miyata of Oita Medical University) from the University of Malaya campus – this is the largest trypanosome parasite of frogs and toads in the world. In addition, Dr Yong has discovered many rare species and other living organisms that are new records for the country.

As a mark of honour and recognition of his contributions in the fields of science, the following species of organisms have been named after Dr Yong by foreign scientists: (1) *Topomyia (Topomyia) yongi* – 1990; (2) *Trypanosoma yongi* – 1992; (3) *Johora hoiseni* – 1992; (4) *Aesalus yongi* – 1993 [now *Echinoaesalus yongi*]; (5) *Pardalaspinus yongi* – 1995; (6) *Duliticola hoiseni* – 1996; (7) *Simulium yongi* – 1997; (8) *Cladomyrma yongi* – 1999; (9) *Manota yongi* – 2006; (10) *Simulium (Gomphostilbia) hoiseni* – 2008; and (11) *Malayozodarion hoiseni* – 2008.

In addition to the Malaysia Toray Science Foundation First Science and Technology Award 2004, Dr Yong has also been honoured with the National Science Award 1995 and the First MPKSN Prize for Advancement/Promotion of Public Understanding in Science and Technology 1996.

DR. HOI WHY KONG

Forest Research Institute Malaysia

"Fuel Technology – Conversion of Biomass Residues into Solid, Liquid and Gaseous Fuels"

Year 1994 MTSF Science & Technology Award Recipient

Dr. Hoi Why Kong obtained his PhD from Aston University, Birmingham, U.K. majoring in Chemical Engineering – Fuel Technology. He was the Senior Research Officer on Wood Energy at the Forest Research Institute Malaysia. Dr. Hoi was the FAO short-term consultant on rural wood-based energy industries for 2 years from 1985 to 1986. He had supervised MSc students and BSc (Hons) students from Twente University, Holland and from Universiti Kebangsaan Malaysia. He was the UNDP consultant on anti-pollution technologies in urban and rural areas in ASEAN Region from 1991 to 1993. He was involved in UNDP project on ruminant feed from oil palm trunk from 1989 to 1992. Dr. Hoi was also the consultant for Goodwood Management on wood incinerator system in October 1992. He had won the Petronas Inventors Award in 1991 and FRIM Inventors Award, First Prize in 1993. He had more than 102 technical publications and submitted patents of his works.

Dr. Hoi was actively in the research of the development of methods to increase wood-wastes utilization by converting into solid, liquid and gaseous fuels. Amongst his most notable achievements are white charcoal technology, anti-pollution combustion kiln systems, wood gasification, activated carbon from palm kernel shells and sago residues and biomass-based absorbents for oil-slick control.

His main contribution was to convert biomass residues into convenient fuels and useful chemical by-products through the major route broadly classified as thermochemical nature. Amongst the numerous biomas thermochemical technologies developed are : improved charcoal production technologies (including white charcoal technology), anti-pollution combustion systems, wood and charcoal gasification and pyrolysis technologies.

In improved charcoal production technology, higher efficiency of charcoal production was obtained by improvement in the system of firing, better heat control. The industry was able to modernize efficiently and produce better and consistent quality charcoal for export. The development of a dense form of high quality white charcoal and charcoal briquettes for numerous applications in non-ferrous metallurgical reduction processes and manufacture of calcium carbide, silicon carbide have contributed to the development of the industrial abrasion and ferro silicon industry in Malaysia.

Research in the development anti-pollution combustion system had resulted in substantial savings in mandatory requirements of pollution abatement equipment. Furthermore the operation under controlled conditions also generated a host of useful materials such as amorphous silica from rice husk and wood ash for mulching purposes.

In wood and charcoal gasification, the development of various throat diameters for different desired hearth, tuyere openings at different levels, insulation mechanism of the reduction zone and a variable horizontal and vertical grates had offered flexibilities of changing gasifier loads through different combinations of throat size, tuyere numbers and diameters. With the elimination of choking problems by ash removal through the variable horizontal and vertical grates, the performance and financial attractiveness of gasification systems in Malaysia is greatly enhanced.

In the development of the patented concept of a double pyrolysis double condensation process, useful data on the relationship between the temperature, rate of heating, retention time and the physiochemical nature of the product had enabled a very important pyrolytic products called oleochemical char absorbents to be produced. This product is very useful as an absorbent for oil slick control and has attracted substantial interest both locally and abroad.

DR. LIM BOO LIAT

Wildlife and National Parks

"Vertebrate Ecology (small mammals, reptides and amphibians), Parasitology of small mammals in relation to zoonotic diseases, Control of Vector-borne diseases and relationship between diseases and the Environment"

Year 1995 MTSF Science & Technology Award Recipient

Born in 1926, Lim Boo Liat is a mammalogist/parasitologist with six decades of knowledge and experience on vertebrate biodiversity, particularly the fauna of Malaysia (both east and west Malaysia). Since 1950 to date, he has published 290 scientific and natural history papers and is often invited in local and international conferences. At the age of 83, he is still active in assisting research studies for under-graduate and post-graduate students at the local universities and involved in conservation of natural history in association with government and non-government agencies.

Some of his pioneer research studies include (1) *Angiostrongylus cantenonsis* (rat-lung worm) the causative agent of human eosinophilic meningoencephilitis, (2) the concepts of parasites as "Ecological labeling of the host" and (3) zoonotic filariasis-*Brugia malayi*. The research study on *Angiostrongylus cantonensis* commenced for the first time in 1962. Through field and laboratory experimentations for a span of six years, this worm was elevated as *Angiostronylus malaysiensis* which received world-wide recognition. This resulted in further studies by local and foreign post-graduate students including genetic studies and epidemiology of the disease in Peninsular Malaysia. In the process of his investigation, he established a new mode of human infection through contaminated lectuce with the parasite which is commonly found in salads. The findings correlated with the seasonal outbreaks of the human disease in New Caledonia where lectuce was grown seasonally.

The identification of the natural infection of parasites of two little known small mammals revealed that the intermediate hosts of the Moonrat are fish, that of the Pentail Tree-shrew, are arthropods mainly leaf and grasshaoppers. The results provided valuable information on the food habit and habitat niches of the definitive hosts, the Pentail Tree-shrew being more arboreal while the Moonrat is more associated in the marshy part of the forest. Thus a new concept was evolved by using endo-parasites of animal hosts as an "ecology label". This concept was widely accepted by parasitologists and animal ecologists in the country as well as worldwide.

Study on microfilarial periodicity of *Brugia malayi* microfilaraemic patients from Bukit Papan of East Kalimantan, Jambi of East Sumatra, Kendari of Southeast Sulawesi Province was carried out from 1980-1982. Three distinct forms of periodicity, namely aperiodic, subperiodic and periodic were seen. The percentage of exsheathed microfilariae in the various forms overlapped and is an unsuitable distinguishing feature, while calculated periodicity indices were found to be more specific. Evidence strongly suggests the possibility of *B. malayi* having non-human primates as its natural hosts in primary forests. The study indicates the adaptability of the parasites to available animal hosts (including man) and anopheline mosquito vectors as the environment changed. With this adaptation, the periodicity of the parasite also changed from aperiodic to highly periodic. It is surmised that the distribution of the various forms of *B. malayi* is related to the landscape ecology, reservoir hosts and mosquito vectors.

Lim Boo Liat started his career at the Institute of Medical Research (IMR) Kuala Lumpur in October 1947. As a laboratory technologist he was involved in field biological research under his mentors, the late Professor J.L. Harrison (Medical Zoologist) and late Professor J.R. Audy (Medical Ecologist) specializing in host-parasite relationship in relation to epidemiology of zoonotic diseases. His knowledge on the ecology of small animals (mammals, birds, amphibians and reptiles) was enhanced by working with renounced scientists who were authorities on herpetology, parasitology and zoology both from local and foreign research institutions. Through these collobarative work, he authored and coauthored 35 scientific papers on various aspects of host-parasite relationship in relation to zoonoses during a 12 year period as a laboratory technician in the IMR.

His first break through was end of 1959 when he received a Sino-Fellowship Trust Award for a one-year course on "Animal Ecology" at Oxford University, England, University of Aberdeen Scotland and the National History Museum London. Following this scholarship, he also received a Hooper Foundation award to the University of California for three months on a parasitology course at the Medical Centre in San Francisco (UCSF) under Professor D. Heyneman, a renounced ecological parasitologist.

Upon his return in 1961, he was upgraded to Experimental Officer of Medical Zoology and in 1965, a new division was created "Division of Medical Ecology" and he was appointed as Head of the Division. The main aim of the Division was to coordinate host-parasite-relationship studies with local universities and also research institutions of South-east Asia (Singapore, Thailand, Indochinese region, Indonesia and also universities and research institutions in England, America and other European countries), but primarily with all the other Divisions at the IMR. Most of the researches were financially supported by foreign grants. At the end of 1969, he was awarded a fellowship grant by the Medical Research Council Award, London for an MSc in parasitology for two years at the University of Aberdeen, Scotland. After completing his study in 1972, he received a study grant by the American National Museum, New York for a study tour on "The taxa of small mammals of Southeast Asia in the museums of New York, Chicago and Smithsonian Washington.

In 1975, he enlisted as a Ph.D student with working on the "Rat lung-worm, *Metastriongylus malaysiensis* a causative agent of human eosinophilic-encephalitic disease, and graduated in August 1977. In the same year, he was seconded to World Health Organisation (WHO) as Senior Scientist in the Rodent Control and Bioliogy and retired in 1987 after 10 years in the Interregional and Regional sections stationed in Jakarta, Indonesia.

Professional activities

Lim boo Liat serves as honourary adviser on animal biodiversity and research activities in international and national research institutions.

1. Honourary consultant on zoology for the Department of Wildlife and National Parks, Kuala Lumpur, since 1989.
2. Adviser to post-graduate students on zoological research activities to University Kebangsaan Malaysia and University Malaya since 1997.
3. Nominated Member of the Expert Committee on "Rodents of Public Health Importance" WHO/VBC/Geneva since 1973.

4. Nominated Member of Expert Committee on "Insectivores" IUCN/Geneva since 1994.
5. Honorary Life and Corresponding Member of the Explorers Club, New York since 1995.
6. Member of the ASM Biodiversity Task Force since 2007.
7. Member of the Task Force on Emerging Infectious Diseases, ASM since 2008.

Professional recognition

For his contribution in the field of biological science the following acarines, helminthes, protozoa, insects and vertebrate animals have been named after him by foreign and local scientists:

Trombiculid mites: *Babiangia booliati* (Audy 1965), *Leptotrombidium (L) limi* (Nadchatram & Traub 1962); Flea: *Medwayellia limi* (Traub 1972); Helminths: *Plasmodium booliati* (Sandosham & Yap 1965), *Heligmonella limbooliati* (Durette-Desset, Diaw & Krishnansamy 1970), *Brienlia booliati* (Ho & Singh 1973); Protozoa: *Sarcocytis booliati* (Dissanaike 1972); Beetle: *Thalliseliodes limbooliati* (Chujo 1965); Lizard: *Dibamus booliati* (Das & Norsham 2003); Snake: *Oligodon booliati* (Leong & Grismer 2004).

Lim Boo Liat was elected Fellow, Zoological Society of London, England (F.Z.S) in 1955 and in 1956 Member, Institute of Biology, London, England (M.Biol.) for his research work on vertebrate zoology. Subsequently he was also awarded with (1) Ahli Mangku Negara (AMN) in 1974 for government services (2) Ordinary Member by the Malayan Nature Society (MNS) in 1977 for services rendered to the MNS (3) Sandosham Gold Medal by the Malaysian Society of Parasitology and Tropical Medicine in 1978 for his parasitological research in Malaysia, (4) Ordinary Member of the American Society of Mammalogists for his zoological research contributions in Malaysia in 2003 and (5) Inaugural Spallazani Award by the North American Symposium, Mexico in 2007 for his pioneering bat research in Malaysia

Education Background

Lower Secondary Education-Junior Cambridge (unfinished due to Japanese occupation in 1942 in Klang High School, Selangor. Obtained MSc. in zoology at the University of Aberdeen, Scotland in 1971 and PhD in zoology at Universiti Sains Malaysia in 1977.

Publications

1. Lim Boo Liat 1979. Poisonous Snakes of Peninsular Malaysia 73 pages. 2nd ed. 1982, 3rd ed. 1991.
2. Lim B.L. 1983. Orang asli Tales (English and Bahasa) (In Bahasa language -2nd ed. 2005).
3. Lim, B.L. and Indraneil Das 1999. Turtles of Borneo and Peninsular Malaysia. 151pp.
4. Norhayati Ahmad, Juliana Senawi & Lim, B.L. 2004. A photographic guide to amphibians of Endau Rompin. 59pp.
5. Norhayati Ahmad, Juliana Senawi & Lim, B.L. 2005. A photographic guide to amphibians of Ulu Muda Forest Reserve, Kedah. 121 pp.
6. Tigga Kingston, Lim B.L. and Zubaid Akbar 2006. Bats of Krau Wildlife Reserve. 145 pp.

DR. ABDUL LATIFF MOHAMAD

Universiti Kebangsaan Malaysia

"Plant Taxonomy, Ethnobotany and Plant Diversity"

Year 1995 MTSF Science & Technology Award Recipient

Dr. Abdul Latiff Mohamad received his MSc and PhD from University of Reading, England.
He joined Universiti Kebangsaan Malaysia as Head of Department of Botany from 1980-1984 and from 1992-1994. He was the Deputy Dean, Faculty of Life Sciences from 1987 to 1991 and was the Professor of Botany.

Since 1980, Dr. A. Latiff had singly embarked on the systematic studies of Malesian Vitaceae. The family is one of the widely distributed and common plants particularly in Malaysia and generally in Malesia. The objectives have been to understand the diversity, infraspecific, interspecific as well as intergeneric variation within, relationship and affinities between taxa and the evolution of the group.

He started contributing his research findings in the phytochemistry and anatomy of the group in the 1980s (Latiff, 1980a, 1980b). Beginning in 1981 onwards, he started publishing systematic treatments of the genera in Peninsular Malaysia (Latiff, 1981; 1982a, 1982b, 1982c, 1982d, 1982e). Two major contributions were made, the recognition of the new genus to science, *Nothocissus* (Miq.) Latiff and the worldwide revision of *Pterisanthes* Blume. In the latter, three new species were described, *P. stonei* Latiff, *P. brevipedicellata* Latiff and *P. sumatrana* Latiff. Subsequently, systematic treatment for *Tetrastigma* was made (Latiff, 1983a) and numerical analysis of the taxa followed (Latiff, 1983b).

The status of two threatened species, one each for *Ampelocissus* and *Pterisanthes* was highlighted (Latiff, 1987, 1989b). A new species, *Ampelocissus madulidii* from the Philippines was described (Latiff, 1988a) and a new record of *Cissus aristolochiodes* for Malesia was reported (Latiff, 1988b). The systematic treatment for Peninsular Malaysia climaxed with the floristic account of the family (Latiff, 1989d). In 1990, two new species, *Tetrastigma megacarpum* Latiff and *T. steenisii* Latiff from Borneo were described.

While studying the Vitaceae, Dr. A. Latiff also studied the genus *Rafflesia* because these parasitic plants grow on the viteceious genus of *Tetrastigma.* In the process, he had described a new species, R. *tengku-adlinii* from Sabah (Mat Salleh & Latiff, 1989) and gave an account on its conservation (Latiff & Mat Salleh, 1991). Lastly, an account of the Peninsular Malaysian rafflesias was given (Wong & Latiff, 1994b).

In 1992, Dr. A. Latiff contributed an account of the genera for the prestigious "Families and genera of Spermatophytes recognized by the Agricultural Research Service" of the United States.

Lastly, the account on seed morphology and inflorescence structures and evolution were made (Latiff, 1993; Latiff 1994), respectively.

Now he is in the process of finalizing the draft of taxonomic revision of the family for *Flora Malesiana* thus making him the sole Malaysian contributor to the Flora. A total of 118 species in 9 genera, comprising *Pterisanthes* (19), *Ampelocissus* (29), *Vitis* (2), *Ampelopsis* (2), *Parthenocissus* (1), *Nothocissus* (4), *Cissus* (18), *Cayratia* (20) and *Tetrastigma* (33) species. With this humble contribution to science, Dr. A. Latiff has been accorded as the Malesian expert in Vitaceae.

Publications

1. Latiff, A. 1994a. Inflorescence structure and evolution in the family Vitaceae. 1994. Malaysian Applied Biology 23 (1&2): 11-22.
2. Latiff, A. On the seeds of fossil and modern taxa of Vitaceae. 1993. Malaysian Applied Biology. 22 (1) : 87-99.
3. Latiff, A. Studies in Malesian Vitaceae. X. Two new species of *Tetrastigma* from Borneo 1991a. Blumea 35 (2) : 559-564.
4. Mat Salleh, K. & Latiff, A. 1989a. A new species of *Rafflesia* and notes on other species from Trus Madi Range, Sabah (Borneo). Blumea 34:111-116.
5. Latiff, A. Studies in Malesian Vitaceae. IX. *Cissus aristolochiodes* Planch., a grape vine new to the Malay Peninsula and notes on other species of *Cissus* L. 1988b. Malayan Nature Journal 42 : 31-35.
6. Latiff, A. Studies in Malesian Vitaceae VIII. A new species of *Ampelocissus* from the Philippines. 1988a. Blumea 33:505-508.
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8. Latiff, A. Studies in Malesian Vitaceae VII. The genus *Tetrastigma* in the Malay Peninsula. 1983a. Gardens Bulletin Singapore. 36(1):213-228.
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12. Latiff, A. Studies in Malesian Vitaceae IV. The genera *Ampelocissus*, *Ampelopsis* and *Parthenocissus* in the Malay Peninsula. 1982b. Federation Museum Journal n.s. 27:78-93.
13. Latiff, A. Studies in Malesian Vitaceae VI. The genus *Cissus* in the Malay Peninsula. 1982a. Malayan Nature Journal. 35(3): 197-207.
14. Latiff, A. Studies in Malesian Vitaceae V. The genus *Cayratia* in the Malay Peninsula. 1981. Sains Malaysiana 10(2):129-139.

PROFESSOR FON WAI CHU

Universiti Malaya

"Theory of Atomic Collision processes Atomic & Molecular Data for Fusion"

Year 1996 MTSF Science & Technology Award Recipient

DR FON WAI CHU FASc is a leading Mathematical Physicist whose research has contributed significantly to the understanding of the mechanisms and physical principles that govern the processes of atomic collisions – excitation, photoionisation, excitation-autoionisation and formation and decays of resonance. His work in the area of Atomic Physics has won him and Malaysia many international distinctions. His contributions and achievements are reflected by the following honours: Fellow of the Institute of Physics (UK), Consultant to IAEA (Vienna), Malaysia Toray Foundation Science and Technology Award 1996 and the National Science Award 1997. He has had vast experience working in collaboration with prominent international scientists and wide exposure to the mainstream international movement in science, which has enabled him to contribute significantly to the promotion of Science and Mathematics in the nation. In particular, he has a keen interest in the promotion of Science and Mathematics at the pre-university level and has actively pioneered and involved himself in activities towards that goal such as his efforts in pioneering the 'Desa Matematik' project.

Publications

1. W.C. Fon. Elastic scattering of electrons and positrons by hydrogen atom via selective expansion method. Jour. SNAS. Vol. 3 supplement 222-225, 1973.
2. W.C. Fon. Excitation of hydrogen atom by electron impact. Malaysian Jour. Science Vol. 3(B), 107-122, 1975
3. W.C. Fon and D.F. Gallaher. Positron-hydrogen elastic scattering with long range force via pseudo-state expansion. J. Phys. B : (Atom. Mole. Phys.) 5, 943-949, 1972.
4. W.C. Fon, K.A. Berrington, P.G. Burke and A.E. Kingston. Electron excitation for the 2^3S, 2^1S, 2^3P and 2^1P states of helium between the n=2 and n=3 excitation thresholds. J. Phys. B. (Atom. Mole. Phys.) 11 325-329, 1978. Ref. Int. Bull. Atom. Mole. Data for Fusion, 1978, No. 4, Art. 484
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7. W.C. Fon, K.A. Berrington and A.E. Kingston. Alignment and orientation parameters for excitation of the ground state of helium to the 2^1P state by 81 eV electron. J. Phys. B: (Atom. Mole. Phys.) 12 L171-3, 1979. Ref. Int. Bull. Atom. Mole. Data for Fusion, 1979, No. 9, Art. 229.
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10. W.C. Fon and K.A. Berrington. The elastic scattering of electrons from inert gases II : Neon. J. Phys. B: (Atom. Mole. Phys.) 14 323-334, 1981. Ref. Int. Bull. Atom. Mole. Data for Fusion, 1981, No.15, Art. 85.
11. W.C. Fon and K.A. Berrington and P.G. Burke and A.E. Kingston. Elastic scattering of electrons by atomic hydrogen at intermediate energies. J. Phys. B: (Atom. Mole. Phys.) 10 1041-1051, 1981. Ref. Int. Bull. Atom. Mole. Data for Fusion, 1981, No.16, Art.73.
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19. W.C. Fon and K.A. Berrington and A.E. Kingston. Electron impact excitation of the $n^3,^1P$ (n=2, 3 and 4) states of helium. J. Phys. B: At. Mol. Opt. Phys. 24 2161-82 (1991)
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21. W.C. Fon, K.M. Aggarwal and K. Ratnavelu. Electron impact excitation of n=2 states of atomic hydrogen at energies just above the ionization threshold. J. Phys. B: At. Mol. Opt. Phys. 25 2625-37 (1992)
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25. W.C. Fon and K.Ratnavelu and K.M. Aggarwal. A 15-state R-matrix investigation of resonances in elastic scattering of electrons from atomic hydrogen at low energies. J. Phys. Rev. A. 47 2968-2977 (1993)

26. W.C. Fon and K.P. Lim. A 19-state R-matrix investigation of resonances in e^--He scattering : IV, 11S-21P differential cross sections and electron-photon coincidence parameters λ and |χ|. J. Phys. B: At. Mol. Opt. Phys. 26 2717-2732 (1993)

27. W.C. Fon, K.P. Lim and P.M.J. Sawey. A 29-state R-matrix investigation of resonances in e^--He scattering at low energies : II, on the convergence of the calculation of 1^1S-$2^3P,^1P$ differential cross sections. J. Phys. B: At. Mol. Opt. Phys. 26 4201-18 (1993)

28. W.C. Fon, K. Ratnavelu and P.M.J. Sawey. Differential cross sections for the electron impact excitation of 33S and 31S state of helium at low energy. J. Phys. B: At. Mol. Opt. Phys. 26 L555-L560 (1993).

29. W.C. Fon, K.P. Lim and P.M.J. Sawey. Differential cross sections and the electron-photon coincidence parameters λ and |χ| for the electron impact excitation 1^1S-3^3P in helium at low energies. J. Phys. B: At. Mol. Opt. Phys. 26 L747-L752 (1993)

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39. A.E. Kingston, W.C. Fon and P.G. Burke. 1s-2s and 1s-2p excitation of hydrogen atom by electron impact. J. Phys. B: (Atom. Mole. Phys. 9 605-618, (1976)
40. K.A. Berrington, W.C. Fon and A.E. Kingston. Electron excitation rates for helium. Mon. Not. Roy. Astro. Soc. 200 347-50, 1982 (Britain)
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DR. KOH CHONG LEK

Universiti Malaya

"Molecular Genetics and Molecular Biology (including recombinant DNA Technology or Genetic Engineering)"

Year 1996 MTSF Science & Technology Award Recipient

Dr. Koh Chong Lek graduated from the Universiti Malaya in 1969. He received his BSc (Hons) from Universiti Malaya, obtained his PhD from Universiti London, England and DIC from the Imperial College of Science & Technology, London, England. He was a lecturer at the Department of Genetics and Cellular Biology, Universiti Malaya from 1973-1984, Associate Professor from 1994 (July) to 1995 and was later a Professor at the Department of Genetics and Cellular Biology. He was a Research Fellow in the laboratories of Dr. Peter M. Bennett, University of Bristol, England from May to July 1985, Professor David H. L. Bishop, NERC Institute of Virology and Environmental Microbiology, England from April to July 1989 and Professor Sir Alec J. Jeffreys, FRS, University of Leicester, England from February to November 1993. He was the Adjunct Associate Professor in the laboratories of Professors Maurille J. Fournier and Thomas L. Mason, University of Massachusetts, USA from February to October 1987.

Dr. Koh's expertise and working experience lies in molecular genetics and molecular biology. Since 1982, he has been giving effective leadership in molecular genetics and molecular biology to scientists in Malaysia. He was the consultant in molecular biology to individuals from the universities, research institutes and private sectors, as well as to a number of government agencies. Through research and collaboration with a number of research groups, undergraduate courses taught in the University Malaya, supervision of postgraduate and undergraduate students, teaching of external courses, molecular biology training courses, invited lectures and seminars, he had actively educated and trained Malaysian and overseas scientists in the use of recombinant DNA techniques in teaching, research and industrial applications. He had promoted the advancement and development of molecular genetics and molecular biology in Malaysia through publications and academic/professional activities. He was involved in a number of research projects which received funding from the Ministry of Science, Technology and the Environment, Malaysia and a couple of external agencies, e.g. WHO and FAO.

Publications

1. Koh C.L., "Recombinant DNA Techniques. A Laboratory Manual for the Training Course," 83 pages, 1989.
2. Koh C.L., "Recombinant DNA Techniques. A Laboratory Manual for the Training Course," 91 pages, 1990.
3. Koh C.L., Lim M.E., and Wong Y.H., "Plasmid-mediated transferable chloramphenicol and tetracycline resistance in Salmonella typhi (Vi phage type 25) isolated in Peninsular Malaysia." Medical Journal of Malaysia, December 1983, Vol. 38, No. 4, p. 320-324.
4. Koh C.L., "Infectious antibiotic-resistance plasmids in Escherichia coli from tidal water in Kapar, Selangor, Malaysia, "Malaysian Applied Biology Journal, December 1984, Vol. 13, No. 2, p. 123-125.
5. Koh C.L., "Detection and purification of plasmids present in Thermus strains from Icelandic hot springs, "MIRCEN Journal of Applied Microbiology and Biotechnology, 1985, Vol. 1, p. 77-81.

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DR. HO CHEE CHEONG

Universiti Malaya

"Colloid and Surface Chemistry of Dispersed Systems, in particular
(1) Natural Rubber Latex, (2) Palm Oil Emulsions,
(3) Tin Tailings Slurry (Ponds), (4) Waste-water Discharge
from factories and mills, & (5) Solid-Phase Immunoassay"
Year 1997 MTSF Science & Technology Award Recipient

Dr. Ho received his BSc (Hons) degree in Chemistry from the University of Malaya in 1968, MSc and PhD from the University of Bristol, England in 1969 and 1972 respectively. He was formally trained in Colloid and Surface Science, the forerunner of nanoscience, during the MSc programme in Bristol. He specialises on rubber latex chemistry and technology, polymer characterization, properties, processing and applications, green materials and green industrial processes.

He started his career as a research officer at the Rubber Research Institute of Malaya in the early seventies before moving to the University of Malaya in 1975. He was a former Head and professor of the Department of Chemistry. After his statutory retirement from the university in 1999, he spent three years as the R&D Director of a rubber glove manufacturing plant before returning to the academic world as the Dean and professor of a private university to establish a new Faculty of Applied Sciences. Dr. Ho has more than thirty-five years' experience as a researcher out of which twenty-eight years were spent in the university environment. His research interests centred on latex technology and palm oil systems of economic importance to Malaysia in the early years. This has since shifted to materials science with emphasis on green materials, clean processes and technology including surface characterization using advanced analytical techniques.

Dr. Ho is actively involved in the promotion of science and technology in schools and colleges, in particular the field of chemistry and polymer science. He and his team organised science camps to train school students and teachers on how to conduct science projects in schools under the Ministry of Education, Academy of Sciences Malaysia (ASM) and Intel. With his vast experience and expertise in the field of chemistry, Dr. Ho is actively serving the Ministry of Human Resources in developing manpower requirement for the chemistry sector, SIRIM on Malaysian Standards development, Department of Safety and Health (DOSH) in addressing safety and environmental issues of handling chemicals. He is currently the chair of the Advanced Manufacturing Sector of the Brain Gain Malaysia Programme of the MOSTI with the objective of establishing a critical mass of Malaysian S&T manpower capable of spear-heading cutting-edge technologies for our industries. He is also a member of the STI group of ACCCIM.

Dr. Ho is a Fellow of the Malaysian Institute of Chemistry and its immediate Past-President. He is a Fellow of the Academy of Sciences of Malaysia and its former council member. He is also a Chartered Chemist and a Fellow of the Royal Society of Chemistry, Britain. He sits in the Council of the Pacific Polymer Federation and is a founding member of the Asian Oceania Green and Sustainable Chemistry Network. He was awarded the DSc degree by the University of Bristol in 1998, National Science Award in 1999, the Malaysia Toray Science Foundation Science & Technology Award in 1997, the IKM Gold Medal in 1999 and the Rotary Research Foundation Gold Medal in 1999.

Since the 1970s Dr. Ho Chee Cheong has successfully pioneered the application of Colloid and Surface Chemistry in the study of natural rubber latex, palm oil mill effluent, palm oil emulsions and ex-mining pond slurry in Malaysia. Dr. Ho was instrumental in introducing the teaching of Colloid and Surface Chemistry**,** the fore-runner of nano-science, to undergraduate curriculum in the University of Malaya in the early 1970s. Dr. Ho has published more than 80 scientific articles in refereed journals in the field of synthetic latex and natural rubber latex properties, palm oil mill effluent treatment and geochemistry of tin-tailings slurry. Over the years, he has delivered more than 90 conference papers, 25 of which were Invited and Plenary lectures presented at international conferences overseas. Dr. Ho has also been frequently invited to present talks at seminars at universities and research centres overseas.

From the late 1980s, Dr. Ho initiated the application of colloid and surface chemistry as a common link to all these diverse disperse systems and to devise synergistic applications among them. These studies resulted in the discovery of new phenomena (heterocoagulation of NR latex with tin tailings) and new synergistic uses of these materials (natural rubber latex and palm oil emulsions), which have direct applications in the industries concerned. These research efforts have resulted in a clear understanding of these complex natural colloidal dispersions, which though of considerable economical values to Malaysia, were hitherto poorly characterized and thus were not well understood in their functions and applications until work was initiated by him.

In the 1990s, the focus of Dr. Ho's research has been shifted to materials science involving synthesis, characterization and applications of new materials. The emphasis was on green materials, clean manufacturing processes as applied to the rubber latex industry. The effects of green new additives on latex were studied using atomic force microscopy. This work is of direct relevance and great importance to the rubber glove manufacturing and organic coatings (paints) industries. The products of both industries depend on the ability of the latex dispersions to form a continuous film with the desired morphology and strength for them to function as a barrier material or protective coating. One of his research findings has been in use for several years now by a multinational company in Malaysia in producing specialty gloves for export and another piece of research finding for new green materials designed for the tire industry (see following patents).

Currently Dr. Ho is the co-inventor of four patents :

1. Japanese Patent 100627 (1994) *Palm oil emulsion and its production method* with Kausar Ahmad as co-inventor.
2. Malaysian Patent PI 9700201 (1997) *Method for the recovery of natural surfactants and dewatered sludge solids from palm oil mill effluent* with Chow Mee Chin as co-inventor.
3. European Patent EP1840161 Publication date: 10/03/2007 Preparation *process of oil extended rubber for tire, oil extended rubber for tire and rubber composition and tire using the same*. Inventors: Sakaki Toshiaki, Ichikawa Naoya, Hattori Takayuki, Ho Chee-Cheong, Choong Dick-Hean.
4. United States Patent 20070232745 Publication Date: 10/04/2007 *Preparation process of oil extended rubber for tire, oil extended rubber for tire,and rubber composition and tire using the same*. Inventors: Sakaki Toshiaki, Ichikawa Naoya, Hattori Takayuki, Ho Chee-Cheong, Choong Dick-Hean.

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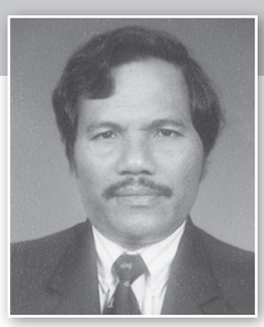
PROFESSOR DR. ABDUL SALAM ABDULLAH



Professor Dr Nordin Lajis
Associate Professor Dr. Mohd Ali Rajion

Universiti Putra Malaysia

"Anti-Nutritional Factors in local feedstuffs"
Year 1997 MTSF Science & Technology Award Recipient

Published information on anti-nutritional factors in general is scantly, particularly, for tropical feeds. Anti-nutritional factors are major causes of economic loss to the livestock industry in the tropics. However, it is difficult to quantify the real latent cost of diminished production, due to physical malformations, abortions, photosensitization and reduction of growth of livestock. Nevertheless, the capacity to cause damaged to livestock by the anti-nutritional factors has been underestimated and has been practically ignored in this country by both the farmers and professionals involved in livestock production.

Research conducted by us over the last ten years has generated a considerable amount of information on the various anti-nutritional factors which are hazardous to our livestock. In addition to the complete description of hepatogenous photosensitization disease in sheep and goats caused by Signal grass (*Brachiaria decumbens*), we have successfully answered many questions pertaining to the anti-nutritional factors in this grass and also in agro-industrial by-products such as the Palm Kernel Cake (PKC), both causing severe hepatic damage and subsequent death of sheep in this country. The toxic compounds involved in Signal grass toxicity, which we have successfully identified and characterized to be epi-sarsasapogenin and epi-smilagenin, have been acknowledged and accepted by the international scientific community in the area of toxicology.

These major findings have contributed significantly to the understanding of hepatogenous photosensitization disease in ruminant animals. At the same time, these findings have also discounted conclusively the earlier suggestion that the fungus *Pithomyces chartarum* may be the causative agent of *Brachiaria decumbens* toxicity in sheep.

Other important achievements include the establishment of practical preventive measures through antidotal approaches and the development and multiplication of *Brachiaria decumbens* resistant flock of sheep. Thus, our investigation has been directed towards the safe utilization of Signal grass by sheep and this has a significant impact on the economics of the developing local sheep industry. This also has a great socio-economic impact on the sheep farmers who are mostly smallholders which will contribute towards the eradication of rural poverty.

Our investigations also revealed that the oil palm fruits accumulate copper as they mature. This contributes to the high copper content in the palm kernel cake which can render it unsuitable for supplementary feeding to sheep. This knowledge would contribute towards the safe utilization of this abundantly available by-product used as animal feed and which represents a valuable export commodity.

Although there have been many publications on various species of poisonous plants in many temperate and tropical parts of the world, there has been little or no documentation of the poisonous plants found in Malaysia. My book on poisonous plats of Malaysia also provides information on poisonous plants which may be found in many parts of this humid tropical region.

Thus, our investigation not only contributes towards the development of scientific knowledge but also contributes towards the advancement of science and the socio-economics of Malaysia which should overall improve the quality of life.

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PROFESSOR YAP HAN HENG @ YAP HUN HIN

Universiti Sains Malaysia

"Medical Entomology especially Biology and Control of Mosquitoes and other household insect pests (Cockroaches and Ants)"
Year 1998 MTSF Science & Technology Award Recipient

Professor Yap Han Heng was born in Kuching, Sarawak. He received his PhD from University Minnesota, St. Paul, USA. He joined Universiti Sains Malaysia, Penang (USM) as a lecturer in 1972 and was appointed Professor in 1990. From 1991 until his retirement, Professor Yap was the co-ordinator of the Vector Control Research Unit at USM.

With more than 30 years of research activities resulting in more than 120 scientific publications, Professor Yap's contributions/achievement in the broad field of Biology, in particular Medical Entomology and Aquatic Biology could be summarized into basic (fundamental) and applied research activities.

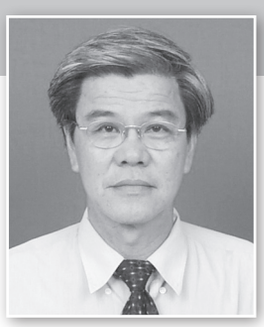
In the field of basic biology, his earlier research activity in the USA as a PhD student and post-doctorate research fellow and subsequently more than 28 years of research activity with USM had resulted in the following contribution :

1. Circadian rhythms of mosquitoes.
2. Enzyme ATPases as an indicator for aquatic pollution.
3. Behaviour of mosquitoes (*Aedes* and their predator *Toxorhynchites*).
4. Indigenous bio-agents for mosquito control (predatory and microbial agents) and Turbellarian worms.
5. Sonic attractancy of mosquitoes.
6. Species composition and distribution of household insect pests (cockroaches and ants)..

In the field of applied biology, his contributions include the following :

1. The development of an effective and efficient surveillance technique (ovitrap) for *Aedes* population in dengue control.
2. Initiation and introduction of biological (microbial) agents as larvicides for vector mosquitoes since early 1980s.
3. Continuous field-oriented research activity on space spray (thermal fogging/ULV) since late 1970s resulted in the introduction of new water-based insecticide formulations for dengue vectors (*Aedes*) control.
4. The first to introduce the concept of integrated control approaches (use of bacterial agents as larvicide and water-based pyrethroids as adulticide) for the control of dengue vectors, *Aedes* species.
5. Establishment of test protocols and conducting efficacy trials which have been essential for the control of Brugian filariasis vectors, *Mansonia* mosquitoes in Southeast Asia.
6. Research on household insect pests (including mosquitoes, cockroaches and ants) and their control have resulted in a series of publications on efficacy and sublethal effect of household insecticide products.

PROFESSOR DR. MAK CHAI @ MAK LIAN FONG



Universiti Malaya

"Plant Breeding and Biotechnology"

Year 1998 MTSF Science & Technology Award Recipient

Prof. Dr. Mak Chai is married with two children. He obtained his secondary education in Sam Tet School and A.C.S. School, both in Ipoh. He continued his tertiary education in University of Malaya where he was awarded the B.Sc. Honours degree in Genetics and M.Agri.Science. He proceeded to University of Saskatchewan in Canada under the Commonwealth Scholarship to complete his Ph.D. in Plant Genetics and Breeding in 1976.
Since then, he had served in University of Malaya as an academician and actively involved in doing research on plant genetics, plant breeding, quantitative genetics, mutation breeding, tissue culture and in-vitro manipulation for crop improvement. Crop plants include long bean, soybean, tomato, eggplant, chilli, oil palm, tapioca, papaya and banana. His more than 20 years of research had resulted in more than 90 scientific publications. In addition, he had made significant contributions in the areas of plant genetics, breeding and in-vitro mutagenesis. These include:

- Genetic studies and breeding for yield improvement in long bean and inter-specific hybridization in the *Vigna* species.
- Genetic studies and breeding soybean for local adaptability and yield stability.
- Breeding for bacterial wilt resistance in tomato.
- Breeding to improve yield and pungency in chilli.
- Quantitative genetics and breeding theory of doubled haploids.

Between 1900 and 2001, his research has focused on banana improvement through induced mutation techniques and biotechnology where he and his research team had developed skills, technology and scientific knowledge, significant not only to banana research but also the banana industry. These include:

- Selection of natural variants and propagation by tissue culture technique.
- Using tissue culture method to generate somaclonal variation for successful selection.
- Of early maturity clone and resistance to Fusarium disease.
- Using gamma irradiation to induce genetic changes in tissue culture plants in order to select for desirable traits and development of technique for mutant selection and early screening for Fusarium wilt resistance.
- Using colchicine to induce changes in chromosome number (or polyploidy induction) of local diploid banana such as Pisang Mas to improve fruit size and productivity.
- Developing an efficient system of plant regeneration through single cell culture (i.e. somatic embryogenesis, cell suspension culture and plant regeneration by using bioreactor)
- Molecular studies of wild banana for disease resistance.

For his expertise in banana breeding and in-vitro mutagenesis, he was invited to serve as Technical Cooperation Expert by International Atomic Energy Agency (IAEA) for technology transfer missions to Sri Lanka, Philippines, Indonesia and Sudan. In addition, he also served as consultant to some Malaysian plantation and biotechnology companies. He had traveled extensively to attend international conferences, expert committee meetings and scientific visits; these include Singapore, Thailand, Vietnam, India, China, Russia, Taiwan, Japan, Australia, USA, Canada, Germany, France, England, Belgium, Czech Republics and Austria.

Since his retirement from University of Malaya in 2001, Prof. Dr. Mak Chai has devoted his time fully in private higher education. He served as President/CEO of SIT International College for 5 years followed by 3 years in KDU College as Strategic Development Manager and Dean of the School of Engineering, Science and Technology. He is currently, the Professor and Director of Foundation Studies in Asia e University.

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PROFESSOR DATO' DR. KHAIRUL ANUAR BIN ABDULLAH

Universiti Malaya

"Parasitology, Immunology and Molecular Biology
especially in the field of Toxoplasma and Filaria"
Year 1999 MTSF Science & Technology Award Recipient

I obtained a Degree of Drs from the Universitas Gadja Mada, Faculty of Medicine in the year 1974. I started my career as a lecturer in 1975 at the Universiti Sains Malaysia. Then in 1978 I was admitted to the School of Public Health at Tulane University, in New Orleans, USA. I completed my MPH (Masters of Public Health) in 1978. Then went on to do my PhD at the Department of Tropical Medicine, Tulane School of Medicine. In 1982 I graduated from the Tulane School of Medicine, USA with a PhD in Immunology. On return to Malaysia, I was appointed as a lecturer in the Department of Medical Microbiology, Faculty of Medicine, Universiti Sains Malaysia. As a lecturer I was involved in teaching Medical Parasitology and Immunology to Medical Students and conducted research on parasitic infections. In the year 1985 I was appointed as an Associate Professor in Medical Parasitology. During this period I taught medical students, supervised postgraduate students, conducted research and was also involved in the setting up of this medical school. In 1986 I was given the Fulbright Scholar award and was placed in the Harvard Medical School as a visiting Scientist, where I conducted research and taught post-graduate students. During this time, the Harvard Medical School was changing its curriculum from a traditional form to an integrated one. With little experience that I had at USM medical school I was able to learn more about the new medical curriculum then known as the new pathway at Harvard Medical School. Never thought that one day I will be more and more involved in the development of medical curriculum.

On return to Malaysia in 1988, I was extremely involved in the reshaping of the preclinical medical programme as the Phase I coordinator, member of the curriculum committee, postgraduate committee, block coordinator, supervised many students at Masters and PhD level. Served many professional organizations and was elected as the vice president and later as the President of the Malaysian Society for Parasitology and Tropical Medicine. I served as an external examiner for many post graduate students and as external examiner for the Medical programme for University of Malaya, Universiti Kebangsaan Malaysia, Universiti Malaysia Sarawak and Universiti Putra Malaysia.

During the 34 years of my service, I have gained enormous experience in teaching Parasitology to Medical, Pharmacy, nursing and biomedical students. The teaching included both theory and laboratory Parasitology.

My research career began in a small scale in 1975. During my career I have obtained many grants both from local and international organization. To date I have published more than 175 publications in learned Journals. In recognition of my research output I was awarded the MSPTM Silver medal in the year 1988. In the year 1998, I was awarded the Malaysia Toray Science Foundation Science & Technology Award for distinguished researcher of the year. Again in the year 2002, the Sandosam Gold Medal (which is the highest award given by MSPTM to any one in the field of Tropical Medicine) was conferred on me for my achievements and contributions in Tropical disease research.

My contribution to management began in 1984 when I was appointed as the Phase I coordinator of the Medical Programme at the USM. Between 1984 and 1994 I held many administrative positions at the School of Medicine and the University. In 1995 I was offered the Chair of Parasitology at University of Malaya so I migrated to Kuala Lumpur to take this new position at the Oldest University in Malaysia. Held the position of the Head of the department for 2 years and I was promoted to the post of Deputy Dean of Academic at the Medical Faculty. During my tenure as the Deputy Dean I chaired many committees and also was an executive member of the Hospital University Board. During this time I was elected to the advisory board of the Perak Medical College and also acted as the chairman for unscheduled University graduates programme. In 2004 I retired from my post and was invited to set up the new Medical School at UiTM. I served the UiTM Medical Faculty as the Deputy Dean of Academic for three years. During this period, I was many times the Acting Dean for the faculty. In 2007, I was invited again to MAHSA to pioneer the Medical Faculty, where I am employed as the Foundation Dean of the Faculty of Medicine. As the foundation dean currently I am planning the building of the Medical Faculty, laboratories, intake of medical students and staff recruitment.

Other positions held currently are: Chairman of The Brain Gain Malaysia for MOSTI, President of The Malaysian Science Association, Council member of the Academy Science Malaysia and Council Member of the FASAS (Federation of the Asian Academies of Science), Member of the Medical Advisory Panel (FOMEMA).

IR PROFESSOR DATO' DR. CHUAH HEAN TEIK

Multimedia University

"Microwave Remote Sensing : Theoretical Modelling, Sensor Development and Applications "

Year 1999 MTSF Science & Technology Award Recipient

Dr. Chuah Hean Teik graduated with a BEng (First Class Honours), MEngSc and PhD degress in electrical engineering, all from University of Malaya, Malaysia, in 1986, 1988 and 1992, respectively. From July 1988-April 1997, he was on the faculty of the Electrical Engineering Department of the University of Malaya. From March-Nov 1994, he was a Fulbright Scholar at the Wave Scattering Research Centre, University of Texas at Arlington, USA. From May 1997 – Jan 2008, Dr Chuah was a Senior Professor at the Multimedia University, during which he held various posts as Vice President (R&D and Academic Development), Dean of Engineering, and Director of Research. From June 2001-Jan 2008, he also assumed the duty of the Penang State Government Professor of ICT in MMU. Since April 2008, he has been appointed as President of Universiti Tunku Abdul Rahman (UTAR) in Malaysia.

His research interests include microwave remote sensing, applied electromagnetics, and wave propagation for indoor and outdoor communications. He has authored/co-authored more than 220 papers in international journals and conferences. He has successfully supervised 14 PhD candidates and 13 Master's candidates.

Dr. Chuah is a Fellow of the Academy of Sciences, Malaysia; the Institution of Engineers, Malaysia; the Remote Sensing & Photogrammetry Society, UK; the Institution of Engineering and Technology, UK; the Electromagnetics Academy, USA; Hon. Fellow of the ASEAN Federation of Engineering Organisations, a Founding Fellow of the ASEAN Academy of Engineering and Technology; and a Chartered Engineer, Engineering Council, UK; and a Professional Engineer, Malaysia. Dr. Chuah is currently the Deputy President/President Elect of the Institution of Engineers, Malaysia, a Board Member of the Board of Engineers, Malaysia, and a Council Member of the Academy of Sciences Malaysia. He is a Senior Member of IEEE, and Hon. Member of the Golden Key International Honour Society. Dr. Chuah serves as reviewer for technical papers submitted to international journals. He is the Chairman of the Expert Review Panel for the Sea-to-Space Brain Gain Programme in Malaysia, a member of the Disciplinary Committee Panel under the Advocates and Solicitors' Disciplinary Board, Malaysia, and a Council Member of the Malaysian Qualifications Agency.

Professor Chuah received the MTSF award due to his exemplary contributions in the area of microwave remote sensing. Since 1986, Dr. Chuah has been involved in microwave remote sensing research, both in the theoretical and fundamental aspects and the experimental aspects. His contributions can be briefly categorized in three areas:

(a) Theoretical Studies on Wave-Matter Scattering Mechanisms in Random Media

(i) His Monte Carlo Scattering and Emission Models and High-order Renormalisation Scattering Model for electrical-space media have been cited and used by researchers in the area. For example, his High-order Renormalization Model and his Monte Carlo Scattering and Emission Models were referred to in the 1990 and 1993 *Review of Radio Science* (a tri-annual publication by the International Union of Radio Science to report advances in various areas of radio science), indicating the significance of his models. In fact, many other groups have used his models in their work. These include the groups from Microwave Systems, SAAB Missiles, Sweden; the China Research Institute of Radiowave Propagation, P.R. China; and Mullard Space Science Lab, University College London, U.K.

(ii) In a dense medium, the conventional far-field approximation is no longer valid. The Dense Medium Phase and Amplitude Correction Theory (DMPACT) developed by Dr Chuah in 1994 introduces the concept of electrical dense medium, and is the first scattering model that could offer self-consistent explanation of measurements from both laboratory and field conditions. It is able to give excellent prediction to both co- and cross-polarized radar backscatter from dense media over a wide range of frequency. It is currently used by researchers in Malaysia, the United States, and New Zealand for tropical forests, sea-ice and snow applications.

(b) Experimental Work

(i) Dr. Chuah has set up laboratory facilities to study the dielectric constants of vegetation samples from the country (such as rubber leaf, oil palm leaf and leaves from tropical forest) at the microwave region. These data can be used by researchers in this area since these are essential input to all theoretical models.

(ii) Dr. Chuah has been instrumental in the design and construction of a microwave anechoic chamber suitable for bistatic radar cross-section measurements in a controlled laboratory environment. Such measurements are very important for characterization of the targets and for validation of theoretical modelling. The Chamber, first of its kind in South-East Asia, has been fully operational since Jan 1997 at the Electrical Engineering Department of the University of Malaya. The system is fully automated and the antenna systems are unique in that the positions of the antennas can be controlled using the automated system. Together with his graduate students, he has managed to design and construct another Multi-purpose Wideband Anechoic Chamber for EMC/EMI and Radar Cross-Section Measurements in Multimedia University in 1999.

(c) Sensor System for Remote Sensing Applications

Dr. Chuah is instrumental in leading a group of researchers in the design and construction of both C-band and a L-band Fully Polarimetric Scatterometer Systems which can be mounted on a boom-truck for field measurements. These systems are used for radar backscatter measurements from earth terrain such as paddy field, rubber and oil palm plantations, and forest stands.

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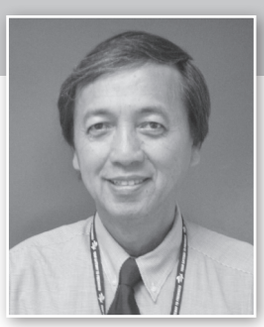
DR. CHAN YING KWOK



Mr. Toh Woon Kong
Mr. Lee Hoon Kok
Miss Rusna Binti Isa

Malaysian Agricultural Research & Development Institute

"Breeding and selection for improvement of Papaya and Pineapple varieties"

Year 2000 MTSF Science & Technology Award Recipient

Dr. Chan obtained his Degree in Agricultural Science from the University of Malaya in 1972, Masters in Horticulture from the University of California, Davis in 1979 and his PhD. in Genetics from the University of Malaya in 1995. He started his research career as a plant breeder at the Malaysian Agricultural Research and Development Institute (MARDI) in 1972. He held the positions of Fruit Breeding Programme Head and Deputy Director of Horticulture Research Centre till his retirement in 2003. He is currently the Senior Vice President of Crop Science and Production Technology at the Malaysian Agrifood Corporation Berhad (MAFC).

Dr Chan has more than 30 years experience in fruit research and development with particular emphasis on germplasm work and breeding and selection of papaya and pineapple. The notable successes of his papaya breeding are the development of Eksotika papaya in 1987 and the Eksotika II F_1 hybrid in 1991. The Eksotika was developed using the backcross method with the local Subang 6 as the non-recurrent parent and the Sunrise Solo as recurrent parent. The Eksotika released in 1987 has most of the outstanding eating qualities of Sunrise Solo but with improved fruit size and local adaptability of the Subang parent. It was, however, susceptible to fruit freckles and poor in keeping quality. Crosses of the Eksotika with a sib line (Line 19) in the backcross population resulted in a F_1 hybrid which had improved fruit cosmetics, heterosis in yield and vigour and improved keeping quality. This F_1 hybrid was named Eksotika II and released in 1991. The backcross and F_1 hybridization methods used by Dr. Chan were the first in the world for papaya breeding. Subsequent to his work, many countries have changed their papaya breeding programmes to F_1 hybridization that provides varietal protection and better yield and adaptation.

In pineapple breeding, Dr. Chan's most notable success was the development and release of the 'Josapine' in 1996. 'Josapine' was so called because it was selected from a cross between the Johore and Sarawak pineapple in a F_1 population of 50,000 progenies. This new hybrid is sweeter (Brix 15-18%) and more aromatic than conventional varieties. It has attractive fruit cosmetics with longer shelf-life and resistance to black heart disorder. 'Josapine' is one of the world's first successful commercial pineapple hybrid and its fruits are now popularly sold in local supermarkets with increasing export markets to Singapore and Hong Kong.

The varieties of papaya and pineapple from Dr. Chan's work had significant economic impacts on the Malaysian fruit industry. The export revenue of papaya before the Eksotika was released in 1987 was negligible, but it climbed steadily ever since and recorded a peak of RM 120 million in 2004, making Malaysia the second most important papaya exporting country in the world after Mexico. The 'Josapine' now is the preferred fresh pineapple variety and had practically replaced the other older varieties. It is now commonly found on the shelves of most supermarkets and served in fruit stalls and restaurants.

Dr Chan has published more than 80 technical papers related to fruit breeding and the fruit industry. He has been nvited to speak on tropical fruit breeding, cultivation and other aspects at local and international forums, seminars and conferences. He was a consultant to fruit development projects in Sarawak and Guinea, West Africa and a member of the Expert Panel for Review of National IRPA projects on fruits in Malaysia. He won the MARDI Excellent Service Awards in 1984 and 1997 and the King's Award Kesatria Mangku Negara (KMN) in 1997. In 2000, he was the recipient of the MTSF Science & Technology Award. He was the bronze medallist at the International ITEX 2004 for innovation and technology achievement and the gold medal at the 2005 MARDI Science and Technology Innovation and Commercialization Exhibition.

Publications

PINEAPPLE

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2. Chan, Y.K. (1991). Evaluation of F1 populations from a 4 x 4 diallel in pineapple and estimation of breeding values of parents. MARDI Res. J. 19: 159-168.
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DR. NG SEIK WENG

Universiti Malaya

"Chemical Crystallography of Organotin Chemicals"

Year 2000 MTSF Science & Technology Award Recipient

Dr. Ng Seik Weng received a BSc (Hons.) from the National University of Singapore (1975), an MSc from the University of Malaya (1978) and a PhD from the University of Oklahoma (1983). He joined the University of Malaya in 1987 and began research on organotin chemicals. His contribution to this field revolves around his discovery that the biocidal activity of such chemicals was significantly improved by the introduction of a 'spacer' unit between the organotin portion and the anionic portion of the molecule. He also addressed the problem of the solubility of bioactive chemicals in water: to render them more soluble, he converted them into an anionic form, and used an ammonium cation as counter-ion to balance the charge. The synthesis led to a new class of monomeric and oligomeric polyanionic organotin carboxylates. From anti-fungal chemicals, he extended his research to anti-tumour chemicals, and a similar method was used to confer higher aqueous solubility of the diorganotin class of compounds. In vitro tests of these compounds showed that their anti-tumour activity exceeded that of neoplastic drugs against cancer cell lines. In collaboration with crystallographers from abroad, he began publishing the crystal structures of organotin chemicals. His work in organotin chemistry won him the Malaysia Toray Science Foundation Award in 2000.

After 2000, Dr. Ng left the chemical laboratory as well as organotin chemistry behind and turned to crystallography. He concentrated on the publication of crystal structures, largely in collaboration with chemists from China who provided the diffraction measurements. Some of the results are reported in high impact-factor journals. The year that he spent in China (April 2003 to March 2004 as foreign professor in Xiamen and Sun Yat-Sen Universities) allowed him to establish extensive collaborative linkages with several universities. Perhaps the most visible acknowledgment of his contribution to crystallography is the award of honorary professorships by universities there - Central China Normal University (2005), East China University of Science and Technology (2008), Fuyang Normal College (2004), Guangdong Ocean University (2007), Guangxi Normal University (2005), Guangzhou University (2007), Harbin Normal University (2006), Heilongjiang University (2004), Jilin Normal University (2007), Luoyang Normal University (2005), Northeast Normal University (2007), Northwest University (2006), Qingdao University of Science & Technology (2004), Qufu Normal University (2004), Shantou University (2004), Shanxi Normal University (2004), Shanxi University (2008), Wenzhou Normal University (2002), Yangzhou University (2007), Xianning College (2006), Yunnan University (2005), Zhejiang Normal University (2007) and Zhengzhou University (2005).

He has published more than 1000 papers in SCI journals. The number of citations to his work, excluding self-citations, exceeds 2000. He is listed among the ten most prolific researchers in Science and Technology Knowledge Productivity in Malaysia Bibliometric Study (2003). His most valuable contribution to crystallography is his manuscript on how to deal with disorder in the analysis of crystal structures. [Some strategies for the refinement of disordered structures by

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DATO' DR. CHOO YUEN MAY

Malaysian Palm Oil Board

"Development of Efficient and Green Processes for the Palm-based Industry"

Year 2001 MTSF Science & Technology Award Recipient

Dato' Dr. Choo Yuen May ***(D.S.P.N., K.M.N)*** was born in Penang, Malaysia. She received her Bachelor of Science as well as Masters of Science Degree from University of Waikato, New Zealand and Doctorate of Philosophy in Chemistry from University of Malaya. Dato' Dr. Choo also holds an Executive MBA from Asian Institute of Management Phillipines.

Dato' Dr. Choo started her career as a lecturer in Universiti Sains Malaysia before she joined Palm Oil Research Institute of Malaysia (PORIM) as a Research Officer in 1982. Throughout her career development in PORIM (later MPOB), Dato' Dr. Choo was promoted to be the Leader of the Processing Research Group (1993 – May 2000), Head of Milling and Processing Unit (June 2000 – June 2004), Director of Engineering and Processing (June 2004 – August 2006). Dato' Dr. Choo has been holding the post of Deputy Director General (Research and Development) since January 2006, overseeing R&D activities of palm oil in MPOB.

To date, Dato' Dr. Choo has spent about 27 years of research on Chemistry and Technology of palm oil. The results of her research findings have culminated in 38 patents and 530 publications in refereed journals, book chapters, conference proceedings/papers, and technical reports.

Dato' Dr. Choo has developed several novel manufacturing technologies / products for the palm based industry. She is the project leader and principal researcher of these projects including commercialization aspect of the projects. These include :

- Production of palm oil methyl esters for applications as biodiesel and oleochemicals. Based on her team's patented technologies, commercial plants for (i) normal palm biodiesel (methyl esters) (pour point 15^0C, 60,000t – 120,000t per annum); 4 in Malaysia, one each in South Korea and Thailand) and (ii) 3 winter grade biodiesel (pour point 0^0 to -21^0 C, capacity 30,000t per annum) have been built and in commercial production.

- Production of palm carotene concentrate and vitamin E concentrate for food, nutritional, nutraceutical and cosmoceutical applications.

- Production of carotene rich red palm oil for food and nutritional applications. (The product is already commercially available in both local and overseas market bearing the trade name CAROTINO).

- Production of monoglycerides from oils and fats for applications as food emulsifiers, surface active agents in non-food applications and anti-microbial agent

- Production of palm-based industrial solvent, degreaser and lubricity additives/ improver for ultra low sulphur diesel.

Besides concentrating her efforts in the development of various novel manufacturing technologies for the palm based industry, Dato' Dr. Choo also carried out basic research in areas such as supercritical fluid technologies for processing of palm oil, organic reactions of palm oil triglycerides, chemistry and properties of palm oil minor components (carotenoid, tocols, sterol, squalene, co-enzyme Q, phospholipids), chemistry of palm based oleochemicals and their derivatives as well as chemical basis of atherosclerosis.

She is also involved in the developmental work on the palm derived lubricating base oil for the 2-stroke motorcycle engine oil which has been commercialized. Dato' Dr. Choo also introduced the concept of growing *E. oleifera* oil palms as a source of carotenoids and vitamin E (tocopherols and tocotrienols). Other areas of R&D which she has directly contributed include second generation palm biofuel, palm derived aviation fuel, palm food grade lubricant (being commercialized) and one-step enzyme catalysed esterification of oils and fats for the production of esters for various food and non food applications which include biofuel.

Her scientific contribution has gained recognition which won her numerous National (19 awards) and International awards (26 awards). She is now Fellows of (i) Academy of Science Malaysia, (ii) Malaysian Institute of Chemistry, and (iii) Malaysian Oil Scientist and Technologist Association.

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DR. TEE E SIONG

Institute for Medical Research
"Human Nutrition"
Year 2001 MTSF Science & Technology Award Recipient

This Malaysia Toray Science Foundation Science and Technology Award has been presented to Dr Tee E Siong, Institute for Medical Research (IMR), on the basis of his prominent role in the advancement of nutrition science and the promotion of healthy nutrition in the country. He is recognized as one of the leading nutritionists in Malaysia and has played a major role in raising the profile of nutrition science in the country. With over 29 years of working experience in the IMR, he has become an expert in many fields of nutrition research including community nutritional status, nutritional value of foods, nutrition intervention strategies and programmes as well as food standards and legislations. Several of his publications and research output have become useful reference materials for researchers and programme managers. He pioneered the setting up of the Malaysian food composition database some 20 years ago and played a key role in the development and establishment of the required analytical methods. His contributions in the development of analytical methods for the analysis of carotenoids and retinol are particularly noteworthy of mention. Based on the nutrient database, he developed the first nutrient analysis software in the country. In the area of nutritional epidemiology, his contributions to the methodologies for the assessment of nutritional status are also noteworthy.

The technical expertise and experience he has gained in the IMR for almost 3 decades have been put to good use through his contributions as a member or chairman of a number of technical committees. Through these committees he has helped formulate nutrition policies of the country as well as a variety of food and nutrition programmes and activities. His expertise has also been recognized in the region and has been invited as temporary advisor to the World Health Organization on several occasions. He has been invited as a member of the Board of Scientific Directors of the International Life Sciences Institute (SEAsia Branch). He has assisted the Ministries of Health Brunei and Laos in conducting nutrition studies. Dr Tee has been invited to make numerous presentations in scientific meetings within and outside the country on a variety of topics. He has also led the country's delegation in several meetings of the FAO/WHO Codex Alimentarius Commission. For over 12 years, he has been one of the prime movers of the Nutrition Society of Malaysia (NSM) and has been the President of the Society since 1996. Through NSM, Dr Tee has been working tirelessly in promoting nutrition science amongst its professional members as well as healthy eating amongst the community. Amongst the most notable NSM projects include a series of community nutrition roadshows, the establishment of the NSM website (nutriweb.org.my) and Bright Start Nutrition, a dedicated programme for toddlers. Dr Tee is recognized for his sincerity and honesty as well as his dedication and excellent work performance.

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PROFESSOR TOU TECK YONG

Professor K.S. Low
Associate Professor S.W. Ng

Multimedia University

"Laser Systems and Applications"

Year 2002 MTSF Science & Technology Award Recipient

Tou Teck Yong was born in the East Malaysian town of Sibu, Sarawak, where he obtained his schooling at the Methodist Secondary School, and thereafter his BSc (Hons) in Physics and PhD at Universiti Malaya, Malaysia in 1982 and 1987, respectively.

He has 2 years of postdoctoral experience at the Institute of Advanced Studies, Australian National University, in magnetic confinement research between 1987-1989, 1 year as research fellow at Institute of Advanced Studies, Universiti Malaya in 1989-1990 where he became lecturer in 1991 and promoted to Associate Professor in 1993. He joined Universiti Multimedia in 1999 as a full professor in the Faculty of Engineering.

He has spent more than 7 years in plasma research, 10 years in laser research and 6 years in silicon wafers, thin film depositions and characterization techniques. He has supervised 16 Masters and 5 PhD students, and currently 3 Masters and 4 PhD students.

He is currently a Fellow of Malaysian Institute of Physics, and Academy of Sciences Malaysia. He has been reviewing manuscripts submitted to the following journals in past 5 years : Optics Letter; Optics Express, Applied Optics, Optics Communication, IEEE Transaction of Plasma Science, J. Polymer B, Microelectronics Reliability, JOSA B, etc. He has also been invited to sit in the organizing committees for some international conferences, which include nanophotonics, intense laser science, etc.

He is a founding member and co-chair of the Asian Intense Laser Network, chaired the 3rd Asian Symposium on Intense Laser in Malaysia in 2007, which brought together the top scientists in Asia in this relatively new, advanced laser science.

He has published more than 66 SCI journal papers, presented some lectures and invited talks in Tokyo University, The Australian National University, Fukui University, Osaka University, and international conferences.

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PROFESSOR DR. ASMA ISMAIL

Dr. M. Ravichandran, Professor Ong Kok Hai

Mr. Zainuddin SA Kader, Dr. Mohd Zaki Salleh

Universiti Sains Malaysia

"Medical Microbiology, Medical Biotechnology, Rapid Diagnosis of Infectious Diseases especially on Typhoid and Paratyphoid fevers and Development of new technology platforms in especially for Rapid Diagnosis of Diseases"

Year 2002 MTSF Science & Technology Award Recipient

Prof. (Dr.) Asma Ismail graduated from the University of Nevada, Reno, USA with distinction in biology, received her MA in Microbiology from Indiana University, Bloomington, USA. and obtained her PhD in the field of Cellular and Molecular Biology at the University of Nevada, Reno, USA in 1986.

Prof. Asma started her career as a lecturer in the Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia in 1986. She was a visiting scientist at University of Tokyo in 1989 and a visiting fellow at the Medical College, St Bartholomew's Hospital in London in 1992. She was promoted to Associate Professor in 1993 and served as Deputy Dean of Administration in 1994. She was promoted to Professor in 2000 and became Deputy Dean of Research in the same year. In 2001, she became the Director for the Centre for Medical Innovations and Technology Development, USM. In 2003, she became the Director, Institute for Research in Molecular Medicine (INFORMM), the first multi-disciplinary cluster based research institute for USM. In May 2008 she was promoted to Deputy Vice Chancellor (Research and innovation) at USM.

Prof. Asma Ismail specializes in the area of proteomics and its application in the rapid diagnosis of infectious diseases, especially typhoid fever. Her studies on specific biomarkers led to the discovery of an antigenically specific 50kDa of *Salmonella typhi*. Prof. Asma is one of the scientists credited with the translation of the scientific discovery into 4 rapid diagnostic kits for typhoid that have been successfully commercialized globally. She has filed for 26 patents world wide of which 7 has been granted. She has helped to create a start up biotech company pioneering in Bio-diagnostics for the country and currently served on its board of directors.

Prof. Asma's R&D achievements and impact of her research at national and international levels have gained more than 57 awards and recognitions at national and international levels including WHO temporary Advisor for vaccine and diarrhoeal diseases since 2002, National Young Scientist Award in 1991, National Inventor Award in 2003, National Innovation Award, 2006 and the National Academic Award for Product and Commercialization in 2007. She was made a Fellow of the Academy of Sciences Malaysia in 2003 and served as its council member since 2007. At the National level, she chairs the Cluster Development Committee for the Nobel Laureate grants in Physiology or Medicine under the Academy of Sciences Malaysia and the Fundamental Research Grant Scheme (medicine) for the Ministry of Higher Education. She is a committee member for the Establishment of Research Universities in Malaysia and is also a member of the Assessment Committee for Research Universities by the Ministry of Higher Education that led to the landmark establishment of Research Universities in Malaysia. She also serves as the chair for the development and direction of R&D towards implementation of the National Strategic Planning for Higher Education and chairs the Select Committee for the National Academic Award, Ministry of Higher Education since 2007.

Prof. Asma is actively involved in research and publication, attaining more than RM 9 million (USD 2.6 million) in terms of grants within the last 5 years, presented more than 100 papers as invited or keynote speaker at national and international levels to share her scientific findings and experience in the commercialization of R&D products.

Achievements regarding winning entry

Typhoid affecting 21 million people annually, is caused by the bacteria *Salmonella Typhi*. The disease is difficult to diagnose since the clinical symptoms mimic those of other fevers common in the region. Current diagnostic methods used lacked sensitivity, specificity, speed and could not be performed in the field. As a consequence 200,000 people die annually mainly among children.

In developing an accurate test for typhoid fever, there is a need to discover antigens specific for typhoid diagnosis. The discovery of the 50 kDa outer membrane protein specific for *Salmonella typhi* was reported in 1991 by Ismail, A et al. This is an important discovery that led to the development of a rapid dot enzyme immunosorbent assay (EIA) kit called *TYPHIDOT* ™ for the rapid detection of specific IgM and IgG to S. Typhi within 3 hours compared to 2-5 days via conventional diagnostic methods. Since typhoid is prevalent in under developed countries, it is imperative that the test developed be highly sensitive, specific, rapid, cost effective and simple to perform. The *TYPHIDOT* ™ test is a breakthrough in typhoid diagnosis since it fulfilled the criteria stated. The use of the dot EIA method paved the way for visual interpretation by eye for typhoid diagnosis rather than using an expensive machine. The commercialization of the typhoid kits since1994, starting with Malaysia and now sold to 18 countries round the world, was an IRPA success story that had established and spearheaded the bio-diagnostic industry in Malaysia. To ensure that the test remained technologically competitive, further research was done to remove total IgG in the serum sample which led to the development of *TYPHIDOT-M* ™ , in 1996, that detects only IgM for the accurate diagnosis of acute typhoid within 3 hours. Further improvement was also done in 1996 to the *TYPHIDOT* ™ test to allow interpretation of test results within 1hour. Since the global bio-diagnostic scene is moving to rapid diagnosis within 5 to 20 minutes, research was done to change the technology platform to the immunochromatography format. This format allowed the test to be performed within 15 minutes, with the added advantage of greater simplicity, highly cost-effective and more importantly without need for cold chain (4^0C) transportation and storage. This IgM test, developed in 2002, for typhoid fever, showed a sensitivity of 100% and a specificity of > 97%.

A similar technology platform is being used to develop another breakthrough in the diagnosis of typhoid carriers via detection of IgA. A DNA detection test called EZ Typhi Carrier DNA has also been developed and tested to be effective for the detection of typhoid carriers. Development of such a test would create an impact not only in public health management but also bring in high economic returns due to the need for such a test world-wide. Work is currently in progress to develop multi-tests, via development of "lab on a chip" using nanotechnology that allowed the detection of 16 -48 tests within 30 minutes. The discovery of the specific antigen and the development of the various kits have led to the filing of 24 patents world wide of which 7 have been attained.

In the quest toward solving the problem for typhoid diagnosis, technology has to be acquired and conquered. The work demonstrates the need to combine fundamental and applied research toward wealth creation for the country, enhancing quality of life of Malaysians and provide help to the underdeveloped countries of the bottom billion. With technology

foresight, the ability to acquire and manipulate technology and imbibing the spirit of global competitiveness, Malaysian scientists will ensure Malaysia as a player in the Knowledge-based economy and prove that Malaysian R&D can bring in respect and profits and not an economic drain.

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DR. LEE HAN LIM

Institute for Medical Research

"Research and Development of Innovative approaches for the Control of Insect Vectors of Public Health importance"

Year 2003 MTSF Science & Technology Award Recipient

Born 25 February 1953 in Kuala Kangsar, Perak, Dr LEE Han Lim received his early education in Kuala Kangsar and Ipoh, Perak. In 1977, he graduated from Universiti Sains Malaysia (USM), Penang with BSc (Hon). Subsequently, he joined the Institute for Medical Research, Kuala Lumpur in March, 1978 as a research officer in the Division of Medical Entomology. He immediately enrolled into the post-graduate Diploma in Applied Parasitology & Entomology (D.A.P. & E.) and graduated in the same year. In 1996, he received his Master of Science from USM, Penang and in 1999 his PhD from the Hamilton University based on his voluminous publications and research experiences. In 1993, Dr Lee was appointed the Head of the Medical Entomology Unit and the WHO Collaborating Center for Vectors, which he is still holding to date. He was also appointed the Deputy Dean of the School of D.A.P. & E. in 1996 and subsequently the Dean in 2004. Dr Lee's main research interest is in the vast field of medical entomology and parasitology. His research focuses on vectors of dengue, chikungunya, malaria, filariasis, Japanese encephalitis; microbial control agents, chemical control agents, insecticide resistance, forensic entomology, maggot debridment therapy, myiasis and transgenic technology. With such wide and varied interests, Dr Lee has published a total of 180 papers in peer-reviewed scientific journals, 85 reports and presented 216 papers in international and local seminars. He is the recipient of 48 major research grants and 38 awards and honours. To date, he has patented and filed 10 patents worldwide.

Research on Microbial Control Agents of Vectors

The world's first anaerobic mosquito microbial control agent, *Clostridium bifermentans* serovar *malaysia* was isolated from a soil sample collected in Malacca in 1990. Isolation of another new serotype of mosquitocidal *Clostridium bifermentans* known as serovariety *paraiba* was also successfully conducted. Isolation of a new serotype of mosquitocidal bacteria-*Bacillus thuringiensis* serovariety *jegathesan* (H-28a28c) was shown to produce novel toxins (9 polypeptides) unrelated to all known serotypes of *B. thuringiensis*. Other isolation included a gram negative mosquitocidal agent, *Burkholderia pseudomallei*, which was reported for the first time and another novel *Bacillus thuringiensis pahangi*. Production of an anti-microbial agent from a local isolate of mosquitocidal *Bacillus sphaericus* was also reported for the first time. Several field trials were conducted to confirm the efficacy of indigenous microbial agents in mosquito control. A technique for the mass-application of mosquitocidal bacteria through ultra-low-volume spraying was also first developed. A related development in application technology was the first discovery that mosquitocidal *Bacillus thuringiensis* and *B. sphaericus* can be effectively applied by thermal *fogging*. Attempts were also made to mass produce indigenous *B. thuringiensis* H-14 and *B. sphaericus* by utilising local organic wastes. Indigenous bacteria exhibiting molluscidal activity against snail of medical importance were also isolated for the first time.

Research on Chemical Control of Vectors

In resistance studies, enzyme microassay techniques for the rapid detection of insecticide resistance in mosquitoes were developed. Rapid monitoring of resistance gene frequency in wild populations of *Culex quinquefasciatus* adults

utilising enzyme microassays was first reported. The occurrence of insensitive acetylcholinesterase-type of insecticide resistance in Malaysian German cockroach was also first detected using enzyme microassays. The effect of ultra-violet light and microwave on the insecticide susceptibility status of insects was studied for the first time. The absorption and translocation route of chemical insecticides in the fronds of *Eicchornia* plants to the root whence the larvae of *Mansonia* mosquitoes (vector of Brugian filariasis) attached was first reported . This can be exploited in *Mansonia* control as the insecticide can be translocated by the plant directly to the larvae. In other studies, the pyrethroid deltamethrin was shown to be able to replace DDT in residual-spraying for the control of malaria, while the adulticidal and larvicidal effect of ivermectin, a filaricidal drug on Malaysian vector mosquitoes was evaluated.

Research on Dengue Vector Biology & Control

Two nation-wide *Aedes* larval surveys in urban towns in Peninsular Malaysia were conducted. In *Aedes* vector surveillance, ovitrap was found to be a more sensitive surveillance tool than larval survey. A dengue outbreak stochastic model useful in the determination of the threshold of dengue transmission and prediction of outbreak occurrence by employing ovitrap survey data was constructed. To monitor the virologic infection in *Aedes* vectors, an ELISA technique to detect dengue antigens in mosquito vectors and therefore the dengue infection rate in mosquito was developed. In studying the emergence of resistance to chemical control agents, low temephos resistance in *Aedes aegypti* in Malaysia was first detected. The effectiveness of other larvicide such as permethrin as an alternative larvicide against container-breeding *Aedes* was evaluated. For the first time the occurrence of transovarian transmission of dengue virus in *Aedes aegypti* under experimental conditions was detected. This finding is subsequently confirmed in field studies. The seroepidemiology of dengue infection in children was conducted in Kuala Lumpur. The findings indicated that children were seroconverted at a rate of 15% annually and the rate of seroconversion was associated with vector density. Other related studies involved the first report that sublethal dosages of malathion did not affect the vectorial capacity of *Aedes aegypti* adults and the development of the dengue virus, while the inhibitory effects of ribavirin, an antiviral agent on dengue virus in *Aedes aegypti* adults was shown.

Other Important Studies

These include: characterisation of arthropod succession in primate carrion and application of these data in forensic entomology, characterisation of protein profiles from Malaysian vector mosquitoes as a first step in understanding human immunological response to mosquito bites, documentation and reporting of human myiasis cases in Malaysia and study on the role of housefly in the transmission of rotavirus.

Commercialisation

Research findings were successfully commercialised. Among these are: Kits for the rapid detection of insecticide resistance in medically important insects (4 kits), insecticidal emulsion paint, a wettable powder of a Malaysian isolate of mosquitocidal *Bacillus thuringiensis* H-14 and *Bacillus sphaericus* and a saliva test kit for the detection of acetylcholinesterase.

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ASSOCIATE PROFESSOR DR ZARITA ZAINUDDIN

Universiti Sains Malaysia

"Neural Networks"

Year 2003 MTSF Science & Technology Award Recipient

Dr. Zarita hails from Taiping, Perak and received her early education at Treacher Methodist Girls' School, Taiping. She pursued her tertiary education at Monmouth College, Illinois, USA in Mathematics where she graduated with Departmental Honors and Cum Laude and was subsequently offered the Universiti Sains Malaysia's (USM) Academic Staff Training Scheme (ASTS) fellowship to pursue M.Sc. in Applied Mathematics at Ohio University, USA. Upon successful completion of her masters program, she began her career as a lecturer with USM in 1981. After three years of service, she was awarded another fellowship by USM to pursue a doctoral degree in Applied Mathematics and now holds a Ph.D. with specialization in Neural Networks.

Dr. Zarita's research interests center on optimization methods in learning algorithms of neural networks, applications of neural networks, mathematical modeling and integration of technology in the teaching of mathematics.

Dr. Zarita's efforts were duly rewarded when she won the prestigious international Commonwealth Association of Science, Technology and Mathematics Educators (CASTME) 2002 award and the prestigious Malaysia Toray Science Foundation Award (MTSF) 2003 in addition to her awards in the International Invention, Innovation, Industrial Design and Technology (I.TEX 2002) and The Ministry of Science, Technology and Environment (MOSTE-2003) innovation competition for the newly proposed Accelerated Learning Methods (ACEL) in Neural Networks. Recently, she was awarded the PERSAMA (*Persatuan Sains Matematik*) Principal Award (Innovation Category) for her Neural Networks Toolbox named AceL Neural Net. In recognition of her excellent achievements, she was twice awarded the USM Excellent Service Award, in 2001 and 2006.

Dr. Zarita has numerous publications to her credit in books, journals and proceedings, both at local and international levels, including articles in International Journal of Control, Neural, Parallel and Scientific Computations and International Journal of Computer Mathematics. She is also the reviewer for neural networks articles for various local and international journals and conferences. Besides being a member of the prestigious neural networks society, the International Neural Networks Society (INNS), she is also a member of the International Scientific Advisory Board for WASET (World Academy of Science, Engineering and Technology). In January 1994, she was appointed as Visiting Scientist at the University of Manchester Institute of Technology (UMIST) and subsequently, in June 1994 and Oct 2003 as Visiting Research Associate at Loughborough University of Technology (LUT) and Universite du Quebec (UQU) a Trios-Rivieres, Canada, respectively, where she conducted joint research in neural networks.

From the very start of the computer era, scientists have dreamed of making computers that can learn and think. With this aspiration, artificial neural network (ANN) models, which draw their ultimate inspiration from neurons in the brain, have been studied for many years.

There are many different neural network architectures designed for different purposes; the most popular being the multi layer perceptron (MLP). The most common way of training the MLP is the Back Propagation (BP) algorithm. The popularity of the BP algorithm can be attributed to its simplicity and generality. The MLP is capable of approximating arbitrary nonlinear mappings. One of the principal concerns in BP learning is the complexity of learning. BP learning is too slow for many applications; further, it scales up poorly as tasks become larger and more complex. The slow speed at which the current algorithm learns has been the greatest single obstacle to the widespread use of connectionist learning networks in real-world applications. Viewed in this light, the approach and emphasis taken in this research endeavor is to investigate factors governing the convergence rate of the BP learning algorithm. The goal is twofold: to develop faster learning algorithms and to contribute to the development of a methodology that will be of value in future studies of this kind.

The reasons for the slow convergence of the back propagation method have been studied. To date, many techniques have been proposed to deal with the inherent problems of back propagation. These techniques can be divided roughly into two main categories; global and local adaptive techniques. Global techniques are algorithms that use global knowledge of the state of the entire network, such as the direction of the overall weight update vector. Most of these techniques have their roots in the domain of optimization theory. The simplest is the first-order method that uses the steepest-descent (SD) direction. An alternative is the conjugate gradient (CG) method, which modifies the SD direction by conjugating it with the previously used direction. Finally, the Levenberg – Marquardt (LM) method is a second – order method that approximates the second derivative using the first-order gradient. Mathematical analysis not only uncovers the detailed computations involved in the back propagation algorithm but also provided important insights into the development of two local adaptive methods, namely, the Dynamic Momentum Factor (DMF) and the Dynamic Learning Rate (DLR). All the above methods have been incorporated into the award winning Neural Networks toolbox, termed, AceL Neural Net.

The effectiveness and fast convergence of the training methods have been demonstrated on several real-world application problems. These include the classification of the iris plant, gender classification of crabs, breast cancer detection and human face recognition. The acceleration methods, namely, Dynamic Momentum Factor (DMF) and Dynamic Learning Rate (DLR), have proven to be very effective and superior in terms of convergence, when tested and compared with the Batch BP on four real world application problems. A speed up of up to 97.08 % and 97.27 % was obtained for the DMF and DLR methods respectively.

After the network has been respectively trained with the batch BP, DMF, DLR, CG, LM and SD methods, the generalization capability of the MLPs on new vector pairs was tested. Although fewer function evaluations are necessary to achieve convergence, both the DMF and DLR showed similar generalization capability compared to the batch BP. In other words, the capability of the networks to recognize input patterns outside the training set is not impaired by the employment of these acceleration methods. Hence, these algorithms are promising in practical applications where generalization is important.

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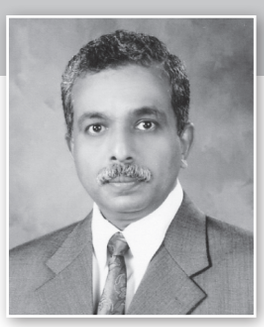
PROFESSOR DR. KURUNATHAN RATNAVELU



Universiti Malaya

"Theoretical Atomic Collision Physics"

Year 2004 MTSF Science & Technology Award Recipient

Professor Dr. Kurunathan Ratnavelu *BSc Hons, MSc (UM), PhD (Flinders), CPhys (UK), MInstP (UK), F.I.P.M (Malaysia)* received his basic university degree with First Class Honours at the Department of Mathematics, University of Malaya in 1982 and with a University of Malaya Fellowship obtained the degree of MSc by Research in 1985. Subsequently with a Flinders University Research Scholarship, he obtained his doctorate in physics from Flinders University in 1990. He is married to Dr. Nirmala Mahadevan and they have two children (a girl and a boy).

On joining the Department of Mathematics in 1989, he initiated research in theoretical aspects of the scattering of positrons and electrons with atoms. He has contributed significantly to the advancement of knowledge in the theoretical understanding of the physical systems involving the scattering of positrons with atoms as well as scattering of electrons with atoms. His development of a realistic ab-initio Coupled-Channel Optical (CCO) method to elucidate positron-hydrogen atom scattering and its extension to other hydrogenic-atoms such as lithium and sodium was a significant contribution to the theoretical development in this field. His joint work on Proton impact on Positronium (Ps) demonstrated the advancements made by theorists in treating charge exchange reactions with the Ps target especially on the production of anti-hydrogen using the charge exchange mechanism which is vital for the testing of the fundamental theories of physics on anti-matter.

Prof. Ratnavelu's contribution to his field is evidenced by his more than 35 international publications in high impact journals such as Journal of Physics B (UK) and Physical Review A. His international recognition can be gauged from his role as a frequent reviewer for the Journal of Physics B (UK): Atomic, Molecular and Optical Physics and the Journal of Condensed Matter (UK) as well as External Examiner for Ph.D. theses in overseas universities. His academic leadership qualities have also been recognized at University of Malaya where he has served as the Deputy Dean in charge of Research and Higher Degree in 2003-2006 and as acting Deputy Dean (1999-2000). He was promoted as Professor in 2001.

For his work, Prof. Ratnavelu was awarded the National Young Scientist Prize in the Strategic Sector by Ministry of Science, Technology and Environment in 1996 and subsequently the 2005 Malaysia Toray Science Foundation Award. In 2006, he was named as one of the 40 Distinguished Alumni of Flinders University for his achievement and contribution in Physics internationally and in Malaysia.

He serves as Honorary Secretary to the Malaysian Institute of Physics. He also serves as an Associate Editor of the Jurnal Fizik Malaysia as well as of the Malaysian Journal of Science Part B: Physical Sciences.

Prof. Ratnavelu's main research interest is in Theoretical Atomic Collision Processes with specific interest in positron collisions with atoms. Among his outstanding contributions are in the recent development and the demonstration of

an optical potential method to positron-hydrogen atom scattering process and its extension to other hydrogenic-type atoms. Another major contribution is his joint theoretical work with Jim Mitroy on anti-hydrogen formation in 1995. His scientific contribution's is evidenced by the international publications; as a reviewer for international journals and my collaboration with eminent international researchers. In Malaysia, the recent Deluxe Edition of the National Citation Indicator for Malaysia (publications analysed with Malaysian addresess) from Thomson ISI for the period 1982-2005 lists Prof. Ratnavelu as the most Highly Cited in Physics within Malaysia with about 178 citations for 24 papers with a citation impact factor of 7.

The following contributions can be considered as among his most significant works:

A. Contribution to Production of Anti-Hydrogen Atom and Low-Energy Positron Hydrogen and Positron-Alkali Atom Scattering

Ratnavelu has worked on the low-energy scattering regime with Jim Mitroy of the Northern Territory University, Australia. Mitroy developed the Momentum-Space Close-Coupling Theory for Positron-Atom Scattering Systems in 1993. Among their major works are: The highly cited work of Mitroy and Ratnavelu J. Phys. B (1995), *Positron-Hydrogen System at Low-Energies, Vol 28,* 287 provided the most comprehensive study of the positron-hydrogen and positronium-proton systems at energies up to the ionization threshold. They undertook the most detailed scan of the cross sections. Their calculation also represented the first attempt at an accurate calculation of the e^+-e^--p system for the Ps(1s)-p entrance channel. This work is considered among the first accurate ever theoretical calculation on a very challenging theoretical problem, the formation of Hydrogen by proton impact with positronium (Ps). The subsequent experimental work by Merrison et al (1996) demonstrated the first experimental observation of charge exchange involving a Ps target. This charge exchange reactions has remained a formidable problem until the advent of theoretical breakthroughs that treated Positronium formation in the same manifold as the atomic target systems. This demonstration is supportive of the production of anti-hydrogen using this reaction. [Direct Citations for Mitroy and Ratnavelu(1995)upto early 2007: 43 Citations Total Estimated Indirect Citations (The citations of articles citing the above work) subtracting self-citations of author and co-author for the above theoretical paper ~ 300 citations. See Holzscheiter MH, Charlton M *Ultra-low energy antihydrogen* in REPORTS ON PROGRESS IN PHYSICS Vol 62 (1): 1-60 JAN 1999. Citations: 60; Merrison JP, Bluhme H, Chevallier J, et al., *Hydrogen formation by proton impact on positronium ,* PHYSICAL REVIEW LETTERS Vol. 78 (14): 2728-2731 APR 7 1997. Citations: 20].

In 1994, Mitroy and Ratnavelu further demonstrated the close-coupling formalism for positron-alkali atoms that allowed the atomic targets and Positronium states to be treated in the close-coupling expansion. Their generalized rearrangement matrices showed that the earlier calculation of Hewitt et. al was not error-free. This technology also allowed practical calculations to be done. Unlike other works, Mitroy and Ratnavelu also incorporated the direct effects of the core electrons into the calculation.

B. Contribution to the Accurate Calculation of Positron Impact Ionization Cross Sections for Hydrogen Atom

The first ever experimental measurement on Positron hydrogen atom scattering was reported in early 1990 (Spicher et al Phys. Rev. Lett Vol 64, 1019 (1990)). There has been a strong justification that the continuum effects are the main contributory aspect in the calculation of ionization cross sections in atomic scattering. Ratnavelu (Australian Journal of Physics, Vol 44,265 (1991)) reported by using the continuum optical model that the ionization cross sections for H measured by Spicher et al are puzzling. It took another experimental work in 1993 (Jones et al, Journal of Physics B, Vol 26, L483 (1993)) to demonstrate that the earlier experiments may be an overestimate. [Direct Citations: 6 citations Total Estimated Indirect Citations (The citations of articles citing the above work) subtracting self-citations of author and co-author f or the above theoretical paper ~ >100 citations].

C. Contribution to the Development And Implementation Of An Optical Potential Method For Positron-Hydrogenic Atomic Systems

In 1997, Ratnavelu (Private Communication) extended the highly successful optical potential model of McCarthy and Stelbovics to study positron scattering by atoms. This was a major development as unlike previous studies that exclude the Positronium channels, this work treated the positron-atomic target channels and the Positronium-proton channels in the same close-coupling expansion. Furthermore, the optical potential was used in the most realistic treatment of positron scattering by atomic hydrogen [Ratnavelu and Rajagopal J. Phys.: At.Mol.Opt.Phys (1999)]. Their main theoretical finding demonstrated accurate ionization cross sections that were in excellent agreement with the latest experimental measurements of Jones et. al. This provided enough justification that the method has much promise in studying positron scattering by atoms.

D. Contributions to the electron scattering by atoms

Professor Ratnavelu had also worked and helped in the development of the Coupled-Channels-Optical Method for the electron-atom scattering systems. This research was considered among the leading studies of the period before the advent of more highly sophisticated methods in the mid-1990s. Among his work, the work on electron-magnesium atom is still being cited by other researchers in the area.

Publications

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PROFESSOR DR. YAAKOB BIN CHE MAN

Universiti Putra Malaysia

"Instrumental methods for Fats and Oil analysis with special emphasis on Palm Oil products"

Year 2004 MTSF Science & Technology Award Recipient

Born on 2nd May 1954, Professor Dr. Yaakob B. Che Man started his modest upbringing and schooling in Kuala Terengganu. He obtained his Diploma in Food Technology from Institut Teknologi Mara in 1976. He furthered his studies in the USA where he graduated with High Honours in B.Sc. and M.Sc. Degrees (Food Technology & Science) from the University of Tennessee, Knoxville, in 1977 and 1979, respectively before gaining his PhD in Food Chemistry from the University of Illinois at Urbana-Champaign in 1988.

He began his career as a lecturer in Universiti Pertanian (now Putra) Malaysia in 1980. Since then, he has served the university in several capacities such as Head of Department of Food Technology and Deputy Dean of the Faculty of Food Science and Biotechnology and currently, the Founding Director of the Halal Products Research Institute, UPM. He is also currently the Honorary Secretary and Fellow of the Malaysian Institute of Food Technology (MIFT), past executive council member of the Malaysian Oils Scientists and Technologists Association (MOSTA), member of the Malaysian Invention & Design Society (MINDS), Professional member of the American Oil Chemists' Society (AOCS) and the Institute of Food Technologists (USA), and a past Chairman of the Food Chemistry and Biochemistry Sub-section, International Society of Food, Agriculture and Environment (ISFAE). He sits on the editorial boards of several international journals. To date, he has more than 200 scientific publications in international refereed journals and book chapters, countless invites to deliver speeches and keynote addresses in scientific meetings and conferences as well as public fora.

Professor Yaakob's main area of research is the chemistry and technology of fats and oils. For several years, he worked on the development of state-of-the-art rapid instrumental techniques for fats and oils analysis, especially palm oil, and of late on halal products authentication. His recent success in the development and optimization of an electronic nose technique for rapid oils and fats analysis in food products was exhibited in the National Museum of Emerging Science & Innovation, Tokyo from June to October 2007. Using just 10 seconds to detect lard (non-halal or impermissible according to Muslim tenets) in foodstuff, Prof. Yaakob iterated that, "the Japanese see this development as unique and unusual because it merges religion and science harmoniously." This work also received an editorial acknowledgement earlier in 2004 by Sensor Technology Alert of USA, for its breakthrough research in sensor technology for monitoring the oil quality.

Over the years, he has received numerous awards and accolades, which include; First Prize UPM Research Award 2000, Outstanding Paper Presentation Award 2000 by the American Oil Chemists Society, USA, listed in the Top 10 Scientists in Malaysia for the year 2003/04 by the Ministry of Science, Technology and Environment (MOSTE), 2004 Al-Khwarizmi International Award, Tehran, 2004 Malaysia Toray Science & Technology Award, 2004 Vice Chancellor Award for Research Excellence from Universiti Putra Malaysia, Saintis Cemerlang Award from the Ministry of Higher

Education in 2005, Gold (2005) and Silver (2006) Medals Awards in Geneva-Palexpo, Switzerland, Best Publication awards from Malaysian Palm Oil Board for the year 2000 to 2006, Anugerah Melayu Cemerlang UMNO 2006, Anugerah Tokoh Akademik Negeri Terengganu 2006, making the list of Top 10 in Malaysia and No. 1 in Universiti Putra Malaysia in "Leading Scientists and Engineers in OIC Countries" in 2006/07 and many more other awards and accolades. For his academic and scientific contributions, he has been cited in The Marquis Who's in the World since 2000. He was also awarded the Pingat Ahli Setia Sultan Mahmud (ASM) in 2000 by the Kebawah DYMM Al-Wathiqu Billah Sultan Mizan Zainal Abidin Ibni Almahum Sultan Mahmud Al-Muktafi Billah.

Professor Dr. Yaakob's research was on the development of rapid instrumental techniques for fats and oils analyses by Differential Scanning Calorimetry (DSC), Fourier Transform Infrared (FTIR) Spectroscopy and Electronic Nose (EN) technology. These includes rapid methods for qualitative and quantitative determinations of various parameters related to oils and fats quality such as determination of FFA, IV, PV, total polar compounds, melting point, cloud point, minor components content, analysis of synthetic antioxidants, monitoring lipid peroxidation and storage stability and transesterification processes, crystals development and solid fat content during processing of oil-based food poducts, detection of food and oil adulterations, and many more. The methods developed were breakthroughs in the field of oils and fats aimed to replace the conventional methods.

The necessity for rapid and reliable assessment of quality is of special importance to the food industry in general and oils and fats in particular. Professors D. Yaakob's studies show that the use of DSC, FTIR spectroscopy and EN techniques can prevent the use of time- and work-consuming chemical methods and eliminate the use of toxic chemicals that are hazardous to the analysts as well as to the environment. Accurate monitoring of oil quality using these state-of-the-art techniques can help avoid either risk to public health or cause financial loses to the food industry. Professor Dr. Yaakob's studies represent recent developments in the use of DSC, FTIR spectroscopy and EN as potential new analytical techniques for fats and oils industry, especially palm oil industry. His studies have contributed to science and technology in the following ways:

- DSC, FTIR spectroscopy and EN techniques represent rapid, accurate, reliable, non-destructive, cost effective and efficient alternative approaches t conventional standard chemical methods for monitoring and quantitative determination of various quality parameters in fats and oils, with no need for delicate skills.
- The new instrumental methods are completely safe and no safety precautions required. The techniques involve small sample size, minimal sample preparation, and absence of toxic and hazardous chemicals.
- These techniques can prevent the use of laborious, time- and work-consuming chemical methods and eliminate the use of toxic chemicals that are hazardous to the analysts as well as to the environment.
- The results in these works represent new techniques useful for the assessment of different quality of fats and oils and their products.
- The techniques developed have the potential to replace the chemical methods for fast and on-line and at-line for assessing fats and oils quality.

- The use of DSC, FTIR spectroscopy and EN encourages many scientists to innovate new techniques to measure the various physical and chemical properties of fats and oils as well as to monitor quality changes during processing. Thus increased application of these instruments in academic research and in fats and oils industry.

Publications

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4. Che Man, Y.B. and Marina, A.M. 2006. CHAPTER 2: Medium-Chain Triglyceride, in *Nutraceutical and Specialty Lipids and Their Co-products*. Edited by F. Shahidi. CRC Taylor & Francis, New York, USA. Pp 27 - 56 (ISBN 157444499).

PROFESSOR DR. WONG CHIOW SAN

Universiti Malaya

"Plasma Technology, Pulse Power & Gas Discharge Physics "

Year 2005 MTSF Science & Technology Award Recipient

Prof. Wong Chiow San is the Leader of the Plasma Technology Research Group at University of Malaya since 1992. He has managed to gather a group of scientists with similar interest from within the institution as well as from other institutions to conduct research in such critical fields as plasma processing of advanced materials and pulsed plasma radiation sources. This Plasma Technology Research Group supports a large group of postgraduate students, with 27 having graduated with MSc and PhD degrees. It is currently still supporting 3 PhD and 10 MSc students.

Prof. Wong is undoubtedly an acknowledged expert in the area of plasma physics and is one of the pioneers in the development of plasma technology in the country. His main research focus is on the important development of various pulsed plasma radiation sources including the plasma focus (neutrons, x-ray and ion beam), the vacuum spark (x-ray and EUV) and the capillary discharge (EUV). His research on plasma focus has the vast potential to be the neutron source for the next generation airport security on neutron activation technique, a very important area in the present era of terrorism. The vacuum spark and capillary discharge are being developed for nanolithography in microelectronic fabrication, another cutting edge technology. For his research, he has many collaborators both nationally and internationally. Prof. Wong and his collaborators have published 156 papers, 81 of which are in peer-reviewed journals. He has been invited to international meetings as keynote and plenary speaker and has a long list of collaborators locally and overseas. He is also involved with many international and local scientific bodies and often asked for his expert opinions by international journals including Applied Physics Letters (AIP), IEEE Transaction on Plasma Science, Plasma Sources Science and Technology (IOP), Nanotechnology (IOP) and Vacuum (Elsevier). He has served as consultant to several major companies including international company such as Ciba Vision.

Prof. Wong is a well-respected scientist not only nationally but also globally. He has gained accolades for his work and championed the cause of plasma research among developing countries. Prof. Wong was elected President of the Asian African Association for Plasma Training (AAAPT), a plasma network of 44 institutions from 24 countries. Another international achievement, which brings great pride to the country, is the recognition of his laboratory by the International Centre for Theoretical Physics to be one of its associated Centres for the training of scientists from developing countries in plasma technology. He has served in the International Organizing/Advisory Committees of various International meetings such as the EEE International Conference on Plasma Science (ICOPS) 2003 held in Korea and the 7th Asia Pacific Conference on Plasma Science and Technology (APCPST) 2004 in Japan. Prof. Wong has many publications to his name and has published his work in such prestigious international journals like Review of Scientific Instruments, Journal of Applied Physics, Japanese Journal of Applied Physics, IEEE Transactions on Plasma Science, Laser and Particle Beams and Physics Letters.

Prof. Wong has made significant contributions in his discipline by promoting and encouraging plasma research in Malaysia. For his contribution, he was made a Fellow of the Malaysian Institute of Physics and a Fellow of the Malaysian Scientific Association and remains as a long-serving Council Member of the Malaysian Institute of Physics.

He has served as the Chief Editor of the Journal Physics Malaysia since 1991. He was the Hon. Secretary of the Organizing Committees of the series of four Tropical Colleges on Applied Physics held in Kuala Lumpur between 1983 and 1990, and the Hon. Secretary of the 5th Asia Pacific Physics Conference held in 1992. In addition, he was the Chairman of the Local Organizing Committee of the International Meeting on Frontiers of Physics held in 2005 in Kuala Lumpur.

Prof. Wong feels that Plasma Technology is critical for the national industry in the 21st century. Instead of sourcing for this from advanced countries, he firmly believes that Malaysia should develop its own plasma technology and Prof. Wong, through his leadership, is proving that this is feasible even for a developing country such as Malaysia.

For his long and distinguished service to the country, Prof. Wong was named one of the recipients of the Malaysia Toray Science and Technology Award for 2005, recognition well deserved and long overdue for his many years of dedicated service in the promotion of his scientific discipline. He was elected a Fellow of the Academy of Sciences Malaysia in 2007.

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PROFESSOR DR. SOH AIK CHIN

Applied Agricultural Resources Sdn. Bhd.

"Plant Breeding & Genetics "

Year 2005 MTSF Science & Technology Award Recipient

Professor Soh, Aik Chin is a leading oil palm breeder and geneticist internationally. Prof. Soh headed Applied Agricultural Resources Sdn Bhd., a R&D company owned by two large plantation companies, Boustead Bhd. and Kuala Lumpur Kepong Bhd., from 2000-2007. He obtained his B.Agric.Sc.(Hons) in 1971 from University of Malaya on the Malaysian Rubber Fund Board Scholarship and Varsity Senior Scholarship awards. After graduation he worked in Felda as a cocoa agronomist for two years before returning to his alma mater to pursue M.Agric.Sc. and became a lecturer. He obtained his PhD at Oregon State University in 1979 on a international student scholarship/teaching assistantship. Upon returning, he joined HRU as an oil palm breeder/geneticist.

Over the years, Prof. Soh has developed: (1) the Dumpy-AVROS semi-dwarf oil palm hybrid variety which facilitates harvesting and extends the economic life of the crop, (2) the further improved Dumpy.Yangambi.AVROS hybrid variety with improved higher yield potential, and (3) high yielding clones from tissue culture These varieties have continued to make significant economic impact on the oil palm industry.

Prof. Soh continues to make valuable contributions to the knowledge and advancement of science and technology, particularly in plant breeding/genetics and tissue culture/biotechnology e.g. devising selection and breeding strategies to further improve oil palm hybrids and clones. These are vital contributions towards the competitiveness of the palm oil industry and the country's economy. He has more than 80 publications, a number in leading international journals. In addition, he has served as editor and reviewer in leading international and national journals e.g. Euphytica, Plant Breeding Reviews, Journal of Oil Palm Research, and as a consultant for Malaysian Palm Oil Board and CIRAD, the French leading international research institute for tropical crops. Prof. Soh has also served in the technical committees of MPOB, MPOA, DOA, SIRIM and National Accreditation Board. He remains as a consultant to his old company.

Prof. Soh was recognized for his achievements and contributions in the following awards:

- Malaysia Toray Science Foundation - Science & Technology Award, the first from the private sector.
- Fellow of Academy of Sciences Malaysia.
- Special Professor in University of Nottingham Malaysia Campus

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DR. CHUA KAW BING

National Public Health Laboratory, Ministry of Health
"Clinical and Molecular Virology"
Year 2006 MTSF Science & Technology Award Recipient

Dr. K.B. Chua graduated from the University of Malaya, Kuala Lumpur, Malaysia in 1979 with a MBBS degree. He sailed through several specialist examinations to obtain his membership in the U.K. Royal College of Medicine (Paediatrics) in 1983 and the Master of Medicine degree in Paediatrics from the National University of Singapore in 1984. Subsequently, he was awarded the Doctor of Medicine degree from the University of Malaya, in 1998; membership of the Royal College of Pathologist, U.K. in 1999 and fellowship of the Royal College of Medicine, Edinburgh, in the same year. His academic pursuit was crowned by a pass with distinction in the Doctor of Philosophy degree he obtained from the University of Malaya in 2002. He obtained his fellowship of the Royal College of Pathologist, UK in 2007.

Dr. Chua's professional career began as a house-officer and medical officer in the University Hospital Kuala Lumpur. Five years later, he left to work as a consultant paediatrician, first with the Chinese Maternity Hospital, Kuala Lumpur, then with the Lady Templer Specialist Centre (formerly Lady Templer Hospital) in Cheras, Kuala Lumpur. With the closure of Lady Templer Specialist Centre in 1986, Dr. Chua practiced as a consultant paediatrician in the Pertama Specialist Centre until 1994, when he returned to his alma mater, the University of Malaya because of his burning passion for learning and research. He was taken in as a lecturer in the Department of Medical Microbiology, Faculty of Medicine, and rose in rank to become clinical virologist in 1996, then consultant clinical virologist and associate professor in 1999. In 2001 he was appointed Professor of Paediatrics and Medical Microbiology in the International Medical University but in early 2003 he accepted an invitation from the Ministry of Health to serve as a consultant clinical virologist in the National Public Health Laboratory where his primary role is to set up a world-class diagnostic virology laboratory and a state-of-the art biosafety level 3 laboratory complex.

Dr. Chua's scientific achievement, discoveries and breakthroughs:

1) **Discovery of Nipah virus and Nipah virus related work**

   The discovery of the Nipah virus was pivotal for the adoption of effective control strategies to end the outbreak after more than 6 months of suffering, anxiety and bewilderment. The isolation of the virus from patients' excretions led to the adoption of extra precautions for health care and public health workers and this averted a potential secondary outbreak among those who were looking after infected patients. The novel approach of collecting urine samples and partially eaten fruits from fruit-bats for virus isolation helped to establish the pteropid fruit bats as the natural reservoir hosts of Nipah virus. Dr. Chua's novel way of urine collection from bats has been adopted by many researchers world-wide as it opened a new gateway to the study of infectious agents carried by bats. Dr. Chua was the first to work out the complex interplay of events leading to the spillover of the Nipah virus from flying foxes to pigs and thence to the human population. The results of this investigation are expected to help prevent future spillover events.

2) Other emerging diseases in Malaysia

In 1996, shortly after starting his career as a clinical virologist, Dr. Chua became the first person in Malaysia to isolate human herpesvirus 6 (HHV6) and human herpes virus 7 (HHV7) from the blood of children who were down with exanthema subitum. In the following year, Dr. Chua was involved in a controversy over the cause of deaths due to hand-foot and mouth disease (HFMD) among children in Sarawak and Peninsular Malaysia. Contrary to prior reports that the outbreak was due to a Coxsackie virus and death was the result of viral myocarditis, Dr. Chua confidently diagnosed acute encephalomyelitis due to EV71 virus and he was subsequently shown to be right. In early 1999, Dr. Chua was called upon to solve the mystery of an outbreak of "febrile illness with joint involvement" in Port Klang. Again, he created history by being the first person in Malaysia to identify the chickungunya virus as the cause of an outbreak. This finding was confirmed by a reference laboratory in Australia. When the virus re-emerged as the cause of another outbreak in Bagan Panchur, Perak, in March 2006, Dr. Chua was able to confirm the etiology within 48 hours and also showed that the 2006 chikungunya virus was genetically closely linked to the 1999 outbreak strain.

In 2000, Dr. Chua established the cause of another outbreak of HFMD with fatal encephalomyelitis as EV71 together with a new variant of echovirus 7. Fuelled by his interest in zoonotic transmission of infectious agents to humans, Dr. Chua systematically studied the microbial flora in bat urine. This effort led him to the discovery of the Tioman virus, a novel *Rubulovirus* in the family of Paramyxoviridae, closely related to the Menangle virus which is another bat paramyxovirus that emerged in New South Wales, Australia, known to cause abortion and stillbirths in pigs and influenza-like illness in humans. With his novel method of collecting bat urine, he was able to isolate the Pulau virus, a novel *Orthoreovirus*. This discovery threw new light on the reservoir host of the Nelson Bay virus, a closely related reovirus isolated in Australia in the 1960s.

He isolated another two more novel viruses (Melaka virus and Kampar virus) from patients with influenza-like illness during his career in the National Public Health Laboratory. Both Melaka virus and Kampar virus are novel orthoreoviruses and closely related to Pulau virus which was isolated from fruitbats. Thus, all these three novel orthoreoviruses underpin the importance and understanding of emerging zoonotic viruses.

3) Development of Diagnostic Virology in Malaysia and the region

Within 2 months of joining the NPHL, he established a high capacity national cell-line bank for diagnostic virology and within 6 months, the diagnostic virology laboratory of NPHL was fully equipped to support national infectious disease surveillance and outbreak investigation. Together with a high capacity diagnostic molecular virology laboratory developed by him, the NPHL was able to rapidly establish the aetiology of a number of infectious disease outbreaks affecting the country since 2003. His innovative cell culture tube, named Jui Meng's (JM) tube, for diagnostic virology, significantly reduced the cost and enhanced the biosafety of work on virus isolation.

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PROFESSOR DR. ROSLAN ABD SHUKOR

Universiti Kebangsaan Malaysia

"Condensed Matter Physics – High Temperature Superconductivity"

Year 2006 MTSF Science & Technology Award Recipient

Professor Dr. Roslan Abd Shukor is currently with the School of Applied Physics, Universiti Kebangsaan Malaysia. He earned his BSc in 1982 and MSc in 1985 in physics from Northern Illinois University, USA and his PhD in Solid State Physics in 1991 from the University of Arkansas, USA.

Professor Roslan has been actively involved in high-temperature superconductor research since he started working on his doctorate dissertation in 1989. Superconductors are materials that can conduct electricity without any resistance and they have many electrical and electronic applications.

Professor Roslan has studied the propagation of ultrasonic waves in a wide range of high-temperature superconductors and showed that the two dimensional character of these materials naturally leads to high-temperature superconductivity. His research has shed light on the interplay of crystal lattice vibrations in the mechanism of these materials. These findings have been published in *Superconductor Science and Technology* in 2002 which has since been cited by researchers in the United States and Europe.

Until now Professor Roslan has published 75 papers in the international journals, 40 papers in national journals and has written and co-edited 16 books. He has been invited to deliver keynote address and plenary papers at international and local conferences.

Professor Roslan serves as an Advisory Board of *Superconductor Science and Technology* since 2004, which is currently the world's highest impact factor superconductor journal. He was a Visiting Fellow at the Department of Physics, Princeton University in 2003. On the education front, he co-trained and led the national team to the International Physics Olympiad since 2002, a world competition for pre-university students.

Professor Roslan was conferred several awards including the National Young Scientist Award by the Ministry of Science, Technology and the Environment in 1999, Ahli Setia Negeri Sembilan by His Majesty, DYMM Yang Dipertuan Besar Negeri Sembilan in 2002 and the Excellent Malay in Education Award in 2006 in conjunction with the 60th anniversary of UMNO Malaysia. For his contribution to research on superconductivity, in August 2004 he was elected as a Fellow of The Institute of Physics of United Kingdom.

Professor Roslan received the MTSF Science & Technology Award based on his work on the elastic properties and the role of phonons in the mechanism of the copper oxide based high temperature superconductors. He has published more than 73 papers in international journals. He studied the propagation of ultrasound waves in a wide range of high temperature superconductors and found that the two dimensional character and the singularity in the density of states at the Fermi level can lead to high temperature superconductivity. Furthermore his study also showed that a

very small electron-phonon coupling (100 times smaller than conventional superconductors) is sufficient to form the superconducting phase. The result has been published in a number of publications for example, R. Abd-Shukor, 2002. Acoustic Debye Temperature and Role of Phonon in Cuprate and Related Superconductors, *Superconductor Science and Technology* (Institute of Physics, United Kingdom) 15(3): 435-438). This paper has been cited together with a similar model proposed by Abrikosov (Nobel Prize 2003) by several groups in the US and Europe.

He is also recognized in his field internationally. He is currently (2004-2008) an Advisory Board Member of *Superconductor Science and Technology* a journal published by The Institute of Physics, UK; currently the world highest impact factor superconductor journal. He was nominated Fellow, The Institute of Physics, United Kingdom in 2004. He was also a visiting fellow at the Department of Physics, Princeton University July-September 2003. On the education front, the candidate co-trained and lead the national team to the International Physics Olympiad, a world competition in physics for pre-university students in 2002 (Bali, Indonesia), 2004 (Pohang, Korea), 2005 (Salamanca, Spain) as well as the up coming competition in July 2006 in Singapore.

Publications

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PROFESSOR DR. HARITH BIN AHMAD

Universiti Malaya

"Photonics and Laser Technologies"

Year 2007 MTSF Science & Technology Award Recipient

Professor Dr. Harith Ahmad obtained his Bachelor's degree in Physics with 1st class honors from the University of Malaya (UM) in 1979. He obtained his Master's degree in ionization physics (high voltage technologies) and PhD in laser technology from the University of Wales, in 1980 and 1983 respectively.

In 1983, Dr. Harith was appointed as a lecturer in UM where he was also actively involved in laser research, and helped to construct the first high-powered Nd:YAG laser in the country. His research also focused on diode-pumped YAG and crystal lasers, optical parametric oscillation phenomenon, solid-state dye lasers as well as titanium sapphire and femtosecond lasers. These efforts helped to kick start solid state laser research and development of photonics in Malaysia with focus on optical fibre technologies, optical amplifiers and other optical components, leading to a joint-venture between the UM and Telekom Malaysia's (TM) R&D division (TM-UM collaboration) for research into optical fibre devices.

By 1998, Professor Dr. Harith has already headed many national level projects such as the IRPA program on Biophysics, the IRPA program on optical communications and the Top-Down Photonics Project under the Ministry of Science, Technology and Innovation. Besides the development of optical fibre devices for optical communications, the Top-Down Photonics Project also involved the development of planar waveguides with a focus on the fabrication and design of new optical devices.

Professor Dr. Harith oversaw the development of UM's Photonics Laboratory to that of world-class standards, having set up the only planar lightwave fabrication facility in the region. Apart from establishing a joint effort with UM, MMU and UiTM to develop silica on silicon optical fibre devices such as the 1x8 splitter and arrayed waveguides, he has collaborated with UKM, UPM, UTM, UiTM, UniMAP and SIRIM on photonics research.

Professor Dr. Harith has produced over 300 internationally recognized journal publications and conference proceedings. As the director of the TM-UM Collaboration, he has obtained 10 patents jointly with TM. He has also acted as a consultant to TM, the Malaysian Investment Development Authority (MIDA), the Malaysian Industrial-Government on High Technology (MiGHT) and Petronas.

DR. THONG KWAI LIN

Universiti Malaya

"Molecular Microbiology of Foodborne and Nosocomial Bacterial Pathogens"

Year 2007 MTSF Science & Technology Award Recipient

Dr. Thong Kwai Lin graduated from the University of Malaya with a BSc Honours in Zoology in 1980. She obtained the Rhode Island Scholarship to do her MSc in Marine Ecology (1984). After completing her Diploma of Education (passed with excellence) in 1985, she worked as a Science Teacher in a private college. In 1990, Dr. Thong joined the UM as a lecturer and at the same time, continued her postgraduate research in Molecular Biology of *Salmonella* and obtained her PhD in 1997. She was promoted to Associate Professor in 1997 and Full Professor in 2003.

Her field of specialization is molecular microbiology of foodborne and nosocomial bacterial pathogens. She is the first in Malaysia to use and establish the technique of pulse-field gel electrophoresis in characterizing bacterial pathogens. She has shortened the original 5-day to 1-day method of DNA preparation. The rapid turn-around time is useful in real-time determination of clusters of strains involved in outbreaks of infectious diseases. Her landmark paper in the Journal of Clinical Microbiology (JCM 1994; 32:113) showed that *S. enterica* serovar Typhi is genetically diverse as opposed to the dogma that *S.* Typhi is homogenous. She also demonstrated that there is a movement of strains among the Southeast Asian countries, strains from fatal cases of typhoid fever was genetically distinct from those associated with mild form of the disease and the possible link in transmission between sewage-contaminated water supplies and human disease. Such information has great impact in the choice of strain for candidate vaccine design and public health.

In the field of molecular diagnostics of *Salmonella*, she has developed a simple test and reagent for simultaneous detection of *Salmonella* spp, *S.*Typhi and *S.*Paratyphi A. This PCR assay can detect the organisms in environmental samples (contaminated food or water) and also clinical specimens (stools). Such innovative test would be useful for detecting carriers and hence reduce the burden of disease.

She has published over 100 papers in refereed national and international journals and proceedings and presented more than 120 papers in conferences/symposia. She has won numerous Awards related to research and teaching activities. These include 11 Gold Medals, 10 Silver Medals, 5 Bronze Medals in various National and International Invention & Innovation Exposition, Excellent Scientist Award from the Ministry of Higher Education (2005), the Medical Association of Malaysia-Infectious Disease Fund Visiting Fellowship (2006) and the 2007 Malaysia Toray Science Foundation Science and Technology Award. She is currently the American Society for Microbiology (ASM) Ambassador to Southeast Asia (2007-2010). In addition, she is a life member of the Malaysian Society for Microbiology, Malaysian Society for Infectious Diseases and Chemotherapy, and the Malaysian Society for Molecular Biology and Biotechnology. She is one of the funding members of the PulseNet Asia Pacific.

Development of PFGE as the Molecular Typing tool for bacterial enteric pathogens

Professor Dr. Thong has pioneered the technique of PFGE in typing bacterial pathogens in Malaysia and trained numerous personnel in institutions of higher learning and public health laboratories. She has shortened the original

5-day to 1-day method of DNA preparation. Her research team was the first to publish the landmark paper in the Journal of Clinical Microbiology (JCM 1994; 32:1135) to show that *Salmonella enterica* serovar Typhi is genetically diverse as opposed to the dogma that *S.*Typhi is homogenous. They also demonstrated that there is a movement of strains among the South east Asian countries and that strains from fatal cases of typhoid fever were genetically distinct from those associate with mild form of the disease. Such information has great impact in the choice of candidate strain for vaccine design. Her expertise was called upon to investigate nosocomial cases involving *Salmonella enteritidis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in local teaching hospitals. By this technique, the sources of infection were successfully identified, thus reducing the burden of disease. She also applied the technique to investigate cases of *Salmonella*-contaminated imported foods. Due to her active involvement and expertise in this area, she was invited by the Association of Public Health Laboratories, Washington, USA to participate in the setup of PulseNet Asia Pacific comprising 12 countries. Since then, she has represented Malaysia in the past five PulseNet Asia Pacific Meetings. She conducted four PFGE training workshops (between 1996 to 2006) and has trained numerous people from the Universities, Public Health Laboratories, Government Hospitals and the Institute for Medical Research. It has benefited a lot of researchers and scientific officers who have used the technique in investigation of many other bacterial pathogens such as *Burkholderia pseudomallei, Vibrio cholera, Vibrio parahaemolyticus, Pasterella multocida, Streptococus peneumoniae, Staplylococcus aureus* and *E.coli, Listeria monocytogenes*, *Enterobacter sakazakii* , *Pseudomonas aeruginosa* and others.

Development of molecular diagnostics for *Salmonella*

Salmonella spp are Gram negative bacteria responsible for over 21 million cases of salmonellosis including gastroenteritidis, typhoid and paratyphoid fevers which pose significant health problems worldwide. The problem is compounded by emergence of multidrug resistant strains. Clinical diagnosis of typhoid and paratyphoid fever is difficult because the signs and symptoms are not unique and overlap with other common febrile illness. Rapid and sensitive laboratory methods are essential for prompt and effective therapy to reduce serious complication, mortality and the carriage state. Existing diagnosis lacks specificity and is time consuming. In cases of outbreaks, a rapid, specific and sensitive method that can detect these pathogens from different sources is therefore desirable. Based on a subtractive hybridisation genomic library developed by Prof. Thong, she designed and patented a pair of oligonucleotide primers for the specific detection of Salmonella *enterica* serovar Typhi. It has won numerous awards including a Gold Medal award in 2005 at the 33rd International Exhibition, New Techniques and Products in Geneva, Switzerland. In addition, through bioinformatics and genomic approaches, Prof Thong and her team further developed a single one-step multiplex PCR that can simultaneously detect *Salmonella enterica*, *S.* Typhi and *S.* Paratyphi A. An internal amplification control is incorporated into the mix to eliminate false negative results. The detection method takes only 4 hours starting from a single suspected bacterial culture as opposed to 3-5 days using conventional culture methods. This Salmonella Triplex PCR prototype was tested to be very specific under laboratory conditions and can be used to detect the DNAs of the organisms from clinical (stools, blood) and environmental (water, food) samples and is especially useful for detecting carriers, an important source of the disease. The significance of this prototype is that it can be used to detect carriers and hence reduce the burden of disease. The innovation would be the first for simple and rapid detection of *Salmonella enterica*, S.Typhi and S. Paratyphi A, the causative agents of salmonellosis, typhoid and paratyphoid fevers, respectively.

PROFESSOR DR G. SURESH KUMAR

Universiti Malaya

"Medical Parasitology"

Year 2008 MTSF Science & Technology Award Recipient

Professor Dr Suresh Kumar's 17 years of pioneering work in Malaysia carried out on *Blastocystis* , a diarrhoeal causing pathogen have enabled him to generate almost 200 publications including conference papers, mostly on *Blastocystis.* His research contributions include the establishment of the parasite's life cycle, the description of its pathogenicity in animal models, investigations into organelle function, metronidazole resistance, the existence of pathogenic and non-pathogenic *Blastocystis* strains, occurrence in populations and accompanying risk factors, its zoonotic potential and its environmental occurrence. A major contribution has been to edify individuals that some *Blastocystis* isolates of humans are pathogenic. This concept is now gaining momentum.

He developed an improved laboratory detection to determine the significance of *Blastocystis* in human and non-human hosts which won the Geneva International Innovation Gold medal for his diagnostic test. Suresh has investigated various aspects of the parasite's biology with 6 international collaborative networks (Europe, USA, Australia, Thailand, Bangladesh, India), and generated a position paper for *Blastocystis* in the WHO World Water Guidelines book.

Prof Dr Suresh and his team is responsible for placing Malaysia in the frontiers of international *Blastocystis* research. His pioneering work has been constantly referred and is one of the persons responsible for the trigger for a global interest in this organism. His contributions have placed the organism to be seriously considered when patients suffer gastrointestinal symptoms. He has explored using all tools and skills and elevated the organism otherwise considered as a commensal (not important organism) to a very important major contributory cause for diarrhoea, bloating stomach and other gastrointestinal symptoms. He wrote the fact sheet for *Blastocystis in* WHO guidelines for water.

Prof Dr Suresh also served as the WHO Temporary Advisor for Waterborne Zoonosis, Shellfish and Water committees and still serves the WHO World Drinking Water Guidelines Committee. He has won several awards in the past including Malaysia's National Young Scientist Award, Malaysia Toray Science & Technology Award, National Innovation Award, Commonwealth Scholar, Malaysian Society of Parasitology and Tropical Medicine Award (MSPTM Silver Medal) , twice nominated for the UN Public Service Award and World Water Award respectively and 4 times winner of University Malaya Excellence Award. He has supervised more than 60 students at graduate and post graduate levels including PhD and Master's levels, has been External Examiner at these levels and served as reviewer for international peer reviewed journals. He was also involved in WHO expert consultation committee meeting for recreational water and organisms.

He has served as the President and currently a member of MSPTM, Fellow of the Royal Society of Tropical Medicine and Hygiene, Fellow of the Malaysian Science Association, Life member of the Indian Academy of Tropical Medicine, Member of the Institute of Biology, UK and Deputy President of the Sathya Sai Baba Central Council of Malaysia. He has authored 4 motivational books and is an international speaker on human values, ethics, strategy, performance management and leadership.

He currently serves as a Professor of Parasitology and Tropical Medicine and is the Head of Parasites South East Asian Diagnostic (Para:SEAD) Laboratory an outcome of the British Council Link between the Department of Parasitology and Scottish Parasite Diagnostic Laboratory , Glasgow . The outcome of this link was very much appreciated by the British Council who hailed the link as a blue ribband quality in terms of the high number of deliverables achieved and was highlighted in the web site of British council accessible to all commonwealth countries.

Prof Dr Suresh is also an international speaker on human values, mind set change and a performance management consultant who advises on productivity and performance to public and private organization. He has given motivational spoken in Australia, India, Europe, UK, USA, Pakistan, Thailand, Indonesia, Switzerland, Philippines and Singapore. He has with another consultant developed a new performance management tool with special focus on strategic development, performance management and vision development so that people do not `parasitize' on existing resources but learn to give.

He has to his credit 10 international collaborations.

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PROFESSOR DR. ABDUL LATIF AHMAD

Universiti Sains Malaysia

"Membrane Separation Technology, Wastewater Treatment, Separation Technology"

Year 2008 MTSF Science & Technology Award Recipient

Professor Abdul Latif is an educator, consultant and outstanding researcher all entwined into one. He received his doctorate in Membrane Technology from University of Wales, Swansea, United Kingdom in 1995. Professor Latif, one of the youngest professors in Universiti Sains Malaysia is currently the university's Dean of School of Chemical Engineering. Professor Latif is a fellow of the Institute of Chemical Engineers, United Kingdom. He is also deputed as Chartered Engineer and member of the European Desalination Society as well as the Malaysian Council Engineering Dean.

Professor Latif is a prominent figure locally and internationally in his research field of membrane technology, wastewater treatment as well as separation process. His unique and innovative inventions have received international recognition including being the sole Asian recipient of the Saudi Arabia Prince Sultan Bin Abdulaziz International Prize for Water, besides being conferred the Malaysian Excellent Scientist Award by the Ministry of Higher Education Malaysia and winning the 20th Khwarizmi International Award from Iran for outstanding research achievement.

Professor Abdul Latif's expertise is evident from the publication of more than 130 technical papers in well established and high impact factor international refereed journals such as Journal of Membrane Science, Environment Science and Technology, Water Research, Separation and Purification Technology and etc.

As a tenacious educator, Professor Abdul Latif has successfully supervised the work of 8 PhD and 25 master students. With his persevering attitude, he is a veritable a source of inspiration to hundreds of his former students spread throughout the globe.

Description of the winning entry, field of specialization, achievements, new discoveries, and significant breakthroughs

Water shortages, deterioration of water quality, and environmental constraints, have led to an increased interest of recovering and recycling water in many parts of the world. In Feb 2002, the Singaporean government started to reclaim water from the sewage water for drinking and general use instead of buying from other sources. According to the "News Straits Times" dated 19 March 2002, Malaysia has never been a smooth flow of water for even a week although Malaysia is a tropical country having ample of rainfalls. The present situation that has required water rationing is a case in point. It is not entirely due to drought that has a short supply of water. Urbanization and pollution are main reasons for water stress. Although water is important for development, ungoverned development has, in turn, deteriorated the quality of water.

In the past forty years, the palm oil has proven to be the most important commodity of Malaysia. Malaysia is one of the world's largest producers and exporter of palm oil. Malaysia currently accounts for 48% of world palm oil production and 58% of world export. However, the rapid growth of the palm oil industry in Malaysia has invited serious water

pollution in the rivers. In year 2005, 14.96 million tones of crude palm oil (CPO) had been produced that resulted in 44.88 million tones of Palm Oil Mill Effluent (POME). With this statistics, the palm oil mill industry in Malaysia is identified as the industry that produces the ever largest pollution load into the rivers of Malaysia.

POME is thick brownish viscous liquid waste which is non-toxic but has an unpleasant odor. It is predominantly organic in nature and identified as one of the world most polluting wastewater. POME has the Chemical Oxygen Demand (COD) reading of 50000 ppm which is ten times higher than that the COD reading of pulp and paper wastewater. Taking the statistic of year 2005, for 44.88 million tones of POME produced, the amount of BOD produced is 1.122 million tones which is equivalent to the waste generated by 61,479,500 citizens of the country (assuming each citizen produce 18.25 kg of BOD every year).

Currently, the majority of palm oil mills have adopted conventional biological treatment of anaerobic or facultative digestion which needs large treatment area (2-3 football fields) and long treatment periods (80-120 days). However, not all of the palm oil mills are meeting the Malaysian Department of Environment (DOE) standards of pollution control under the Environmental Quality Act (1974). The final discharge always has high turbidity and color which definitely damage the delicate marine ecosystem and leave the marine ecosystem in the rivers and the fishery based economy unprotected.

Facing the problems of water shortages and deterioration of water quality, the needs of environment conservation in order to ensure continuous clean supply of raw water for the future generation are indeed very important. On the other hand, the palm oil is the most important commodity of Malaysia to sustain the development of the country. At the same time, the palm oil mill industry in Malaysia is the industry that produces the ever largest pollution load into the rivers of Malaysia. In order to prevent water pollution caused by the POME discharge without jeopardizing the continuous growth of palm oil as the most important commodity of Malaysia, a clean technology which is environmentally viable has to be invented.

Therefore, the goal in the current research invention is to introduce a POME treatment method which is the most cost effective invention and economic method in treating the POME with Zero Discharge Approach. The finding from this research shows that there claimed water from POME using the current treatment system can be enhanced to become drinking water which meets the United State Environment Protection Agency (USEPA) drinking water standard. Results also prove that the sludge generated can be recovered as high grade organic fertilizer. This treatment system is the first and only one ever done in the world which proves to have numerous advantages compared to the conventional treatment method (biological based treatment system).

Most importantly, this invention has eliminated the ever largest pollution load into the rivers of Malaysia and this has solved the most severe water pollution problem in Malaysia. Besides, current cost effective invention had greatly encourage the water reuse and recycle with innovative-creative ideas and technology. This project has been classified as of national interest and is the first of its kind in the world. This recognition is given due to its ability to win many gold medals at Malaysia as well as international level research exhibition.

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MTSF SCIENCE & TECHNOLOGY
RESEARCH GRANT

LIST OF GRANTEES FROM 1994 TO 2008

SUMMARY OF THE COMPLETED RESEARCH PROJECTS

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 1994 (RM325,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Dr. Fashihuddin Badruddin Ahmad (replaced Dr Muney ak Serit) and co-researchers, Universiti Malaysia Sarawak	Prospecting for Pestical Compounds from Plants against Insects	RM47,500 (*) Completed
Professor (Dr.) Rahmah Noordin and co-researchers, Universiti Sains Malaysia	Identification of Putative Protective Third stage Larvae Antigens of *Brugia malayi*	RM45,000 (*) Completed
Associate Professor Dr. Kim Kah Hwi and co-researchers, Universiti Malaya	Electrophysiological and Neuropharmacological Studies on Purified Toxin from Plants, Marine Fishes, Algae, Snakes and Bacteria	RM35,000 (*) Completed
Associate Professor Dr. Hj Wan Mahmood Mat Yunus and co-researchers, Universiti Putra Malaysia	Photoacoustic Investigation of Surface Plasmons Polaritons in Silver Interfaces	RM50,000 Completed
Associate Professor Dr. Mahiran Basri and co-researchers, Universiti Putra Malaysia	Designing Enzyme to improve its Stereo-selectivity for use as Bio-Catalysts in Organic Synthesis	RM50,000 Completed
Associate Professor Dr. Shahidan B. Radiman, Universiti Kebangsaan Malaysia	Phase characterizations of Ternary Surfactant Mixtures	RM50,000 Completed
Professor Dr. Roslan Abd Shukor and co-researchers, Universiti Malaysia Sarawak	Formation and Optimization of Thallium Cuprates High Temperature Super-conductor	RM47,500 Completed

(*) Funded by Toray Science Foundation, Japan

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 1995 (RM355,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Dr. Ruslan Abdullah and co-researchers, Universiti Kebangsaan Malaysia	Genetic Transformation of Rice for Tungro Virus Resistance	RM50,000 (*) Completed
Associate Professor Dr. G. Suresh Kumar and co-researchers, Universiti Malaya	Immunological and Pathological Studies in *Blastocystics Hominis*	RM45,000 (*) Completed
Associate Professor Dr. Omar Bin Shawkataly, Universiti Sains Malaysia	Reactivity of some Group 8 Cluster Carbonyls towards small molecules	RM40,000 (*) Completed
Associate Professor Rahmah Mohamed and co-researchers, Universiti Kebangsaan Malaysia	Studies on the Protease Gene of *Pseudomonas pseudomallei*	RM55,000 Completed
Dr. Farah Diba Abu Bakar and co-researcher, Universiti Kebangsaan Malaysia	Molecular approaches in the control of the Fungal Plant Pathogen, *Glomerella cingulata*	RM45,000 Completed
Associate Professor Dr. Nakisah Mat Amin and co-researcher, Universiti Putra Malaysia	The mechanism of action of some medicinal plant extracts on *Acanthamoeba castellanii*	RM40,000 Completed
Associate Professor Dr. Rubiyah Bte Yusof and co-researchers, Universiti Teknologi Malaysia	An intelligent based self-tuning PID Controller	RM40,000 Completed
Dr. Sheila Nathan and co-researchers, Universiti Kebangsaan Malaysia	Studies on DNA Repair Related Disorders	RM40,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 1996 (RM275,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Associate Professor Dr. Kurunathan Ratnavelu, Universiti Malaya	Elastic Scattering of Positrons (Electrons) by Bound Atoms	RM45,000 (*) Completed
Miss Kamisah Bt Mohamad Mahbor and co-researchers, Advanced Material Research Center, SIRIM Berhad	Development of Polymer Electrolyte	RM45,000 (*) Completed
Dr. Yeap Guan Yeow and co-researcher, Universiti Sains Malaysia	A Study on the new Langmuir - Blodgett (LB) Thin Films	RM45,000 (*) Completed
Associate Professor Dr. Norazmi Mohd Nor and co-researcher, Universiti Sains Malaysia	Construction of Recombinant M. Bovis BCG containing the Erythrocyte binding Antigen (EBA) of *P faciparum* - Potential use as a Malarial Vaccine	RM50,000 Completed
Dr. Ang Hooi Hoon, Universiti Sains Malaysia	Studies on the medicinal properties of Eurycoma Longifolia Jack (Tongkat Ali)	RM45,000 Completed
Dr. Mohd Danial Yahya and co-researchers, Universiti Kebangsaan Malaysia	Development of Anti-Tumour Heteroconjugates using Novel Ferrocene/Ferrocenium Compounds	RM45,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 1997 (RM330,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Dr. Ayub Mohd Yatim and co-researchers, Universiti Kebangsaan Malaysia	Effects of L-Carnitine on the Metabolisms and Detoxification of Aflatoxin B1 in freshly isolated Hepatocytes	RM50,000 (*) Completed
Dr. Iskandar Idris Yaacob and co-researcher, Universiti Malaya	Synthesis of Magnetic Oxide Nanoparticles in water-in-oil Microemulsions for High Density Magnetic Recording Devices	RM40,000 (*) Completed
Dr. Siti Aisyah Alias and co-researcher, Universiti Malaya	Integration of Ultrastructural and Molecular approach in Delineating selected Marine Fungal Phylogenies	RM30,000 (*) Completed
Miss Sharifah Nora Asfiah Bt Syed Ibrahim and co-researchers, Universiti Sains Malaysia	Marine chemical Ecology of Associated Fauna and Effects of Environmental Stressors on the Associations	RM50,000 Completed
Dr. Peh Kok Khiang and co-researcher, Universiti Sains Malaysia	Development and in vivo evaluation of a Buccal Bioadhesive Drug Delivery System	RM40,000 Completed
Associate Professor Dr. Jafri Malin Bin Abdullah and co-researcher, Universiti Sains Malaysia	Molecular Studies of Genetic changes in the Tumorigenesis of Meningiomas and Gliomas in the East Coast of West Malaysia	RM30,000 Completed
Professor Dr. Son Radu and co-researcher, Universiti Putra Malaysia	Prevalence and Molecular characterization of Enterohemorrhagic *Escherichia coli* 0157:H7	RM30,000 Completed
Dr. Wan Kiew Lian and co-researcher, Universiti Kebangsaan Malaysia	Expressed sequence tags : An approach to Gene discovery in *Eimeria tenella*	RM30,000 Completed
Dr. Zamri Zainal, Universiti Kebangsaan Malaysia	Molecular Biology of Chilli (*Capsicum Annum*) Fruit ripening	RM30,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 1998 (RM295,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Dr. Mahanem Mat Noor, Universiti Kebangsaan Malaysia	Characterization of Putative Fusion Protein involved in Mammalian Gamete Fusion	RM40,000 (*) Completed
Miss Ling Shui Nyuk and co-researcher, Universiti Malaya	Molecular characterization of a multiple Antibiotic Resistance Transposon from *Salmonella typhi*	RM40,000 (*) Completed
Miss Soon Siew Choo and co-researchers, Universiti Malaya	Epitope Mapping of the Tropomysosin Allergen of House Dust Mites *Dermatophagoides spp*.	RM35,000 (*) Completed
Dr. Mohd Zaid Abdullah and co-researchers, Universiti Sains Malaysia	Development of Microwave Borehole Tomography (MBT) System for Geophysical Applications	RM25,000 (*) Completed
Associate Professor Dr. Sahrim Hj Ahmad and co-researchers, Universiti Kebangsaan Malaysia	Microwave Magnetic and Dielectric Properties of Ferrite Thermoplastic Natural Rubber (TPNR) Composites	RM35,000 Completed
Dr. Hasidah Mohd Sidek and co-researchers, Universiti Kebangsaan Malaysia	The Role of Tyrosine Phosphorylation in Malarial Infection	RM35,000 Completed
Associate Professor Dr. Noorlidah Abdullah and co-researchers, Universiti Malaya	Application of Biotechnology in strain selection and development of selected edible Mushroom for commercial production	RM30,000 Completed
Dr. Rahizan Issa, Institute for Medical Research	Detection and Characterization of Endonuclease Activity of Mycobacterial Inteins	RM30,000 Completed
Associate Professor Dr. Rusli Daik and co-researcher, Universiti Kebangsaan Malaysia	Synthesis of Photoluminescent Polymeric materials and its Application for Optical Fibre Sensor Development	RM25,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 1999 (RM280,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Miss Kim Lian Hua and co-researcher, Universiti Malaya	Cell Death and Proliferation in Hodgkin's Lymphoma and related diseases	RM30,000 (*) Completed
Miss Nazni Bt Hj Wasi Ahmad and co-researchers, Institute for Medical Research	Detection of Bancroftian Filarial Worm Infection in urban Culex Quinquefasciatus Adults and the susceptibility status of Cx. Quin. to Insecticides	RM30,000 (*) Completed
Dr. Tengku Sifzizul Bin Tengku Muhammad, Universiti Sains Malaysia	Molecular Cloning and Mapping of the 5' Flanking and Promoter of Regions of Bovine Lipoprotein Lipase	RM30,000 (*) Completed
Dr. Fong Mun Yik and co-researchers, Universiti Malaya	Molecular approach to determine the target site of the drug atovaquone in *Toxoplasma gondii*	RM30,000 (*) Completed
Dr. Nhareet Somchit and co-researcher, Universiti Putra Malaysia	Mechanism of Toxicity and Apoptosis of Nonsteroidal Anti-Inflammatory Drugs	RM25,000 (*) Completed
Dr Hirzun Mohd Yusof, Universiti Putra Malaysia	Development of geneticaly engineered Saccharomyces Cerevisiae by fusion of α-amylase and glucoamylase gene with surface coat protein for sago starch hydrolysis	RM35,000 Aborted
Miss Tey Wan Chee, Universiti Malaya	Molecular Characterization of a le*u* β-Complementing DNA from *Thermus ruber* in *Escherichia coli*	RM30,000 Completed
Miss Azlina Bt Ahmad and co-researchers, Universiti Sains Malaysia	Red Blood Cell Cytoskeletal Proteins in β-Thalassemia trait associated resistance to Malarial Infection	RM30,000 Completed
Dr. Mohd Khadri Shahar and co-researcher, Institute for Medical Research	Laboratory and Field Studies of Sandflies in relation to Bionomic and Infection of *Leishmania Parasites*	RM20,000 Completed
Dr. Aileen Tan Shau Hwai, Universiti Sains Malaysia	The development and research into the production of fast growing Triploid Oysters for Malaysian Food Industry	RM20,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 2000 (RM300,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Dr. Choong Chee Yen and co-researchers, Universiti Kebangsaan Malaysia	Establishment of CdNA library for sex determination in Calamus manan	RM30,000 (*) Completed
Dr. Ishak Ahmad and co-researcher, Universiti Kebangsaan Malaysia	Surface modification of the Aramid Fibres to improve Adhesion to Natural Rubber and Thermoplastic Natural Rubber (TPNR) Matrix composites	RM30,000 (*) Completed
Associate Professor Dr. Kamaruzzaman Bin Yunus and co-researchers, Kolej Universiti Sains & Teknologi Malaysia	Sedimentary record of Paleoproductivity in the Mangrove Forest Sediments	RM30,000 (*) Completed
Miss Shamine d/o Jairaman and co-researcher, Universiti Malaya	Identification of markers involved in histological progression and transformation of follicular lymphoma	RM30,000 (*) Completed
Dr. Kalaivani Nadarajah and co-researcher, Universiti Kebangsaan Malaysia	Cloning of Movement Protein (MP) from Malaysian strains of Cucumber Mosaic Virus (CMV) and its transformation into Tobacco Plants in the Antisense Orientation	RM30,000 Completed
Dr. Tan Eng Lai and co-researcher, Universiti Malaya	A study on the DNA Sequence variation of the Epstein-Barr Virus (EBV) oncogene, LMP-1, in relation to Nasophoryngeal Carcinoma (NPC) in Malaysia	RM30,000 Completed
Dr. Chan Yee Peng, Universiti Malaya	Determination and Analysis of Nucleotide sequences of Nipah Virus isolates	RM30,000 Completed
Miss Faridah Bt Salam and co-researcher, Malaysian Agricultural Research & Development Institute	Development of Diagnostic Enzyme Immunoassay Screening Kit for the detection of Chemical Residues in Livestock Products	RM25,000 Completed
Dr. Choo Quok Cheong and co-researcher, Universiti Sains Malaysia	Regulation of Nitrogen Fixation (nif) Genes from *Paenibacillus azotofixans*	RM25,000 Completed
Mr. Ho Wei Seng and co-researcher, Universiti Kebangsaan Malaysia	Effects of selective logging on Genetic diversity of Shorea curtisii and Shorea singkawang in Tropical Forests	RM20,000 Completed
Mr. Liew Hong Hooi and co-researchers, Universiti Kebangsaan Malaysia'	The role membrane bound proteins in Plasmodium Berghei infection	RM20,000

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 2001 (RM235,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Miss Lim Chui Hun and co-researcher, Universiti Sains Malaysia	Molecular characterization of Human Promoter elements of Peroxisome Proliferator Activated Receptor-Gamma (PPARy)	RM30,000 (*) Completed
Mr. Tan Chin Jye and co-researcher, Universiti Malaya	Chemical & Biological characterization of Indigenous Actinomycetes isolated from marine organisms of West Coast of Peninsula Malaysia	RM30,000 (*) Completed
Miss Tai Yan Chin and co-researcher, Universiti Malaya	Characterization of Genetic alterations in the Lymphoma of Mucosa-associated Lymphoid Tissue (MALT)	RM30,000 (*) Completed
Professor Dr. Mohd Azib Salleh (replaced Dr Hairul Azman @ Amir Hamzah B Roslan) and co-researchers, Universiti Malaysia Sarawak	Characterization of the Starch Biosynthesis Pathway of Sago Palm	RM30,000 (*) Completed
Dr. Geeta Selvarajah, INTI International University College	A Genetic characterization of *Jasminum* species found in Malaysia	RM20,000 Completed
Dr. Mariana Ahamad and co-researcher, Institute for Medical Research	Distribution of Mites, Weevil and their allergen level in Rice and its raw processed products in Malaysia	RM20,000 Completed
Dr. Ho Chai Ling and co-researcher, Universiti Putra Malaysia	Genetic engineering of seaweed for better properties of Alginate : Molecular cloning of mannuronan C-5 epimerase from Brown alga, *Sargassum binderii*	RM20,000 Completed
Dr. Norazizah Shafee and co-researcher, Universiti Malaya	Investigation of the role of divalent cations in Dengue Virus-induced apoptotic pathway	RM20,000 Completed
Miss Chee Hui Yee and co-researchers, Universiti Malaya	Molecular Investigation of Dengue Virus-Cell Interaction	RM20,000 Completed
Dr. Alexander Chong Shu Chien and co-researchers, Universiti Sains Malaysia	Characterizations and isolation of useful substances from Mucus secretion of a fish species demonstrating “milking” behaviour	RM15,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 2002 (RM235,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Dr. Lee Ping Chin and co-researcher, Universiti Malaysia Sabah	Serine/Threonine Kinases and their inhibitors in Mycobacterium and Streptomyces	RM50,000 (*) Completed
Miss Wee Yong Chui and co-researchers, Universiti Malaya	Haematological and Molecular characterization (Prevalent Study) of Alpha Thalassaemia in Malaysia	RM40,000 (*) Completed
Mr. Yong Thian Khok and co-researchers, Multimedia University	Conducting and Transparent Thin Films on Plastic Substrate for Organic Light-Emitting Devices	RM35,000 (*) Completed
Dr. Yeoh Fei Yee and co-researchers, Universiti Sains Malaysia	Synthesis and characterization of Advance Nanostructure Pyroelectric Smart material	RM25,000 (*) Completed
Miss Noor Arniwati Binti Mat Daud and co-researchers, Universiti Kebangsaan Malaysia	Generation and analysis of EST in Blood Cockles, Anadona Ganosa exposed to heavy metals	RM30,000
Mr. Eugene Ong Boon Beng and co-researcher, Universiti Sains Malaysia	Cloning, expression and characterization of the *Bacillus subtiles* alsR Regulatory Protein	RM30,000 Completed
Mr. Sim Lim Chong and co-researcher, Universiti Sains Malaysia	Fabrication of new generation Thermal Interface materials, to be used in the Electronic Industries	RM25,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 2003 (RM185,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Mr. Ho Kian Kheong and co-researcher, Universiti Malaya	Chromosomal abnormalities in confined Placontal Mosaicism and Uniparental disomy associated with pregnancy loss	RM30,000 (*) Completed
Dr. Siti Nursheena Mohd Zain and co-researcher, Universiti Malaya	Susceptibility and Molecular characterization study of the Plant Nematode Disease towards cultivated and wild Banana plants	RM30,000 (*) Completed
Miss Ang Lee Fug and co-researchers, Universiti Sains Malaysia	Investigation of Chitosan as an Immobilization Matrix for Enzyme	RM25,000 (*) Completed
Miss Ida Shazrina Ismail and co-researcher, Universiti Sains Malaysia	Molecular characterization of Bovine Peroxisome Proliferator activated Receptor-Gamma2 (PPARy2) Promoter	RM25,000 (*) Completed
Miss Danley Loh and co-researchers, Universiti Sains Malaysia	Studies on secretion system of Pichia pastoris: The role of signal peptides in facilitating secretion of product payload	RM25,000 Aborted
Dr. Sharida Binti Fakurai and co-researcher, Universiti Putra Malaysia	Mitochondria ultrastructural study following diclofenac and ibuprofen treatment	RM30,000
Miss Chee Jiun Yee and co-researchers, Universiti Sains Malaysia	Production and characterization of Novel Polyhydroxyalkanoate (PHAs) from locally isolated micro-organisms	RM20,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 2004 (RM260,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Miss Salwana Binti Md Hassan and co-researchers, Universiti Malaya	Molecular analysis of Burkholderia pseudomallei	RM35,000 (*)
Miss Yew Saw Peng and co-researchers, Universiti Sains Malaysia	Molecular characterization of Polyhydroxyalkanoate Biosynthesis Genes of Cyanobacteria : Towards Photosynthetic Production of Bioplastics	RM30,000 (*) Completed
Dr. Sim Yoke Leng and co-researcher, Universiti Malaya	Kinetics and mechanism of Cleavage of N-Substituted Phthalimide and Phthalamic acid in mixed Aqueous-Organic Solvent	RM30,000 (*) Completed
Miss Chew Guat Siew and co-researcher, Universiti Sains Malaysia	Differential regulations of PPARs family in Murine J774.2 Macrophage cell line by Cytokines : Molecular mechanisms of Atheroscelerosis	RM25,000 (*) Completed
Miss Mok Pooi Ling and co-researcher, Hospital Universiti Kebangsaan Malaysia	In vitro expression of Erythropoietin by Human Mesenchymal Stem Cells	RM35,000 Completed
Miss Christabel Loni Jiram and co-researchers, Universiti Malaya	Biological and Chemical Diversity of Actinomycetes from Coral Reefs Marine Organisms of the East Coast of Peninsular Malaysia	RM35,000 Completed
Dr. Latifah Binti Saiful Yazan and co-researcher, Universiti Putra Malaysia	The mechanisms of Damnacanthal-induced Apoptotic cell death in the T-Lymphoblastic Leukemia cells (CEM-SS)	RM35,000 Completed
Miss Goh Fen Ning and co-researchers, Universiti Malaya	Development of a rapid detection and quantification assay for *Legionella pneumophila*	RM35,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 2005 (RM320,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Mr. Yong Kien Thai , Universiti Malaya	Taxonomic revision and cladistic analysis of Family Orthotrichaceae (musci) in Peninsula Malaysia and adjacent regions	RM30,000 (*)
Miss Maria Lourdes T. Lardizabal and co-researcher, Universiti Malaya	Toxicology Studies of Plant extractives from *Azadirachta Excelsa* (Jack.) Jacobs against House Flies (Musca domestica L.)	RM25,000 (*) Completed
Miss Nazura Binti Zainuddin and co-researchers, Universiti Malaya	Isolation and characterization of biologically active metabolites from Marine Fungi	RM25,000 (*) Completed
Associate Professor Dr. Mahanem Mat Noor (replaced Mr Lai Wei Hong), Universiti Kebangsaan Malaysia	Molecular characterization of Putative M1 Protein involve in Sperm-Egg Fusion	RM20,000
Professor Dr. Mary Anne Tan Jin Ai (replaced Ms Rita Ling Hui Lian) and co-researcher, Universiti Malaya	Oxidative stress and antioxidant status in β-Thalassaemia major patients at the University of Malaya Medical Centre	RM20,000 (*) Completed
Miss Nor Hayati Binti Abdullah and co-researchers, Forest Research Institute Malaysia	Chemical analysis of Prismatomeris Malayana and its anti-inflammatory activity	RM20,000 (*) Completed
Dr. Hairul Azhar Abd Rashid (replaced Dr Sulaiman Wadi Harun) and co-researchers, Multimedia University	Thulium-doped Fiber-amplifier : Fundamental Studies	RM30,000 Completed
Dr. Teh Geok Bee and co-researcher, Universiti Tunku Abdul Rahman	Investigation of Silicon Nanocrystals : Synthesis, Structural and Properties characterizations	RM20,000 Completed
Dr. Sreeramanan Subramaniam and co-researcher, AIMST University	Genetic Engineering of Phalaenopsis Orchid for Fungal Disease Resistance	RM20,000 Completed
Miss Sew Yun Shin and co-researchers, Malaysian Agricultural Research & Development Institute	Molecular farming of Transgenic Papaya for production of recombinant Hepatitis B Virus Surface Antigen (HBsAg)	RM20,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 2005 (RM320,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Dr. Chow Wen Shyang, Universiti Sains Malaysia	High Performance Recyclable Poly (Butylenes Terephthalate)/Organo-Montmorillonite Nanocomposites	RM15,000 Completed
Dr. Hii Yii Siang and co-researcher, Universiti Malaysia Terengganu	The study of a Symbiotic Bacterial Interaction in Biodegradation of Tapis A Crude Oil	RM15,000 Completed
Miss Lee Hooi Ling and co-researchers, Universiti Sains Malaysia	Synthesis and characterization of Inorganic-organic Nanocomposite comprising metal chalcogenicles (CuS and CdS)and Mesogenic Diols Liquid Crystals as well as their Polymers for photonic applications	RM15,000 Completed
Mr. Hee Chee Seng and co-researchers, International Medical University	Comparison of the relationship between the expression of IL-12 Beta 2 Receptor (IL-12β2R) and CD30 on surface of T-Lymphocytes and disease manifestation in systemic Lupus Erythemathosus (SLE) and Rheumatoid Arthritis (RA) patients from Hospital Seremban - A Pilot Study	RM15,000 Completed
Miss Chai Mee Kin and co-researchers, Universiti Tenaga Nasional	Trace analysis of organic by gas chromatography and liquid chromatography	RM15,000
Miss Josephine Liew Ying Chyi and co-researchers, Universiti Putra Malaysia	Structure and Electron-Phonon Interaction studies in CuSe and SnSe based semi-conductor compounds	RM15,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 2006 (RM290,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Miss Rozilawati Binti Harun and co-researchers, Institute for Medical Research	Studies on Chikungunya Virus Infection in Mosquitoes	RM30,000 (*)
Dr. Chew Kerlit and co-researchers, Universiti Tunku Abdul Rahman	A study on the Sensing Mechanisms of solid state Chemical Gas Sensors for High Temperature Operations	RM30,000 (*) Completed
Miss Ng Woan Shien and co-researchers, Universiti Malaya	Molecular Taxonomy and Phylogenetic analysis of Malaysian Sargassum Species (Sargassaceae, Fucales)	RM30,000 (*)
Dr. Ir Cheong Kuan Yew and co-researchers, Universiti Sains Malaysia	Development of Silicon Carbide (SiC) Nanowire for Nano-Electronic Applications	RM25,000 (*) Completed
Miss Chew Guat Siew and co-researcher, Universiti Sains Malaysia	In search of signal transduction pathways that mediate the Cytokine inhibitory effect on PPARα gene expression in Liver : Towards the elucidation of molecular mechanisms of the development of atherosclerosis	RM30,000 (*) Completed
Miss Cindy Teh Shuan Ju and co-researcher, Universiti Malaya	Development of DNA and Protein based Assays for Salmonella Enterica Serovar Paratyphi A	RM30,000 Completed
Dr. Mohd Taufik Hidayat Baharuldin and co-researchers, Universiti Putra Malaysia	A study on Cannabinoid Receptor Activation: A potential combination Analgesic Therapy	RM25,000
Miss Lim Ya Li and co-researchers, Universiti Putra Malaysia	Study on diversity in Pomela Phizosphere and their potential as Biological Control Agents	RM25,000
Dr. Wong Nyet Kui, Universiti Malaysia Sabah	Glycomics study on Immunosuppressive Glycoproteins : Tamm-Horsfall Glycoprotein and Uromodulin	RM25,000
Mr. Cheah Tead Weng and co-researcher, Universiti Kebangsaan Malaysia	Production of very long chain poly unsaturated Omega 3 and Omega 6 Fatty Acids in Maize	RM20,000
Dr. Phebe Ding and co-researcher, Universiti Putra Malaysia	Betacyanin Pigments and colour expression in red-fleshed Pitaya (*Hylocereus polyrhizus*)	RM20,000 Completed

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 2007 (RM305,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Dr. Ng Yong Foo and co-researcher, Universiti Kebangsaan Malaysia	Taxonomy and Biology of pests thrips (Thysanoptera) on Orchids (Orchidaceae) from Peninsula Malaysia and development of identification system in CD-ROM for diagnostic tool	RM30,000 (*)
Mr. Daicus Anak Belabut, Universiti Malaya	The Ecology and Biology of Genus Mcrophyla Tshcudi, 1838 in the Peninsula Malaysia	RM25,000 (*)
Dr. Chan Kok Gan, Universiti Malaya	Molecular Studies on Quorum Quenching System in Soil Bacteria	RM25,000 (*) Completed
Dr. Tai Cheh Chin and co-researcher, Universiti Malaya	Detecting and monitoring Haematigenous Pathogens in Peri-Prosthetic infection of Hip and Knee joints	RM25,000 (*)
Dr. Ha Sie Tiong and co-researcher, Universiti Tunku Abdul Rahman	Synthesis and Liquid Crystal properties of some Benzothiazole derivatives	RM20,000 (*)
Mr. Mahenderan Appukutty and co-researcher, Universiti Teknologi MARA	Effect of exercise and probiotics supplementation on immune response to Tetanus Toxoid in a mouse model	RM20,000 (*)
Miss Lim King Ting and co-researchers, Universiti Malaya	Determination of the Beta-Lactam Resistance Mechanism in *E.*coli and Klebsiella sp.	RM30,000 Completed
Miss Wong Shew Fung and co-researchers, International Medical University	Production of recombinant allergens for the detection and diagnosis of House Dust Mite (HDM) Allergies	RM30,000
Mr. Lee Lin Kiat and co-researcher, Universiti Malaya	Epstein-Barr Virus latent membrane proteins in Hodgkin's Lymphoma Immunology and survival	RM25,000
Mr. Tay Kheng Soo and co-researchers, Universiti Malaya	Degradation of Biorecalcitrant Pharmaceuticals and Personal Care Products (PPCPs) by Ozone Oxidation	RM20,000
Miss Teh Ser Huy and co-researchers, Universiti Malaya	The production of recombinant Erythropoietin in Pichia Pastoris by fermentation	RM20,000
Mr. Yam Mun Fei and co-researchers, Universiti Sains Malaysia	Development of a new and more sensitive Analgesymeter for screening Analgesic, Anti-inflammatory and Anti-Arthritic Drugs	RM20,000
Miss Tiong Kai Hung and co-researchers, International Medical University	Cytochrome P4502A6 (CYP2A6) : in vitro studies on Genetic Polymorphism and drug-flavonoid interactions	RM15,000

MALAYSIA TORAY SCIENCE FOUNDATION
SCIENCE & TECHNOLOGY RESEARCH GRANT

LIST OF GRANTEES FOR YEAR 2008 (RM318,000)

Name of Main Researcher Name of University/Institution	Title of Research Project	Grant Amount/ Research Status
Dr. Yap Seong Ling and co-researcher, Universiti Malaya	Investigation of the X-ray and EUV emission of vacuum spark discharges	RM60,000 (*)
Mr. Loong Shih Keng and co-researchers, Universiti Malaya	Quantitative estimation of Chikungunya Virus Replication Kinetics in different cell lines	RM30,000 (*)
Mr. Raja Kumar Vadivelu, Universiti Tunku Abdul Rahman	Multi-electrode recordings from organotypic cortical slice cultures : spontaneous and evoked activities	RM26,000 (*)
Dr. Lim Chan Kiang and co-researcher, Universiti Tunku Abdul Rahman	Synthesis of New Xanthone Analogues and their Pharmacological activity	RM25,000 (*)
Dr. Wee Suk Ling and co-researcher, Universiti Kebangsaan Malaysia	Floral odour composition of *Rafflesia cantleyi* (*Rafflesiaceae*) and its ecological significance in pollination biology	RM25,000
Dr. Chong Tet Vun and co-researcher, Malaysian Agricultural Research & Development Institute	Identification of Allelochemicals of Dicranopteris Linearis	RM25,000
Dr. Zulhilmi Bin Ismail and co-researchers, Universiti Teknologi Malaysia	Flood water level influenced by trees and vegetation along the edge of flood plain in case of river flooding	RM25,000
Miss Lim Pei San and co-researchers, Universiti Tunku Abdul Rahman	Phylogenetic diversity of Nitrogen fixation Genes in Microbial community of diverse Malaysia soil environment	RM22,000
Miss Kee Chin Hui and co-researcher, Universiti Malaya	Synthesis and manipulation of unusual stilbenes and dendrimers	RM20,000
Mr. Mohd Hairul Ab Rahim and co-researchers, Universiti Putra Malaysia	Screening and identification of fragrant-related cDNAs in Vanda Mimi Palmer by differential hybridization	RM20,000
Dr. Ramesh T Subramanian , Universiti Tunku Abdul Rahman	Novel poly (vinyl chloride) based composite polymer electrolytes consisting of Ionic liquid in an approach towards environmentally friendly energy sources	RM20,000
Mr. Nik Azmi Bin Nik Mahmood and co-researcher, Universiti Teknologi Malaysia	Bioconversion of Ferulic Acid to Vanilin by using locally *Bacillus sp.*	RM20,000

Dr. Fashihuddin Badruddin Ahmad
(replaced Dr. Muney ak Serit)
Dr. Laily B Din
Mr. Othman B Bajo

Universiti Malaysia Sarawak
"Prospecting for Pesticidal Compounds from Plants against Insects"
Year 1994 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

This screening program started in 1995. The initial program was targeted in developing methods for insecticidal screening especially for termites and mosquitoes. Methods were developed successfully. Based on the methods developed various plants especially in the selected families such as Piperaceae, Lauraceae, Leguminosea, Annonaceae and Rutaceae have been screened in order to evaluate their potential to develop insecticidal compounds. Up to date more than 100 species of plants have been screened and about 30 species showed some good insecticidal properties. Some of the species have been selected for further studies and various biological active compounds have been successfully isolated and evaluated for their insecticidal properties.

Materials and Methods

Plants used in this program have been collected throughout Sabah and Sarawak. Voucher specimens have been prepared for the purpose of identification. Voucher specimen was deposited at UNIMAS Herbarium and also Forestry Herbarium. All plants were dried and used in the extraction process.

Extraction and Fractionation

Air dried samples were extracted with methanol or ethanol at room temperature. The extract was concentrated and partitioned with hexane, dichloromethane, chloroform and ethyl acetate. All the fractions were used for bioassay using developed methods.

Purification of Crude Extract

Each of the partitions was evaporated to dryness using rotorvapour. Purification of each partition was performed with column chromatography using silica gel (70-230 mesh, Merck) and preparative thin layer chromatography (precoated silica gel plate, silica gel 60 F_{254}, 1 mm thick, Merck). Thin layer chromatography were performed on precoated silica gel plate (silica gel 60 F_{254}, 0.25 mm thick, Merck) (Houghton and Raman, 1998). The details on the isolation and structural elucidation are given in the appendix for the paper prepared for publication.

Extraction of essential oil

The essential oil from some aromatic plants were also extracted and screened for their insecticidal activities. The plant materials were subjected to water distillation in Clevenger-type apparatus for 8 h. The oily layers obtained were separated and dried over anhydrous magnesium sulfate. The yields were averaged over three experiments and calculated based on dry weight of the plant materials.

Analysis of the oils – The oils were analyzed on a Shimadzu GC 14A chromatograph equipped with a FID detector using a DB-5 capillary column (25 m x 0.25 mm, 0.25 µm film thickness). The operation parameters were : nitrogen as carrier gas at 50 cm/s, injector and detector temperatures were maintained at 250°C. The column was programmed initially at 75 °C for 10 min, then 3 °C/min to 210 °C and held for 1 min. The oils were also examined using a DB-1 stationary phase column (25m x 0.25 mm, 0.25 µm film thickness) programmed from 60 °C for 10 min, then 3 °C/min to 180 °C and held for 10 min. Peak areas and retention times were measured by electronic integration. The relative amounts of individual components are based on peak areas obtained, without FID response factor correction. Temperature program linear retention indices of the compounds were also determined relative to n-alkanes (Kovats, 1965).

The oils were also analyzed by GC/MS with a Hewlett-Packard GC-MSD 5890 series 2 mass spectrometer (70eV direct inlet) on a BPX5 column (30m x 0.25mm, 0.25 µm film thickness) with similar condition as described in GC programs for DB-5. The constituents were identified by comparison of their retention indices with literature values and their mass spectral data with those from the Wiley mass spectral database, and in some cases by co-chromatography on the different columns with authentic samples (McLafferty and Staufer, 1989; Davies, 1990; Adams, 2001).

Insecticidal Activities

Biological activity for the pure compounds was screened against the second instar of *Aedes aegypti* and pseudogates of termites *Schedorhinotermes sarawakensis*. The methods adopted from Bandara *et al.* (2000) was used to determine the biological activity against *A. aegypti* while for antitermites activity the methods described by Serit *et al*. (1992, 1996) was used throughout the screening program. About 20 ppm of the pure compounds were prepared in suitable solvents and used for the biological activity on *A. aegypti* while concentration of 5000 ppm was used for antitermites activity. Active extracts were tested at lower concentration by serial dilution using the concentrated solutions. The number of larvae dead was recorded at 24 h intervals. For *A. aegypti* the lethal concentrations (LC_{50}) were determined while for antitermite assay the mortality rate as well as the rate of food reducton was determined.

Results and Discussion

Although more than 100 plants species were screened for their biological activities only 30 showed some strong insecticidal activities. Details of the active plants are given in Table 1. The table clearly shows members in the Annonaceae, Piperaceae, Lauraceae, Rutaceae and Zingiberaceae are good candidates for developing environmental friendly insecticidal. Some of the plants which showed strong activities include *Piper sarmentosum*, *P.umbellatum*, *P. longamentum*, *Goniothalamus velutinus*, *G. kinabaluensis*, *G.uvariodes*, *Litsea nidularis*, *Citrus sinensis* and *Hypnea pannosa* (marine algae). To develop such compounds more comprehensive studies are required.

All the manuscripts are prepared in the forms of publication have been submitted or will be submitted for publication as follows :

- Antitermite properties of *Piper* spp. Extracts
- Characterization of Bioactive compounds from Marine Algae, *Hypnea Pannosa*

- The essential oils profile of selected *Piper* species
- Phytochemical and biological studies on *goniothalamus woodii*
- Phytochemical and biological studies on *Piper Macropiper* Pennant
- Screening active compounds from Methanol extracts of *Nepenthes ampullaria* Jack and *N. Mirabilis* (Lour) against *Schredorhinotermes Sarawakiensis* Holmgren (Rhinotermitidae)
- Screening active compounds from two *Piper* species (*P. Sarmentosum* and *P. Betle*) against Pseudogates of *Schedorhinotermes Sarawakiensis* Holmgren (Termitidae)

Table 1 : Plants with strong insecticidal activities

Family	**Plants Species**
Annonaceae	*Artabotrys roseus* *Goniothalamus uvarioides* *G. kinabaluensis* *G.macrophyllus* *G.velutinus* *Polyalthia sumatrana*
Lauraceae	*Cinnamomum javanicum* *Cinnamomum paiei* *Dehaasia incrassata* *Litsea nidularis* *L kunstleri* *L.elliptica* *Phoebe opaca*
Menispermaceae	*Fibraurea tinctoria*
Moracea	*Antiaris toxicaria*
Piperaceae	*Peperomia candida* *Piper sarmentosum* *P. umbellatum* *P.longamentum* *P.macropiper* *P.muricatum* *P.porphyrophyllum*
Rubiaceae	*Uncaria borneensis*
Rutaceae	*Citrus aurantifolia* *C.grandis* *C.simensis*
Zingiberaceae	*Boesenbergia stenophylla* *Hedychium borneenses* *Zingiber cassumunar* *Z.kelabatianum*

Professor (Dr). Rahmah Noordin
Professor Khairul Anuar Abdullah
Miss Noor A'shikin Azahri
Professor C.P. Ramachandran

Universiti Sains Malaysia

"Identification of Putative Protective Third Stage Larvae Antigens of *Brugia malayi*"
Year 1994 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Lymphatic filariasis caused by *Brugia malayi* and *Wuchereria bancrofti* infect about 120 million people worldwide. Third stage infective larvae (L_3) of the parasite enters the body through the bite of an infected mosquito. Identification of L_3 antigens that is involved in the host antibody response towards the invading parasite is important in the understanding of infection and immunity in lymphatic filariasis. Thus, this study was conducted to investigate *B. malayi* L_3 antigenic components that are recognised by endemic area individuals, in order to identify potential protective epitopes.

Infective larvae collected from infected mosquitoes were suspended in phosphate buffered saline, then homogenised, sonicated and concentrated to about 1000 µg/ml. A total of 200 serum samples from a brugian filariasis endomic area in a state in North Eastern Malaysia were divided into the following groups :

1. Individuals with circculating microfilariae (n=36). Majorities were asymptomatic; some had acute symptoms and a few demonstrated minor leg edema. However, it could not be ascertained whether the edema were due to the infection or to other non-specific causes.
2. Amicrofilaraemic individuals with elephantiasis (n = 10).
3. Amicrofilaraemic individuals from the endemic area who demonstrated no anti-filarial IgG4 antibodies towards microfilaria and adult worm antigen i.e. optical densities obtained were zero or less (n = 50).
4. Amicrofilaraemic individuals in the endemic areas with high titres of the anti-filarial IgG4 antibodies (O.D. above 1.200; n = 20).
5. Healthy city volunteers i.e. non-endemic normals (n = 50).
6. Soil-transmitted heminth infected individuals living in non-endemic areas (n = 34).

Phast Electrophoresis System was employed for running of precasted 10% - 15% gradient SDS-PAGE gels and for electroblotting. The protein bands were transferred onto a PVDF membrane and the strips were then successively incubated with 1% blocking solution at room temperature (rt) for 30 minutes, human sera (1:100 dilution for 3h, rt) followed by mouse monoclonal anti-human IgG4 antibody conjugated to horseradish peroxidase at 1:1000 dilution for 30 minutes, rt. In between each incubation steps, the strips were washed twice using Tris buffered saline containing 0.05% Tween 20 (10 mins/wash) followed by 2 times washing (10min/wash) in 0.5% blocking solution. Luminol chemiluminescence detection was then used to develop the blots. The approximate molecular weights (MW) of the antigenic bands were determined using a digital image analyzer. The MW of the antigenic epitopes displayed by the positive blots were MW of 191 kDa, 143 kDa, 80 kDa, 59 kDa, 43 kDa, 25 kDa, 15 kDa and 14 kDa. Out of these,

the most dominant band observed was 43 kDa and the other prominent bands were 14 kDa, 59 kDa and 80 kDa. The most consistently observed band was the 43 kDa and 14 kDa, followed by the 15 kDa and 59 kDa.

28/36 (78%), 1/10 (10%) and 16/20 (80%) of sera from individuals with microfilariae, elephantiasis patients and amicrofilaraemic individuals with high titres of anti-filarial IgG4 antibodies respectively demonstrated antigenic epitopes. 8/36 (22%), 9/10 (90%) and 4/20 (20%) of the above sera groups respectively were unreactive. The elephantiasis patient serum that reacted to the L_3 antigen exhibited bands of MW of 43 kDa, 59 kDa and 80 kDa. Sera from soil-transmitted helminth infected individuals, city dwellers and endemic areas individuals with no anti-filarial IgG4 antibody were not reactive.

Studies of antibody response towards L_3 antigens is important in our understanding of host response to filarial infection and in elucidating epitopes of potential protective value. Protective immunity against primary infection or protection against repeated infections may occur in individuals living in an endemic area. In the initial part of the study we investigated Western blot reactivity of L_3 soluble antigens when probed with all four IgG isotypes. Anti-human IgG4 was found to be most reactive and thus was used for the rest of the study. We observed that there exist two groups of microfilaraemics with regard to anti-filarial IgG4 response to L_3 antigens. The majority (78%) was reactive to the L_3 antigens with dominant antigenic epitopes of MW 43 kDa, 14 kDa, 15 kDa and 59 kDa. It is interesting to observe that 22% of microfilaraemics were unreactive to L_3 epitopes although many prominent antigenic bands were observed with all microfilaraemic sera in Western blots using adult and microfilaria antigens. There was no correlation between the Western blot results and age of the individuals, thus difference in exposure to L_3 cannot explain these observations.

Similar results were also seen in the group of amicrofilaraemics with very high levels of antifilarial IgG4 antibodies to soluble antigens and thus likely to be actively infected. However, we could not ascertain whether the results may be correlated with duration of infection or the strain of *Brugia malayi* (i.e. periodic or subperiodic). In the group of the chronic elephantiasis patients, 90% of the patients were unreactive to the L_3 antigen; this is not unexpected since it has been shown that antibody response to IgG4 is reduced in chronic stages of the disease.

This study demonstrated that the establishment of active infection, as evidenced by the circulating microfilaria, was not correlated with recognition of soluble L_3 epitopes. Therefore, the somatic antigen of L_3 did not seem to confer protection against establishment of *B. malayi* infection and may only be an indication of the degree of exposure to L_3 antigens. In conclusion, our results showed that a proportion of microfilaraemic individuals does not seem to recognize L_3 somatic antigens. This was also observed in amicrofilaraemics with strong evidence of active infection. Thus, IgG4 antibody response to somatic L_3 does not seem to be protective against establishment of *Brugia malayi* infection. Thus, in the attempt to elucidate infective larvae protective epitopes against filarial infection, the focus should probably be on investigations of surface and not somatic antigens of L_3.

Publication

Rahmah Noordin, Khairul Anuar Abdullah, Noor A'shikin Azahri and CP Ramachandran. Antibodies to somatic L_3 antigen not protective against *Brugia malayi* infection. Southeast Asian Journal of Tropical Medicine & Public Health 1999; 30(4):678-681.

Associate Professor Dr. Kim Kah Hwi
Associate Professor Mohd. Afandi Muhamad
Associate Professor Dr. Cheah Swee Hung

Universiti Malaya

"Electrophysiological and Neuropharmacological Studies on Purified Toxin from Plants, Marine Fishes, Algae, Snakes and Bacteria"
Year 1994 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Recent research on marine toxins has been widely carried out to determine the mechanism of their actions to improve the treatment of poisoning caused by marine animals. Simultaneously marine toxins have been developed as new tools in the study of electrophysiological and biochemical properties of ionic channels in excitable membrane. In Malaysia, there are a number of marine fishes, plants, snakes, algae and bacteria that produce toxic effect and even cause death in those who come in contact with them. Every year the number of victims is increasing; unfortunately in many cases there are not rational treatment available, since it is not exactly clear how these toxins act in the body. In this study, we investigated the mechanism of action of these toxins in the body using lectrophysiological methods.

Objectives

This study aimed to identify the toxicity effects, mechanism of action, ionic mechanism and type of receptor biding sites of the purified toxins.

Methodology & Results

a) **Identified the neuroactive peptides**

In present study, we investigated the effect of some neuropeptides on the membrane potential of 30 identified giant neurons of the African giant snail. Neuropeptides examined were

(1) neuropeptides proposed as neurotransmitter in mammal such as met-enkephalin, neurotensin, luteizing hormone-releasing hormone, glutamine-luteizing hormone-releasing hormone, oxytocin and arginine-vasopressin,
(2) neurpeptides proposed as neurotransmitter in invertebrate such as proctolin and FMRF-amide,
(3) venom neuropeptides such as bombesin, ranatensin, vespakinin X and vespakinin M. Out of the 13 neuropeptides examined, neuropeptides induced excitatory effect on one neuronal membrane potential were Metenkephalin (fast and transient excitation on v-RPLN), ranatensin (v-LPSN), Argvasopressin (PON) and proctolin (RAPN). FMRF-amide induced inhibition effect on 10 neuronal membrane potentials (TAN-1, TAN-2, d-LCDN, RAPN, d-LPeLN, v-RPLN, d-PeNLN, R-PeNLN, L-PeNLN and INN). Whereas, oxytocin induced excitatory effect on 8 neuronal membrane potential (v-RPLN, v-LCDN, d-VLN, RAPN, PON, d-RPLN, v-LPSN and VIN) and induced inhibition effect on 2 neuronal membrane potentials (TAN-2 and TAN-3).

b) **Effects of toxins from fishes such as jellyfish, puffer fish, cat fish and scombotoxic fish on neuronal membrane potential of *Achantina fulica***

The present study investigated the effect of toxins extracted from jellyfish, puffer fish, cat fish and scombotoxic fish on the membrane potential of TAN. The jellyfish toxin had no effect on the recorded TAN intracellular membrane potential. This observation suggested that the toxicity expressed is not due to their effect on the central nervous system. Puffer fish toxin had a marked and rapid inhibition of TAN action potentials very similar to tetrodotoxin. However, the effect persisted even after an hour of continuous washing, whereas spike inhibition by tetrodotoxin will disappear after few minutes of wash out. Cat fish toxin had a depolarizing effect on TAN membrane potential, leading to increased frequency of spiking. This depolarizing effect was slightly decreased in presence of 50% calcium and the effect disappeard in calcium-free media. The effect was not altered in presence of 10^{-4}M phosphodiesterase inhibitor, isobutymethyxanthine (IBMX). Possibly, the depolarizing effect of the extract could be attributed to involvement of extracellular or intercellular calcium without involving cAMP. Introduction of scombotoxic fish toxin induced a slow inhibition of TAN action potentials generation. The effect was slightly reduced in presence of 200% potassium, while in presence of 50% potassium or IBMX in normal saline the effect was slightly increased. These observed effects may be attributed either to direct effect on potassium channel, or calcium activated potassium channels.

c) **Toxicity, pharmacological and electrophysiological studies of *Limacia scandens***

A crude extract of *Limacia scandens* injected intravenously as a single bolus induced a dose-dependent increase in arterial blood pressure in anaesthetized rats and cats. Pre-treatment with a non-specific α-blocker phentolamine (10^{-5}M blocked this effect, whereas the β blocker propanolol (10^{-5}M) did not. The extract also reduced intestinal motility and this response could be blocked by pre-treatment with phentolamine (10^{-5}M) and the specific α_1- blocker, prazosin (10^{-5}M). In superfused rabbit aorta preparations, it induced an increase in contractions. This effect was blocked by pre-treatment with prazosin (10^{-5}M), whereas the α_2- blocker yohimbine (10^{-5}M) had only a slight effect. The effects of NA on superfused aortic strip contractions were similar to the extract. Electrophysiological studies on the tonically autoactive neuron (TAN) of the snail *Achatina fulica* revealed that crude extract of *Limacia scanden*s induced excitatory response which were similar to those of serotonin (5-HT) stimulation. Studies with different ionic compositions of the bathing saline revealed that this excitatory effect of *Limacia scandens* could be attributed either to the release of endogenous serotonin or inhibition of serotonin reuptake in the CNS.

d) **TF (G) Fraction of Japanese Habu Snake Venom (*Trimeresures flavoviridis*) (Tf) induces an excitatory effect on periodically Oscillating Neuron of *Achatina fulica***

Of 10 fractions that were screened, only the TF (G) induced excitatory effect on the periodically oscillating neuron (PON). The TF (G) induced excitatory effect remained unchanged in presence of IBMX, forskolin or H-7. Thus, cAMP, Ca^{2}+/calmodulin and Pk. C are not involved in increasing intracellular Ca^{2+}. The effect of TF (G) was abolished in Ca^{2+}-free saline. This indicates that a voltage-dependent membrane-bound Ca^{2+} channel is involved. The response induced by TF (G) is not altered in the presence of TEA and remained unchanged in $C1^{-}$ -free saline. Thus, tentatively we can conclude that the excitatory effect of TF (G) on PON is due to an increase in Ca^{2+} conductance. The possible involvement of cGMP needs to be investigated.

e) **Ionic mechanisms associated with the action of Gamma-Aminobutyric Acid (GABA) on *Achatina fulica* neurons**

The actions of GABA on 34 identified giant neurons in *Achatina fulica* were investigated using conventional electrophysiological recording techniques. Results obtained from this study indicate that GABA induces 3 different responses in *Achatina fulica* neurons, similar to those observed on the neurones of Aplysia and Helix.

1) GABA A receptor. GABA induced a rapid and transient hyperpolarizing response on 8 neurons *f* TAN, TAN-1, TAN-2, LAPN, v-RPLN, d-LPeLN and d-LBMN. This effect was mimicked by muscimol and enhanced by pentobarbitone. Picrotoxin reduced this response. In C1 -free saline a depolarizing response was observed and this suggests a role for C1in the generation of the above response.
2) GABA B receptor. GABA induced a slow hyperpolarizing response on 6 neurons – RPeNLN, LPeNLN, RAPN, LVMN, RVMN and BAPN. This effect was mimicked by baclofen and antagonized by pharcofen. In C1 –free, Ca^{2+} free and 20% Na+ saline, the response remained unchanged. On the other hand, the response was slightly reduced in 200% K^+ and slightly enhanced in 50% K^+ saline. On the basis of this observation, the slow hyperpolarizing response induced by GABA could be attributed to K^+.
3) GABA C receptor. GABA induced a depolarizing response on 7 neurons – FAN, v-VNAN, d-RPeAN, v-RCDN, d-LBAN and d-LBPN. Muscimol induced a similar response. This effect remained unchanged in both Ca^{2+} -free and C1 –free saline. However, in 20%Na+ saline, this excitatory effect was abolished. Thus, it could be postulated that Na+ may be involved in the depolarizing response by GABA.

f) **Multiple Receptor Sites for a Molluscan Tetrapeptide Amide (FMRF-Amide) in *Achatina fulica***

Using a glass microelectrode filled with 3M potassium acetate and conventional electrophysiological techniques, the intracellular neuronal potentials were recorded. The amplified signals were videotaped on a video recorder via a two-channel PCM A/D adaptor. The effects of FMRF-amide on the 34 identified neurons were then investigated. In addition, the experiments were repeated following a change in the ionic composition of the bathing medium to study effects of the amide on ionic permeabilities. FMRF-amide induced a rapid and transient hyperpolarizing response on RPeNLN (right pedal nerve large neuron), LPeNLN (left pedal nerve large neuron) and TAN-2 (tonically autoactive neuron 2). The effect was present in 200% K^+, 20% Na+ and Ca2+ -free saline. However, in C1 –free saline there was a transient rapid depolarizing response. This would suggest that C1 was responsible for the hyperpolarizing response. FMRF-amide induced a depolarizing response in INN (intestinal nerve neuron) and this remained unchanged in Ca^{2+} -free saline. However, in 20% Na+ saline, the depolarizing response was abolished and this would suggest the involvement of Na+ ions in the observed depolarizing response. FMRF-amide induced a slow hyperpolarizing response in TAN (tonically autoactive neuron), d-LCDN (dorsal left cerebral distinct neuron), RAPN (right anterior pallial neuron), d-LPeCN (dorsal large pedal constantly firing neuron) and V-RPLN (ventral right parietal large neuron). This response remained unchanged in C1 –free, Ca^{2+} free and 20% Na+ saline. It was slightly reduced in 200% K^+ and slightly enhanced in 50% K^+ saline. It would thus appear that the slow hyperpolarizing response induced by FMRF-amide in the above neurons is potassium-dependent. The 3 different responses induced by FMRF-amide in *Achatina fulica* neurons are comparable to its effects on the neurons of the

gatropods Aplysia, Helix and Euhara. In the absence of definitive patch-clamp studies, it is suggested that FMRF-amide produces its effects via multiple receptor sites, analogous to its action on *Helix* neurons.

Conclusion

The studies showed that some of the potential compounds could be developed into useful clinical drug.

Publications

1) Kim Kah Hwi, M.A. Muhamad, Cheah Swee Hung, and A. Raman. MULTIPLE RECEPTOR SITES FOR A MOLLUSCAN TETRAPEPTIDE AMIDE (FMRF-AMIDE) IN *Achatina fulica Ferussac*. Journal of the University of Malaya Medical Centre (JUMMEC) 1996 1:33-38.
2) Kim Kah Hwi, Cheah Swee Hung, M.A. Muhamad., and A. Raman. THE IONIC MECHANISMS ASSOCIATED WITH THE ACTION OF GAMMA-AMINOBUTYRIC ACID (GABA) ON *Achatina fulica Ferussac* NEURONES. The Malaysian Journal of Medical Sciences. 1997; 2:28-37.
3) Kim Kah Hwi and Wong Bee Lay. PHARMACOLOGICAL, ELECTROPHYSIOLOGICAL AND TOXICITY STUDIES OF LIMACIA SCANDENS *LOUR*. (Menispermaceae). Journal of Ethnopharmacology 1998; 62:137-148.

Associate Professor Dr. Hj Wan Mahmood Mat Yunus
Dr. Mohd Maarof Moksin, Dr. Zainal Abidin Talib
Dr. Azmi Zakaria, Dr. Zadan Abdul Wahab

Universiti Putra Malaysia

"Photoacoustic Investigation of Surface Plasmons Polaritons in Silver Interfaces"
Year 1994 MTSF Science & Technology Research Grant Recipient

A method for characterization thin metal films using surface plasmon resonance is presented. The optical permittivities and thickness were determined by fitting the experimental data to the Fresnel equation. In this measurement, we found that the real part of the permittivity value for gold film is independent on the film thickness, but the imaginary part of the optical permittivity tend to be slightly thickness dependence. The real part of the optical permittivities measured at four wavelengths (i.e. red, orange, yellow and green) is in good agreement with the data reported in texts book. This surface plasmon resonance technique is also reported as a sensitive optical method for sensing technique. In the present work, the surface plasmon resonance optical sensor has been tested for various samples, such as ethanol, D-Glucose, hevea latex and honey at different concentration. Theoretically, the resonance condition is determined by dielectric constants of the sample in contact with thin gold film of the sensing element. The detection sensitivity and limit for the respective liquid are discussed.

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"Designing Enzyme to improve its Stereo-selectivity for use as Bio-Catalysts in Organic Synthesis"
Year 1994 MTSF Science & Technology Research Grant Recipient

The understanding of the mechanism of drugs, pesticides and hormones interaction on a molecular level has led to the awareness of the significance of using one enantiomer over the other of these compounds for efficacy and safety, as the other enantiomer may give undesirable effects. Enantiomers can be prepared by separation of the racemic compounds by chemical methods. However, the laborious steps and the expensive and sometimes toxic chemicals needed to produce enantiomers meant much higher production costs. In this project, we have developed an alternative method of using enzyme which was designed to produce enantiomers via asymmetric synthesis. Our results suggested that we have successfully designed the enzyme to aquire high stereo-selectivity by: 1. Chemical modification to increase the hydrophobicity of the enzyme by using modifiers of high molecular weight or/and increasing the degree of modification of the enzyme. 2. Immobilizing the enzyme on different supports 3. Bioimprinting the enzyme using the substrate and subsequently inducing and maintaining the enzyme using hydrophobic solvents and 4**.** Using organic solvents of higher log P in the reaction systems.

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"Phase characterization of Ternary Surfactant Mixtures"

Year 1994 MTSF Science & Technology Research Grant Recipient

Introduction

Phase diagram studies of many surfactants (anionic, cationic, nonionic and zwitterionic) in water and water-oil system have been intensely investigated during the last two decades. During the 90s research on binary mixture e.g. anionic-non-ionic and other permutations have been explored. For example, doping of nonionic surfactants in water with anionic surfactant were investigated to find out their effect on critical point shifts as well as degree on interactions. Cationic-anionic mixture is known to form vase-shaped phase diagram (1,2) and regular solution model is able to explain this phenomena. In earlier studies ternary and quaternary system studies were confined to the number of solvents i.e. surfactant-water-oil-alcohol. These investigations led to understanding of bicritical and tricritical points, Lifshitz point and percolation transitions as well as new microemulsion morphologies e.g. sponge-phases. Our main objective of using ternary mixtures (anionic-cationic-non-ionic) has been mimic the complexities found in ternary solvents (water-oil-alcohols) and to resolve phases and transitions base on phase diagram studies with some physico-chemical characterization. Earlier studies on ternary surfactant mixture by us (3,4) showed that cubic, lamellar and hexagonal phases are maintained like in the binary mixture or even single surfactant system. In this sense, the middle of the Gibbs phase triangle would represent a "truly" ternary mixtures and this is where either regular solution theory or others can be tested. In our investigations, we also studied binary systems where their phase diagram were not known. This slowed us down; also not all binary systems of interest are fully given in terms of their phase diagram in water. Some are investigated up to 60% concentration or so. Others include alcohol as a pseudo-phase. Of course the full complexity of the phases (this will be carried out even after this report for many years to come) will be borne out when both ternary surfactant mixtures and the three-solvents are mixed. Increased complexity can also be introduced by adding water-soluble polymers or polyelectrolytes.

Objectives

It had been known that surfactant micelles, microemulsions and polyaphrons can be used in nanotechnology development for e.g. preparation of superconductors and other ceramics. Our objectives have been three-fold:

1. to obtain general characteristic of ternary mixtures as obtained from phase diagram mapping. This ternary mixture behaviour would be understood from their single or binary mixture behaviour (by studing the phases formed at the corners and edges of the Gibbs phase diagram).
2. to characterize phase stabilities of phases formed particularly single phases. This was made using turbidity, conductivity and other physico-chemical methods.
3. attempt to look at possible new applications e.g. in liquid crystal phases or middle-phase microemulsion formed.

Methodology & Materials

Our methodology had been fairly limited to several lab-based methods of conductivity, UV and IR-spectroscopy,

turbidimetry and optical and phase-contrast microscopy. All the surfactants were considered pure (99% or above) and were brought directly either from Fluka, BDH, Sigma or Nikko Chemicals. Borosilicate glass vials were used and sometimes Pyrex glasses where heating of samples are required.

Innovative approaches

Kinetic effects are known to be important in these mixtures. For example, equimolar catinic and anionic molecules could form coacervates (wax crystals) that melt reversibly (outside water) but does not dissolve without heating. Thus, cationics and anionics should not be added together but only after non-ionics are added first with stirring. If oil or other solvents are to be added, it is always better to dissolve the third surfactant in the solvent that is to be added to the aqueous phase. Also, better mixing with less time can be obtained by rolling the final solutions instead of shaking or even vortexing.

Outcomes and discussions

The following phase diagrams and phases found in the mixtures had been characterized :

1. 2-butoxyethanol + Sodium bis-2 ethylhexyl sulfosuccinate (AOT) + Didodecyl dimethyl ammonium bromide (DDAB) in water.
2. Dodecylethyl ammonium bromide (DEAB) + sodium bis-2 ethylhexyl sulfosuccinate (AOT) in water.
3. Hexadecyl trimethyl ammonium bromide (HTAB) + sodium salicylate (NaSal) + the Synperonic Series NP5, NP10 and NP30 in water (500mg/5ml water).
4. Synperonic NP5 + Dodecyl dimethyl ethyl ammonium bromide (DDEAB) = 1:1 + water + n-Heptane.
5. Hexadecyl trimethyl ammonium bromide (HTAB) + Sodium salicylate (NaSal) + Dodecyl dimethyl ethyl ammonium bromide (DDEAB) or ethylene glycol monobutyl ether (EGME) (500mg/in 5ml water).
6. Hexadecyl trimethyl ammonium bromide (HTAB) + sodium salicylate (NaSal) + Benzyl dimethyl hexadecyl ammonium chloride (BDHDAC) (100mg/1ml water).
7. Distearyl dimethyl ammonium bromide (DSDAB) in water-n-heptane (50-50 by volume).
8. Sodium bis-2 ethyl hexyl sulfosuccinate (AOT) + Sodium dodecyl sulfate (SDS) (=1:1) in water + n-Heptane.
9. Sodium bis-2ethyl hexyl sulfosuccinate (AOT) + Tween 20 (=1:1) in water + n-heptane.
10. Tween 40 + sodium bis=2ethyl hexyl sulfosuccinate (AOT) + didodecyl dimentyl ammonium bromide (DDAB) in water (50% volume) – formamide/ formamide/ formamide (50%)-heptane/water (50% volume)-heptane.
11. Dicyclohexylamine (DCHA) + Sodium bis-2 ethyllhexyl sulfosuccinate (AOT) + dodecyldimethyl ammonium bromide (DAB) in water.

Unresolved problems and future directions

The work performed in this report mainly concentrates on phase diagram mapping. Polarising microscopy studies merely differentiates lamellar phases from hexagonal phases. Even the L_3-phase found in the Tween-40 + AOT + DDAB (500mg) in 5ml water showed different textures with slightly differing compositions. We are currently using a Gray Level Co-occurrence Matrix analysis to classify these textures and perform a contour mapping of similar textures (7). However, these textures are due to the different types, densities and interactions of the defect structures and

microscopy studies need to be extended with other structural characterization methods : Nuclear Magnetic Resonance to determine the nematic/smectic directors, differential scanning calorimetry to determine phase transition temperatures, videoscopy and freeze-fracture electron microscopy to determine dynamics and types of defects that gave the textures and small-angle X-ray or neutron scattering to determine symmetry of arrangements (especially cubic and nematic/ smectic phases). Clearly, conductivity and turbidity studies alone could not characterize fully the phases or study possible transitions. This will be pursued by other techniques where possible in the future.

Summary and conclusion

Several important findings can be highlighted and in many cases need further study for good publications :

1. Cubic, lamellar and possibly hexagonal phase formation are determined by the majority surfactant species.
2. Addition of either-based Synperonic series breaks the worm-micelle structure and in the case of Synperonic NP5 into two different micelle types (possibly a dilute and a semi-dilute region).
3. Some middle-phase microemulsion can also be formed from Synperonic mixed with surfactants.
4. Surfactants that prefer form spherical micelles will typically do so even in mixtures.
5. Non-soluble surfactants can be induced into middle-phase microemlusion formation by heating to higher temperatures.
6. Mixtures of the same ionic type will destablize formation of single phases.
7. Kinetics effects are important when for some surfactant mixtures – this led to two distinct microemulsion phases.
8. The sponge phase can only occur in water, showing the importance of hydration forces.
9. Middle-phase microemulsion can also be formed from slightly less polar solvent.
10. Corrosion inhibiting surfactants and possibly other specialty surfactants can be mixed to get a good formulation for coating, etc. by finding the right composition for texture and rheology.

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"Formation and optimization of thallium Cuprates High Temperature Super-conductor"
Year 1994 MTSF Science & Technology Research Grant Recipient

Introduction

At the forefront of high temperature superconductivity research, synthesizing of new materials and understanding the basic mechanisms of these novel materials have been a worldwide interest. Superconductors are materials which allow electricity to flow without any resistance. This unique feature is important in many applications such as electronics, communications, transportation and medical equipment.

This research project is regarding the thallium cuprate superconductors. Some examples of the thallium cuprate superconductors are $Tl_2Ba_2Ca_2Cu_3O_{10-\square\square}$(2223), $Tl_2Ba_2CaCu_2O_{8-\square\square}$(2212) and $TlSr_2(Ca,Cr)Cu_2O_{7-\square}$ (1212). These perovskite-like layered structure cuprates are studied intensively due to their ability to superconduct at high temperature. The thallium-based materials can form many superconducting phases with various numbers of Cu-O layers. The Cu-O layers are believed to be responsible for high temperature superconductivity. In this work the Tl-1212 type phase superconductor is studied intensively.

Ultrasonic velocity and attenuation measurements are useful in studying the bulk properties of superconductors. Although there is a fair number of reports of ultrasonic measurements on the La-based, Y-based (and other rare-earths) and Bi-based high temperature superconductor reports on the Tl-based and Hg-based materials are very few or even non-existent. This may be due to the difficulties in preparing high quality samples where ultrasonic echoes can be observed. In this project ultrasonic shear velocity and attenuation of bulk $TlSr_2(Ca,Cr)Cu_2O_7$ and $(Tl,Bi)Sr_2(Ca,Cr)Cu_2O_7$ high temperature superconductors have been measured between 80 K and above 200 K at 8 MHz.

Objectives

The objective of this research is two folds; (i) better understanding of the mechanisms of high temperature superconductivity via elemental substitutions and ultrasonic studies and (ii) determining the optimum methods of formation of the thallium cuprates superconductors. The optimum preparation method and starting composition to obtain higher transition temperature and high purity superconductor is also studied. Materials characterization includes electric, magnetic, ultrasonic attenuation, elastic constants and structural related properties. Through this research we obtained information regarding the importance of ionic radius and the optimum average Cu valence and their roles in the superconducting mechanisms of thallium cuprates HTSC.

Materials Synthesis and Experimental Details

Superconducting samples were prepared by solid state reaction methods with high purity oxide components by employing the precursor method. The materials that were synthesized in this project are:

$(Tl_{1-x}Pb_x)Sr_2CaCu_2O_7$, $(Tl_{1-x}Bi_x)Sr_2CaCu_2O_7$, $(Tl_{0.8}Bi_{0.2})Sr_2(Ca_{1-x}Cr_x)Cu_2O_7$,
$(TlCd_{0.2})Sr_2(Ca_{1-x}Cr_x)Cu_2O_7$, $(TlSc_{0.2})Sr_2(Ca_{1-x}Cr_x)Cu_2O_7$
$(Tl_{0.8}Zr_{0.2})Sr_2(Ca_{1-x}Cr_x)Cu_2O_7$, $(Tl_{0.8}Ce_{0.2})Sr_2(Ca_{1-x}Cr_x)Cu_2O_7$
and $TlSr_2(Ca_{1-x}Zr_x)Cu_2O_7$.

The ultrasonic velocity and attenuation were measured using a Matec 7700 system which utilizes the pulse-echo-overlap technique. The sample was bonded to the transducer using Nonaq stopcock grease. The ultrasonic waves were propagated along the direction of pressing using an *X*-cut (longitudinal) or a *Y*-cut (shear) quartz transducer at 5-10 MHz. The temperature dependent ultrasonic attenuation and sound velocity measurements were performed in an Oxford Instruments liquid nitrogen cryostat model DN 1711 where the temperature was changed at a rate of about 1 K/min. during warming and cooling. No thermal expansion correction was made. A typical ultrasonic echo train is shown in Figure 1.

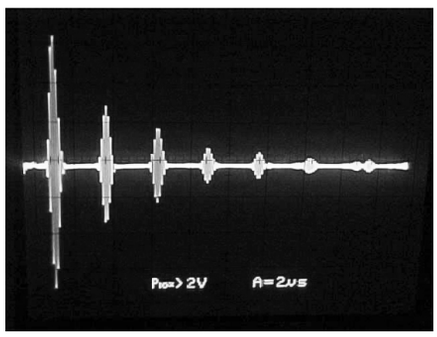

Figure 1. A typical echo pattern of an ultrasonic pulse travelling through a copper oxide based ceramic superconductor.

Outcome and Results

Formation of Tl-1212 Phase Superconductor

A host of the derivatives of the thallium-1212 cuprates high temperature superconducting materials have been synthesized. From the variation of the transition temperature as the materials were doped with several elements, three important findings are obtained:

i) The optimum Cu valence for the highest superconducting transition is between 2.2+ and 2.3+. The undoped materials are in the hole over-doped state with Cu valence of 2.5+. Although this valence state can be represented as exactly half-filled band and supposed to be metallic, it is actually a Mott-Hubbard insulator. By adjusting the Cu valence through elemental substitution, high temperature superconductivity can be initiated.

ii) Our analysis indirectly showed that Bi in the Tl-1212 type is in the mixed state Bi^{+3}/Bi^{+5} whereas Pb exists in the Pb^{+4} state. The valence state of Cr at the Tl site is expected to be > 4+ and the valence state of Zr at the Tl site is estimated to be 4+.

iii) For the formation of the thallium-1212 type phase, the ionic radius of the substituted elements plays a more important role than the electronic configurations. This can be an important consideration in the search for new materials.

Softening tendency

A large thermal hysteresis was observed in the $TlSr_2(Ca_{0.7}Cr_{0.3})Cu_2O_7$ (density 3.74g/cm^3) but very much suppressed in the $(Tl,Bi)Sr_2(Ca,Cr)Cu_2O_7$ (density 5.40 g/cm^3) sample. Shear velocity hysteresis in these materials is very much determined by the density and microstructure and may not be directly related to high temperature superconductivity. A pronounced change in the shear velocity at about 160 - 190 K signifying a softening tendency was observed in the $(Tl,Bi)Sr_2(Ca,Cr)Cu_2O_7$ material.

The thermal hysteresis in the shear velocity is due to microstructure effect such as grain size and not directly related to high temperature superconductivity because a more uniform and smaller grain size sample showed no appreciable hysteresis. The general trend of lattice stiffening as the temperature was lowered in the Tl-1212 sample which is also observed in the R123 (superconducting and non-superconducting) materials is an intrinsic feature of these copper oxide based ceramics and may not be directly related to high temperature superconductivity.

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Universiti Kebangsaan Malaysia
"Genetic Transformation of Rice for Tungro Virus Resistance"
Year 1995 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Plant breeders and genetic engineers share the common goal of plant improvement. While plant breeders traditionally use selective breeding for varietal enhancement, genetic engineers continue to develop techniques for the isolation and insertion of genes for desirable traits. Genes unavailable to a particular plant species due to sexual incompatibility may be obtained from other organisms and transferred into the plant of choice. Several steps using both molecular and cellular biology techniques are involved in producing a genetically engineered plant. A trait must be chosen and the gene(s) encoding the trait identified and isolated from the donor organism. The functional gene must include regulatory regions in order to be correctly expressed in the target plants. The isolated gene must be transferred directly or inserted into vectors, which then delivers the gene into the plant cells. For successful transformation, DNA sequences introduced into the plant cell must be inserted into the plant genome, expressed and maintained throughout subsequent cell divisions. Finally, the transformed plant cells must be regenerated into whole plants.

Objectives

One of the main threats to rice production is high losses due to Rice Tungro Bacilliform Virus (RTBV). Thus, the objective of this project was to transfer protease gene isolated from RTBV into local commercial varieties in our effort to minimize losses following infestation of RTBV.

Methodology

This research uses a protease gene isolated from local RTBV isolates, which was cloned into pBI121, giving rise to a new recombinant plasmid, pBI240E. pBI240E is a gift from Dr . Ismail Ahmad, School of Bioscience and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia.

Basically, there were 2 stages of activities involved. First, pBI240E was transferred directly into selected target tissues or through *Agrobacterium tumefaciens*. Protocols developed from this project were the first to describe the possibility of transforming indica rice using *Agrobacterium tumefaciens* and *A. rhizogenes*. The second phase involves the selection and regeneration of complete transgenic plants from putative transformed tissues carrying the protease gene. To date, plants regenerated from this project have been evaluated up till the 6^{th} generation. This is to study the stability of the transgene in its new environment. From the analysis made, it can be concluded that the transgenes were stably transformed into the plants, and most importantly are transmitted to the subsequent generation, an important prerequisite for effective gene manipulation in crop improvement. The analysis of transgenic plants and the functionality of the transgene in its new environment are however, not within the scope of this project and would not be mentioned in this report.

Results

Since the project was a pioneer work on attempts to manipulate resistance against RTBV in rice, most of the findings are focused on the development of new technologies. They include :

- Development of *Agrobacterium*-mediated gene transfer for indica rice. Rice is a monocot and had been shown to be recalcitrant to infection by *Agrobacterium*. Thus, with the development of such technology, the protocol developed could be exploited to be used in gene manipulation for rice improvement, not necessarily on resistance against RTBV but also other stresses such as against pests and diseases, yield improvement and also for the production of value added products.

- The development of gene transfer system mediated by particle bombardment for rice, an important monocot. In addition to the *Agrobacterium*-mediated gene transfer system developed, this approach provides a more rapid means of introducing foreign gene into difficult monocot such as rice.

- Plant regeneration from putative transformed target tissues. Though this is not new, but regeneration of transgenic plants from putative transformed callus is still not efficient. In addition, the production of transgenic plants has always been marred by the production of chimearic plants alongside the transgenics. Thus, a tight selection scheme is necessary to ensure the production of only true transgenics from putative transformed target tissues.

These new discoveries were then employed in other projects related to gene manipulation for crop improvement, either it be rice or other crops such as oil palm. In addition to technology development, the project also contributed to human resource development, where several theses have been produced from students involved (see list of publications).

Unsolved problems and future directions

It is quite unfortunate that the project was only up to the production of putative transgenic plants carrying protease gene isolated from RTBV. Thus, it is not possible to assess the transgenic plants produced whether they are resistant to RTBV or otherwise. The analysis of the transgenic plants on its effectiveness against RTBV would require an additional 3-5 years before the plants are ready to be supplied to the rice growers.

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Associate Professor Dr. G. Suresh Kumar
Dr. Mak Joon Wah
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Universiti Malaya

"Immunological and Pathological Studies in *Blastocystis Hominis*"
Year 1995 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

a) H.I.V infected patients – 189 patients infected with H.I.V. were assessed for *Blastocystis hominis* using the *in vitro* culture technique. The technique was reported to be the most effective method to detect the parasites in the stool. 29% of the population was infected with the parasite. Paper was published in International Medical Research Journal. This mode of detection when used at Scotland during my Commonwealth Fellowship (Feb 14th 2000-Feb 14th 2001) detected 2.8% *Blastocystis* in patients suffering from gastrointestinal and other related symptoms.

b) Stools from animal handlers from 4 different research institutions including the National Zoo were assessed for *Blastocystis hominis*. A high prevalence of 44% shows that the infection could be zoonotic. This important finding has been published in Parasitology Research. It alerts the international community to be careful while handling animals or being in close proximity with pets.

c) A total of 1,041 stool specimens were received during the two-year period of study, at the Department of Parasitology, Faculty of Medicine, University of Malaya, Kuala Lumpur. The stool specimens were screened for all the pathogenic parasites, bacteria and viruses. *Blastocystis hominis* was the commonest parasitic infection, occurring in 59 of the 1,041 stool specimens examined. *Giardia* was present in 6 patients and *Trichuris* in 6 others. *B. hominis* was present as the only pathogen in 24 stool samples. Diarrhoea was the leading symptom and was presenting 20 of 24 *Blastocystis* infected patients. The next common symptom was abdominal pain, occurring in 12 patients, followed by bloated stomach in 6 cases, fever in 5, vomiting and nausea in 4, heartburn in 3 cases. A few non-specific symptoms like anorexia, headache, fatigue, backache and myalgia was manifested in a few cases. The parasite was highest in the age group 20-40 years. This study provides evidence to incriminate *Blastocystis* as a diarrhoea causing pathogen, as we could detect no other pathogen in these 20 *Blastocystis* infected patients. The findings have been published in International Medical Research Journal.

d) We have extensively studied the prevalence of the parasite in immigrant workers and showed that the size of the parasites of the Indonesian isolate of *Blastocystis hominis* were 3-5 um whereas larger vacuolar forms measuring 10-15 um were seen in Malaysian and Bangladeshi isolates. The small forms show a distinct growth profile and show higher resistance to drugs compared to other isolates. The findings have been published in International Health and Tropical Medicine.

e) The note worthy contribution of the study was to assess the pathogencity of the infection by correlating the shedding patterns of the parasite with clinical signs and symptoms. We demonstrated that there was an irregular shedding pattern of the cystic and the vacuolor forms of the parasite. The important clinical finding which clearly points out that for a proper diagnostic procedure, stools must be collected more than once from the same patient in order to eliminate the cause of the symptoms to the parasite. The high shedding of cystic stages without any

clinical signs also show that there exists a phenomenon of active cyst passers who would be major sources for transmission. The paper has been published in Parasitology Research.

f) The prevalence of *Blastocystis* in lab-bred animals was reported in Tropical Biomedicine. The paper highlights the importance of completely treating the animals before experimental animal inoculation.

g) We reported in South East Asian Journal of Tropical Medicine and Hygiene that *Blastocystis* was prevalent in cockroaches. This was another finding contributing to the understanding of the mode of transmission.

h) We reported in Parasitology Research for the first time that the parasite was seen in house lizards and described a novel mode of reproduction.

i) A good cryoprotectant was needed to maintain the parasites for long periods. There is still a difficulty in selecting a good cryoprotectant to preserve the extremely fragile parasites. We compared the cryoprotective ability of two cryoprotectants, mannitol in glycerol with and without DMSO. The cryoprotectant using DMSO favoured better yield when resuscitated in IMDM medium with 20% horse serum. This was reported in the International Medical Research Journal.

j) We reported for the first time in Parasitology Research the use of foetal calf serum as an excellent cryoprotective agent where we managed to get a yield as high as 90%. We are currently maintaining the isolates using this cryoprotectant.

k) Serum of infected patients showed antibody response to *Blastocystis*, as detected by the enzyme-linked immunosorbent assay (ELISA) and Western blotting. Reactivity was common against proteins of MW70 and 64 kDa, with lesser reactivity against the 57 kDa protein (findings not published yet).

l) Studies in experimental animals : Susceptibility studies were attempted using different mice and rat strains. Wistar rats were found to be the most suitable animal model for *Blastocystis* infection. Infected Wistar rats showed decreased gain in weight, which was noticed in the second week of infection, which recovered during the fourth week post-infection. Histopathology was performed on tissue sections taken from different regions of the intestinal tract such as ileum, colon, caecum and rectum. Macroscopically, there was no significant change in the appearance of the mucosal surfaces of the rectum, caecum and colon. Histology of tissue sections from infected rats showed varying degrees of lymphoid tissue hyperplasia in the mucosa of the colon, caecum and rectum. The luminal content of these sections contained sloughed off musocal cells and cell debris. The lymphoid tissue hyperplasia seen in the mucosa of the stressed rats, was more vast, as compared to the rats which were not stressed. The rats which were injected intracaecally with *Blastocystis* culture showed very diffuse lymphoid follicular hyperplasia, whereas the ones which were injected with the *Blastocystis*-free bacterial culture from the same isolates showed an inflammatory reaction which was relatively mild.

Humoral immune response was detected in the infected rats, using the enzyme linked immunosorbent assay (ELISA) and Western blotting. Immunoblots showed antibody detection starting from the seventh week, reactivity was more intense in the tenth week post-infection and remained for a few weeks. The molecular weights of the proteins in the antigen are 79, 70, 60, 39, 35 and 31 kilo-daltrons. A protein of MW 70 kDa was the common *B. hominis* antigen. The electrophoresis was performed on a Mini-Protean II Electrophoresis cell (Bio-Rad). Both, high and low molecular weight markers were used to indicate the molecular weights of the protein bands, and electrophoresis of the *Blastocystis* antigen was carried out using a 10% gel separation mix, run at 70 volts using a Hoeffer power pack. The gel was then stained using the Silver Staining Method.

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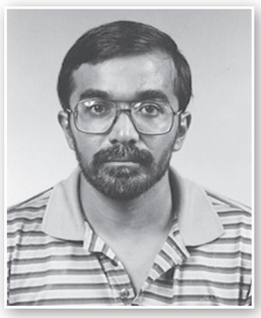
Associate Professor Dr. Omar Bin Shawkataly

Universiti Sains Malaysia



"Reactivity of some Group 8 Cluster Carbonyls towards small molecules"
Year 1995 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

There is currently much interest in the extended interactions that are possible between ligands and metal carbonyl clusters. Many of these interactions are not possible in mononuclear chemistry. The chemistry of $Ru_3(CO)_{12}$ has been explored to a much greater extend am any of its simple substituted derivatives (Bruce, 1982). An area of considerable interest has been the synthesis and reactivity of derivatives of Ru_3 clusters containing group 15 ligands, because of their possible application to catalysis. However, their study has been greatly hindered by the difficulty in getting high yield of these derivatives. High temperature are required for $Ru_3(CO)_{12}$ to react with various tertiary phosphines, and the usual products from such reactions are $Ru_3(CO)_9(PR_3)_3$ (Shawkataly, 1987).

The development of a '*mild*' route to carbonyl substitution by Bruce and co-workers (Bruce, *et al*, 1983) which uses sodium benzophenane ketyl (BPK) to induce specific carbonyl substitution of group 8 metal clusters. This substitution involves an electran-transfer-catalysed (ETC) process. The BPK route have made available a wide range of derivatives of group 8 carbonyl clusters in very high yield and allows their reactivity to be further explored.

Objectives

The aim of this research is to synthesize new and novel cluster complexes and to fully characterize these new complexes. It was also hope the reactivity of these new complexes can be further studied.

Methodology

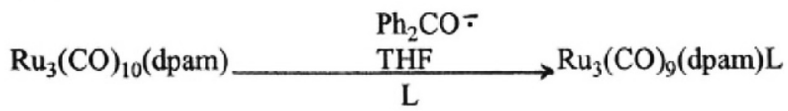
The group 8 metal carbonyls (in this study, I have concentrated on $Ru_3(CO)_{12}$) was purchased from Aldrich. The ligands used were mainly supplied by Strem, Pressure Chemical Company, Fluorochem, BDH, Fluka and Aldrich. All the solvents used were dried prior to use (Perrin, *et al*, 1980). The derivatives were synthesis by the BPK route (Bruce, *et al*, 1983).

$$Ru_3(CO)_{12} \xrightarrow[L\text{-}L]{Ph_2CO^{\cdot-},\ THF} Ru_3(CO)_{10}(L\text{-}L)$$

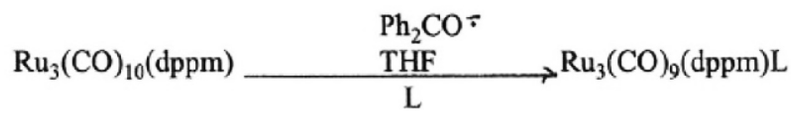
L-L = dppm, dpam, ARPHOS

All the reactions were carried out under an inert atmosphere of dry-deoxygenated nitrogen gas using Schlenk condition (Shriver and Drezdzon, 1986).

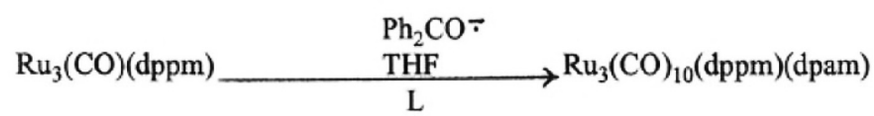
The following reactions were undertaken :



L=PPh_3, $P(OMe)_3$, PCy_2Ph



L = $AsPh_3$, $P(OMe)_3$, $P(OPh)_3$, PCy_2Ph



The products were purified by separation using column chromatography (Florisil) or preparative thin layer chromatography (Kieselgel $600GF_{254}$, 0.5mm. thick).

The products were characterized by IR, H^1, C^{13} and P^{31} NMR, mass spectrometry, CHN analysis and also melting point.

Results

Many new and novel derivatives of $Ru_3(CO)_{12}$ have been synthesized. They were characterized by spectroscopic analysis. Single crystal X-Ray diffraction studies were done on THREE new clusters and ONE ligand. Some of the results have been published (O.b. Shawkataly, *et al*, 1998). The 2 structures that are still unpublished are (i) ARPHOS and (ii) $Ru_3(CO)_{10}$ (ARPHOS).

Summary and Conclusion

This research project has managed to synthesis and characterizes some new and novel cluster complexes. However, this grant is now finished and it is hope that the reactivity of these new cluster complexes can be studied. These new clusters complexes contain mixed ligands and should give modes of bonding when subjected to heating and also hydrogenation reaction (Shawkataly, 1987).

Publication

Synthesis and spectral studies on $Ru_3(CO)_{12-n}$ (μ-$Ph_2AsCH_2AsPh_2$)(L) (n=3, L : PPh_3, PCy_2 Ph, $P(OCH_3)_3$); n = 4, L = $Ph_2PCH_2PPh_2$) : crystal and molecular structures $Ru_3(CO)_9$ (μ-$Ph_2AsCH_2AsPh_2$)(PCy_2 Ph) and $Ru_3(CO)_8$ (μ-$Ph_2AsCH_2AsPh_2$)(μ-Ph_2, PCH_2PPh_2) by Omar bin Shawkataly, K.Ramalingam, S.T.Lee, M.Parameswary, H.K.Fun and K.Sivakumar, *Polyhedron*, 1998, 17, 1211.

Professor Dr. Rahmah Mohamed
Professor Dr. Sheila Nathan
Professor Dr. Mohammed Noor Embi

Universiti Kebangsaan Malaysia
"Studies on the Protease Gene of *Pseudomonas pseudomallei*"
Year 1995 MTSF Science & Technology Research Grant Recipient

The objective of this study was to define the role of protease in pathogenesis of *Burkholderia pseudomallei* (previously known as *Pseudomonas pseudomallei*). An initial study was set up to determine the presence of protease gene sequences in the genome of this organism. Ten probes whose sequences have close homology to known protease gene sequences were identified by the DNASIS programme and synthesized. The rationale for selection is based on close homology between the species (60%) as reported by Letoffe et. al. (1993). Probes synthesized from the elastase gene sequence of *P. aeruginosa* were also utilized as elastase demonstrated similar biochemical activity with protease of *Burkholderia pseudomallei* (Sexton et.al.1994).

Genomic DNA from a human isolate of *B. pseudomallei* was digested with several restriction enzymes (Bam H1, SphI, XbaI, KpnI and Bgl II) and blotted onto nylon membrane. Hybridization signals at 4kb were detected by probes PA1 and PA3 synthesized to the elastase gene sequence of *P. aeruginosa*. Results from this study demonstrated the presence of protease gene sequences similar to that of elastase in *B. pseudomallei genome*.

In order to further investigate the role of protease in virulence of the organism, it is imperative that the gene be cloned and its recombinant product analyzed for biological relevance. Cloning of the protease gene was undertaken in 1996 and completed by the end of the year. Cloning was performed in a strong expression vector pLITMUS-28, which has a T7 promoter, and DNA fragments were cloned into BamH1 sites. Out of the 11,029 transformants screened for protease activity on 3% milk agar, 122 colonies displayed a transparent zone of lysis denoting protease activity.

Further screening utilizing colony hybridization with biotinylated probes PA1, PA2, PA3 and PA4 showed 107 colonies displaying positive signals with one out of four probes. Further characterization demonstrated one recombinant colony designated B1 displayed a positive signal by all the four probes. Southern blot analysis of B1 has shown that probe PA3 gave positive signals at 3.7kb and 1.8kb. Recombinant B1 did not display homology with the metalloprotease of *S. marcescens*. Recombinant B1 carried an insert of 3.7kb and we believe it contains the putative protease gene of *Burkholderia pseudomallei*. The findings from this preliminary search for protease genes was later expanded and resulted in the identification of a novel *B. pseudomallei* extracellular calcium-dependent serine protease involved in the virulence of the pathogen. The protease is capable of completely digesting a number of physiologically important human proteins.

Publication

Jessmi M. L. Ling, Sheila Nathan, Lee Kok Hin and Rahmah Mohamed. 2001. Purification and characterization of a *Burkholderia pseudomallei* protease expressed in recombinant *E. coli. Journal of Biochemistry and Molecular Biology,* Vol. 34, No. 6, pp. 509-516.

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Professor Mohammed Omar

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"Molecular approaches in the control of the Fungal Plant Pathogen, *glomerella cingulata"*
Year 1995 MTSF Science & Technology Research Grant Recipient

Plant diseases caused by *Colletotrichum gloeosporioides* (teleomorph *Glomerella cingulata*) occur on a wide range of plant species and have been recorded world wide in the tropics and subtropics both as pre-harvest and post-harvest causes of crop loss. This fungus penetrates the host by mechanical pressure exerted by a specialised structure called appressorium together with enzymic breakdown of plant polymers. The main aim of this long-term research project is to study the pathogenicity factors of *G. cingulata* which will eventually lead to the development of control measures against the plant pathogen using molecular approaches. The research was carried out by isolating and characterizing strains of *G. cingulata* via morphogenesis and virulence studies, characterizing the cutinase gene of a *G. cingulata* strain by cloning and sequencing the gene from the genomic library, characterizing the cutinase cDNA amplified from the cDNA of cutin induced cultures, determining the three dimensional protein structure of cutinase, constructing an intron-less cutinase gene in *Escherichia* and *Pichia* expression systems and conducting preliminary studies on cutinase gene disruption. Studies were carried-out on 9 Malaysian strains (*C. gloeosporioides*) and one New Zealand strain (*Glomerella cingulata* ICMP 11061). A synchronous appressoria induction assay was carried-out using wax extracted from papaya fruits and rubber leaves. A probe for the cutinase gene was constructed by cloning a cutinase PCR product. The recombinant plasmid containing the cutinase PCR fragment, pFD1, was used to screen the *G. cingulata* ICMP 11061 genomic library. Nucleotide sequence data of the 5.8 kb genomic DNA insert containing the cutinase gene has been characterised. Nucleotide sequence data revealed that the cutinase gene codes for a putative 224-amino acid protein encoded by two exons of 189 bp and 486 bp, separated by an intron of 52 bp. A cDNA clone was prepared from RNA of papaya cutin induced cultures using RT-PCR, the sequence of which was used to confirm the presence and position of the intron. Potential DNA segments for cutinase transcription start, transcription factor binding sites, TATA box and polyadenylation sites, have been identified. The cutinase gene is present in a single copy in the genome of *G. cingulata* ICMP 11061 and the putative protein product is 24 – 99% identical at the amino acid level to other fungal cutinases. An intron-less cutinase gene generated by SOE PCR was successfully cloned into *Pichia pastoris* and *Escherichia coli* expression vectors. Preliminary studies on the disruption of the cutinase gene show that out of 650 transformants resistant to hygromycin, three lacked esterase activity and thus this indicates that their cutinase gene might have been disrupted. The three dimensional protein structure of *G. cingulata* cutinase as determined by protein homology modeling showed that the protein is ellipsoidal and has a central β-sheet consisting of five parallel strands surrounded by 5 helices. The amino acid residues participating in the catalytic triad and oxyanion hole have been determined and are located at one extremity of the protein. The information gathered from this study has provided the platform for further studies that are currently on going (Section B: Projects IRPA 09-02-04-001 BTK/TD/004, IRPA 09-02-02-0040-EA136, IRPA 09-02-02-0105-EA257 and ST/25/01) to elucidate the characteristics and role of cutinase in greater depth, and to study other pathogenicity factors in this important fungus. This project has produced one article in an international journal and 7 articles in proceedings. Two MSc. students carried out parts of this project and have graduated.

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Dr. Abdul Manaf Ali

Universiti Putra Malaysia

"The mechanism of action of some medicinal plant extracts on *Acanthamoeba castellanii*"
Year 1995 MTSF Science & Technology Research Grant Recipient

Introduction

Most free-living amoebae (FLA), such as *Acanthamoeba* spp are facultative pathogens. In their natural habitat, they feed mainly on bacteria and particulate matters but if they accidentally invade the human's body, they become pathogens. Human keratitis caused by these amoebae, have been diagnosed and reported worldwide including in Malaysia. *Acanthamoeba* keratitis is difficult to treat since this amoeba is resistant to most drugs available in the market especially at later stage of infection. Sensitivity of amoeba isolates to ten drugs was tested in vitro by Hay et al., (1994). Only chlorhexadine and polyhexamethylene biguanide showed amoebacidal activities. Other drugs were not effective on the examined isolates. Current medication for successful treatment of *Acanthamoeba* keratitis are chlorhexidine and propamidine (Mohamed Kamel et al., 2000) but these synthetically prepared drugs are not readily available in this country. Therefore, search for a new anti-Acanthamoebic compound from local plants become an urgent matter. Malaysia has diverse bio-resources including medicinal plants that are known to have bioactive compounds against activity of many pathogenic microorganisms. In this study, two pure compounds and crude extract of *Muraya koenigii*'s bark were investigated for their potential as new anti-*Acanthamoeba* agents. Mechanism of action of these compounds on *Acanthamoeba* was the focus of this study.

Objectives

The mechanism of action of the compounds extracted from *Muraya koenigi*i's bark viz it's crude extract and it's two pure compounds, labeled as MK1 and MK2 on *Acanthamoeba castellanii in vitro* were examined by looking at 1) EC_{50} values of each compound including the mixture of the two compounds and the crude extract, tested on amoeba 2) its action on activity's of amoeba's proteinases 3) its action on the formation of mitotic microtubules in amoeba 4) changes in morphology of *Acanthamoeba* cells after treatment with the compounds.

Methodology

A. castellanii is a clinical isolate (obtained from The Institute of Medical Research, Kuala Lumpur) and was cultured axenically in 5% (w/v) Mycopeptone medium, at 30° C and was harvested at its log phase growth for experiment with the plant extracts.

Pure compounds (MK1 and MK2) and the crude extract of *Muraya koenigii* 's bark (prepared and supplied by Professor Dr. Mohd Aspollah Sukari, Department of Chemistry, University Putra Malaysia). Twenty four well plates were used for testing the various concentration of plant extracts (450, 45, 4.5 and 0.45 ppm) against the amoebae. Appropriate volume of the extract solution and amoeba suspension were added to the wells. The plates were incubated for 3 days before staining with 0.5% eosin. The actual optical density of the resulting solution in treated wells was obtained by subtracting the optical density of the solvent of each extract (used as control).

In the rest of experiments, only pure compounds at concentrations of 45 ppm and 450 ppm were used. After treatment with the compounds, the morphology of amoeba cells was observed both by light and transmission electron microscopy. The amoebae were also processed for proteinases analysis and for microtubule detection.

For proteinases analysis, the amoeba samples were subjected to polyacrylamide gel electrophoresis (PAGE) using the SDS discontinuous buffer system (SDS-PAGE as described by Lockwood et al., (1987). After removing SDS, the resolved gels were incubated overnight in buffer containing inhibitors (PMSF (50 μM), Leupeptin (50 μM), and Antipain (50 μM)), MK1 (45 ppm, 450 ppm) and MK2 (45 ppm, 450 ppm), shaking at room temperature. Proteinase activity was identified by the presence of clear bands caused by gelatin hydrolysis on Coomassie Blue-stained gels. Bands were labelled A, B, C, etc in order of increasing mobility. The bands which were absent compared with the control gel were considered inhibited by the inhibitors or by the plant extracts. To roughly estimate the relative molecular weight of each proteinase band (M_r), prestained individual markers ranging from 200-kDa to 14.3-kDa (SIGMA) was used as protein standards.

Microtubule staining of amoeba cells after treatment with MK1 and MK2 was carried-out following Yumura and Fukui techniques (1987). The observation of the microtubules in amoeba cells was done under a LEICA DMLD fluorescence microscopy.

Results and Discussion

The presence of anti-amoebic activities in compounds extracted from *Muraya koenigii*'s bark was evident from their EC_{50} values, changes in the amoeba cell morphology both by light and transmission electron microscopy and inhibition of proteinase enzymes in amoeba. The EC50 values for MK1, MK2, mixture of MK1 and MK2 and crude extract obtained in this study are 1.18ppm, 3.04 ppm, 31.22 ppm and 85.33 ppm, respectively. Based on the EC_{50} values, MK1 is the most potent compound and has the strongest anti-Acanthamoebic activity, followed by MK2, mixture of MK1 and MK2, and the crude extract. Even though MK1 and MK2 are potent compounds when acted individually, their mixture proved to have antagonistic effect, so their anti-amoebic activity is reduced. The crude extract of the plant seems to have very low anti-amoebic property against *A. castellanii*.

Amoeba cells, after treatment with MK1 and MK2, were found to be smaller in size with abnormal acanthapodia formed on the cell's surface compared with control cells to suggest during treatment with the compounds, the amoebae prevent taking in unwanted molecules (MK1 and MK2) across their cell's membrane by reducing their surface area for absoption. By TEM observation, increased number of mitochondria and decreased number of food vacuoles in MK1 and MK2-treated cells were noticed compared with control cells. A mitochondrion is an organelle which involves in ATP production in cells and its presence in high number in MK1 and MK2-treated cells is to indicate that these cells require more energy supply than the untreated cells in order to survive in culture medium containing the two compounds. These amoebae were also observed to have lesser number of food vacuoles compared with control cells to indicate in unfavourable conditions, the amoebae 'eat less' to avoid unwanted molecules will be accumulated in their cells.

A. castellanii seems to posses three proteinase bands on gelatin-SDS-PAGE gels. The bands were labelled as band A (M_r ~ 97.4 kDa), B and C (their M_r are between 43 kDa and 29 kDa). Band B has the highest activity observed on the gel and band A has the lowest activity and was not consistently observed on the gel. Both bands B and C were partially inhibited by PMSF, Leupeptin and Antipain so can be categorized as serine or cysteine proteinases (North, 1989). MK1 (45 ppm and 450 ppm) seems to completely inhibit both bands B and C enzymes. Similar inhibition on the enzyme activities was observed for MK2 (45 ppm and 450 ppm). The results obtained from this study suggest that both MK1 and MK2 inhibit the activity of band B and C proteinase enzymes in amoebae. The involvement of proteinases in amoeba pathogenesis has been reported. Proteinases are believed to play an important role in determining the pathogenicity of the amoeba *Entamoeba histolytica* (Tannich et al., 1991). Aldape et al., (1994) have proposed that one protease (a cysteine proteinase) released by *Naegleria fowleri* has contributed to tissue destruction and hence facilitated host invasion by the amoeba.

Further investigation on the role of bands B and C enzymes in *A. castellanii* is suggested. These enzymes were almost fully inhibited by MK2 and MK1 but partially inhibited by Antipain, PMSF and Leupeptin suggesting the enzymes are of serine proteinases. Leher et al. (1998) have reported that lysis of the corneal epithelium in *Acanthamoeba* keratitis is due to a serine proteinase. The actual mechanism of how MK2 and MK1 inhibit the activity of the B and C proteinase enzymes during amoeba pathogenesis is not clear.

Microtubules, after staining with anti-α-tubulin and anti-β-tubulin have been detected in *Acanthamoeba* cells, both in control and treated cells (with MK1 and MK2). The results of this study indicate that two compounds extracted from *Muraya koenigi*i's bark seem did not inhibit the polymerization of the microtubules in dividing amoeba cells. The detection of the microtubules (or a centrosome or Microtubule organizing center (MTOC) where bundles of microtubules originate from) in amoeba cells was possible despite of the presence of MK1 and MK2 due to the cells which had survived after treatment with the compounds can still undergo cell division.

Conclusion

The results of this study indicated that

1. Compounds extracted from *Muraya koenigii's* bark viz MK1, MK2 and its crude extract have anti-Acanthamoebic activities.

2. Mechanism of action of the compounds on *Acanthamoeba* cells observed in the present study i). The compounds affect the amoeba growth. The EC_{50} values of MK1 and MK2, Mixture of MK1 and MK2 and its crude extract are 1.18 ppm, 3.04 ppm , 31.22 ppm and 85.33 ppm, respectively, ii). MK1 and MK2 caused inhibition both bands B and C proteinases enzymes. Based on inhibitor studies, these enzymes may be categorized as serine/cysteine proteinases, iii). MK1 and MK2 did not inhibit the polymerIzation of microtubules (or formation of a centrosome or MTOC) since the microtubules and its related structures can still be visualized in cells treated with the two compounds iv). MK1 and MK2 cause the size of amoeba became smaller, and with abnormal acanthopodia,

decreased number of food vacuoles but increased number of mitochondria to suggest the physiology and biology of amoeba are affected by these compounds which result in the reduction of number of amoebae as observed in the present study.

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2. Nakisah M. A. 2001. Studies on the mechanism of actions of some medicinal plant extracts on *Acanthamoeba castellanii* A final report submitted to Malaysia Toray Science Foundation, Mac, 2001. 25 pp.
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Associate Professor Dr. Rubiyah Yusof
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"An intelligent based self-tuning PID Controller"
Year 1995 MTSF Science & Technology Research Grant Recipient

Introduction

In the control field, researchers and academicians are mainly engaged in trying to improve the performance of control systems to increase productivity and efficiency. Improving control performance actually involves the development of more robust, adaptive or self-organizing controllers with minimum operator interaction.

Our research are primarily involved in improving the state of existing controllers for industrial applications either through the development of new algorithms or/and modification of existing methodologies or/and the use of new emerging tools such as AI tools in an existing practical environment. This field of research stands as one of the most concentrated field in the world and much effort has been given by researchers around the world and also grants by governments and industries. However in Malaysia, grants for such leading edge research has not been well given due to the nature of Malaysia as a developing country where concentration is mainly towards applied industry based research. To meet both international and local research objectives, we hope to develop a practical intelligent based self-tuning controller which can be applied and tested on a physical pilot process plant. Also, in Malaysia such research has not been given due recognition yet due to the high technology and academic knowledge that are required. However, as we aspire to be among the researchers who are internationally recognized, this field of research is undertaken.

Furthermore, most industries in Malaysia except very large industries are still using very conventional controllers such as PID controllers. And some small and medium sized industries are still even using manual control. These type of controllers, although can perform reasonably well to some extend, still have a distinct limitation. For example, PID controllers are fixed tuned in nature. Therefore, wherever there is a change in the operating point of the process through disturbances other environmental factors, the controller has to be re-tuned each time. The re-tuning process is a time consuming activity and usually has to be performed by experienced field engineers or operators. For manual controllers, the industry would need a large number of skilled operators and engineers. This would result in a very labour dependent industry and a serious problem would result if there is an adequate supply of labour or job-happing situation arises. Furthermore, manual control may not be as accurate as automatic control and this may result in degradation of control system performance and increase in production cost.

In view of these factors, our research group proposed to carry out a research in developing an intelligent self-tuning controller which can perform better than the conventional PID controllers and easily used by the local industries.

Objectives

The objectives of the research can be utilized as follows :

1) to explore various Artificial Intelligence Technique that can be used as self-tuning control.
2) to develop a simplified intelligent self-tuning control that can be easily applied and can perform well above average controllers as well as very robust.

3) to implement the developed algorithm/controller to a laboratory scaled industrial process to show the effectiveness of the controller.

Methodology

i) Development of an adaptive general regression neural network (GRNN) for modeling of dynamic plants.

ii) Tuning of a neuro-fuzzy controller by Genetic Algorithm.

Results

The research explores new approaches to modeling as well as improving the control of complex control systems using artificial intelligence technique. The results of the research have been published in four international journals. The method of modeling dynamics of plants using artificial intelligence technique i.e. adaptive general regression neural network is a novel contribution to the field as it is able to model the plant dynamics quite accurately under noisy environment. The tuning of neuro-fuzzy controller using genetic algorithm is also a novel breakthrough for research in this area. The method has indeed simplified the tuning process of the powerful neuro-fuzzy controller by using genetic algorithm. This is because genetic algorithm is an optimization method which is capable of generating optimized tuning parameters. Hence, the tuning procedures which is rather troublesome and time consuming can be simplified.

Unresolved problems and future direction

The research findings have been validated by some simulated examples and also applications on a laboratory scaled process. The method has not been tested on real control systems. Therefore, the success of the methods if applied to a real system is unknown at this stage. One of the ways to resolve this problem is to try this method on several laboratory scaled processes and validate the results. This would create more confidence in the effectiveness of the method.

Conclusion

The research fund has been used partly to finance this research and the results are quite successful. A new method of modeling plan dynamics has been introduced which can give an accurate model of the dynamics even under noisy environment. The research also develops a new approach of tuning a neuro-fuzzy controller using genetic algorithm. The method proves to be successful when experimented on some simulated plant models and laboratory scaled process.

Publications

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2. "Adaptive general regression neural network for modeling of dynamic plants" Proceedings of Institute of Mechanical Engineers, Vol. 213, Part 1, March 1999.
3. "Adaptive neuro-fuzzy control system by RBF and GRNN neural networks"' Journal of Intelligent and Robotics Systems, 23:267-289, 1998.
4. "Tuning of a neuro-fuzzy control system with an application to a coupled-tank control systems", International Journal of Engineering Applications on Artificial Intelligence, Vol. 11 (1998) pp. 517-529, Pergamon Press.

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"Studies on DNA Repair Related Disorders"
Year 1995 MTSF Science & Technology Research Grant Recipient

Rationale

The possibility of the existence of individuals with DNA repair disorders leading to the onset of skin cancer is evident from the startling numbers of Malaysian people suffering from skin disorders, malignant or otherwise (Hussein, unpublished). The increase in cancer induction in Malaysia is proof of a growing threat to society as a result of the nation's progressive industrialization and affluent life style. Thus, we would expect a concomitant increase in mutational frequencies due to the carcinogenic effects of chemicals including cigarette smoke (passive or direct), industrial waste products and exposure to more intense ultraviolet radiation. Reported data from the Dermatology Clinic Hospital Kuala Lumpur indicated that the number of patients referred to the clinic increased from 1991-1995. Although there was no significant difference between males and females, Chinese people were more susceptible with Indians the least and the appearance of tumours was more pronounced in middle to old age patients.

Objectives

1. To perform molecular epidemiological studies in the Malaysian population affected with skin disorders, especially those at higher risk, to monitor the prevalence of these DNA repair problems in Malaysia.
2. To monitor the occurrence of potential carcinogenic mutations based on the current dosage of PUVA administered, and to predict the applicability of higher doses.

Methodology

Tumour samples were obtained from skin patients with their consent and with the approval of a medical ethics committee. The diseases under investigation include mycoses fungoides, basal cell carcinomas, squamous cell carcinomas, psoriasis, vitiligo and the occasional case of Xeroderma pigmentosum. Patient background information was acquired through a prepared questionnaire and with the patients' consent. Identification of mutations was performed in an established marker, the *hprt* gene, by PCR amplification and sequencing. Screening for mutated tumor-suppressor *p53* gene by single-stranded conformation polymorphism (SSCP) via PCR, sequencing and protein analysis with anti-p53 antibodies was also carried out to establish alternative protocols. An i*n vitro* DNA repair assay system based on *Schizzosaccharomyces pombe* cell free extracts was used to monitor the biochemical and molecular mechanism by which cAMP-dependent PKA regulates DNA repair.

Results

Unique cell lines were successfully established from various cancerous and non-cancerous skin disorders and were successfully maintained at the Cell Culture Laboratory, Centre for Gene Analysis and Technology, UKM. The techniques of Polymerase Chain reaction (PCR) and Single-Stranded Confirmation Polymorphisme (SSCP) were also established and routinely used to amplify the biomarkers of interest (*hprt* and *p53*) as the approach to identify mutations with the gene sequences. Single base changes in these genes were investigated by PCR-SSCP and subsequent sequencing followed by comparing the mutations with published information to determine the presence and type of mutations.

These mutations in the Malaysian population of skin disorder patients were analysed for similarities to those reported elsewhere or if they were novel mutations.

Preliminary PCR-SSCP analysis on vitiligo, psoriasis and basal cell carcinoma samples did not demonstrate any differences when compared to normal samples. There was no evident conformational polymorphism between all the samples. Preliminary data acquired at the time of completion of this project was on exon 5 for one sample each of basal cell carcinoma, psoriasis and a wild type (normal) sample. The generated data was compared to that of the reported *p53* exon 5 sequence (NCBI) and 100% homology was demonstrated for the wild type and psoriasis sample. On the other hand, a single base change (T-A) was observed for the basal cell carcinoma sample at position 26 of the open reading frame resulting in the change of amino acid sequence from a Tyrosine to a Phenylalanine. There would be considerable value in incorporating measures of an individual's ability to repair DNA damage in epidemiological studies of cancer. First, it would establish the importance of DNA repair capacity as a risk factor for different cancers; second it may help to identify individuals at increased risk of particular cancers; and third it may increase the accuracy in estimating the effects of a particular exposure (Hall *et al*., 1994). The use of specific and sensitive assays will assist greatly in understanding the interplay of host susceptibility and exposure in determining the risk of a given cancer as well as establishing the importance of susceptibility factors in their own right.

In conclusion, the project has successfully completed the following:

- Establishment of novel fibroblast cell lines from various skin diseases and cancers
- Use of p53 as a biomarker is suitable for epidemiology studies
- Establishment and optimization of DNA-based mutation detection techniques that can be adapted for use in diagnostic and genetic testing facilities in hospitals

We conclude that the Malaysian population is most likely more resistant to cumulative doses of PUVA based on current treatment protocols and therefore, we support the recommendation of higher doses for more effective control of the disease.

Future directions

The data acquired can be correlated to known extraneous factors and established if the continuous long term use of PUVA as treatment for skin disorders is inducing mutations in these patients and thus predisposing them to more serious forms of cancer. The expected outcome of the data obtained will allow the identification of patients afflicted with various DNA repair deficiency disorders by using a screening method aimed at early detection of potential carcinogenesis and / or birth defects.

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Associate Professor Dr. Kurunathan Ratnavelu

Universiti Malaya

"Elastic Scattering of Positrons (Electrons) by Bound Atoms"
Year 1996 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

In 1949, DeBeneddetto *et al.* observation of two non-colinear gamma rays from the annihilation of a thermalised positron with an electron in a solid marked the beginning of solid-state investigations with positrons. This field of human endeavour has seen spectacular growth in the second part of the last century (Schultz and Lynn 1988) especially in the experimental field.

The need for reliable theoretical description of the interactions of positrons implanted into the subsurface regions of solids has grown with the development of positron implantation spectroscopy as a tool for non-destructive evaluation of layered structures, for the depth profiling of subsurface defects, and for other surface and near-surface studies [Schultz and Lynn 1988]. The main theoretical approach is to use Monte-Carlo simulations, where positron trajectories for a large number of positrons interacting with the target atoms via both elastic and inelastic processes are simulated. These simulations need a good and accurate description of the elastic and inelastic cross sections. The standard technique is to use the partial-wave expansion with appropriate potentials to calculate the elastic cross sections [see papers by Williamson and co-workers].

Objective

To develop an optical potential method that can be used to study positron (or electron) scattering from bound atoms.

Methodology

It aim was to develop a useful and rigorous optical potential to calculate the elastic cross sections for positron (or electron) scattering from bound atoms. Thus, the project was initiated with a comprehensive study of various polarization models that are currently used in positron-atom and electron-atom scattering problems.

The studies have suggested that the short-range correlation effects must be included and we have attempted studies on a number of scattering problems to illustrate our progress in this endeavor.

As for testing of models, the electron-atom scattering systems were used as there are many experimental measurements and theoretical studies available for these systems.

Results

The objective of the project has been achieved with the development of an optical potential method (OPM) for positron (or electron) scattering from bound atoms. The use of this optical potential model has been tested for electron scattering from sodium atom Na (unbound) where there are experimental measurements and rigorous theoretical models like the close-coupling method for gauging the strength or weaknesses of the OPM. The results suggest that the polarization model used in the OPM describes the elastic scattering of electrons from Na quite well. This provides indirect support that the OPM can be reliably used for scattering from bound atoms. Nevertheless, the ultimate aim is to explicitly incorporate the short-range correlations in the OPM.

This work has to be followed up as I believe there are some significant results that can be gauged using the simple OPM as compared to other sophisticated theoretical methods that are available for electron or positron scattering from atoms.

Positron scattering from Bound atoms

The work on positron scattering from bound atoms such as silicon and germanium has been studied using the OPM. These are described in the papers by Natchimuthu and Ratnavelu (2001). However, there is a lack of experimental measurements for positron or electron scattering from bound atoms. That was the reason we have tested our models in the free atom case (as in Na) and obtained very useful and significant results that provide indirect support of the OPM proposed here.

Electron Scattering from Bound atoms

The work on electron scattering from bound atoms such as Si, Ge and In were published {See Appendix on List of Publications}. Further work is being attempted to show the potential of the OPM in the corresponding electron scattering from free atoms. I am also looking forward to pursue the DFT approach to allow for the short-range effects.

Major Milestones Achieved

1. The P2 polarization model in the OPM has shown excellent promise in its ability to incorporate the long-range polarization quite accurately. This has been tested in the corresponding electron scattering from free Na atom and shown good agreement with the available experimental measurements. This unexpected spin off from our work on bound atoms has to be published and work is under progress.

2. The development of a general scattering code for the OPM scattering model has been well tested and will be used for further work in this field.

The success of the project has so far achieved a publication in the Jurnal Fizik Malaysia (1999), two conference Proceedings presented at the International Meeting on Frontiers of Physics [Kuala Lumpur, October 1998] and at the Malaysian Science and Technology Congress {Symposium A} in October 1999.

Future Plans

The funding of this project by MTSF has further helped in nurturing the research interest of the Principal Investigator in a different direction. We intend to follow on through the use of Density Functional Theory (DFT) to develop the correlation potential of the Lee-Yang-Parr (LYP) model or other variants for scattering problems.

Publications

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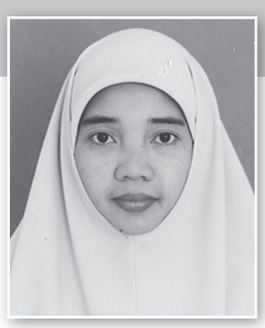
2. K. Ratnavelu, N. Natchimuthu and K.K. Rajagopal, "Optical Potential Study of Positron Scattering by Hydrogenic-Type Atoms", Proceedings of the Malaysian Science and Technology Congress'99 Symposium A (25-27 October 1999 Kuala Lumpur).
3. M. Z. M. Kamali and K. Ratnavelu, "Elastic Scattering of electrons by bound indium atom", Malaysian Science and Technology Congress'99 Symposium A (25-27 October 1999 Kuala Lumpur).

Miss Kamisah Mohamad Mahbor
Miss Salina Sharifuddin
Miss Kalsom Mohd Ghazalli



ADVANCED MATERIALS RESEARCH CENTER,
SIRIM BERHAD

"Development of Polymer Electrolyte"
Year 1996 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Polymer electrolytes are expected to be good candidates of solid electrolyte because of their elastomeric and adhesive properties, which can lead to a very good electrochemical interfaces (Rezrazi et al. 1994). Besides, it shows good mechanical properties, easy in fabrication into film, broad window stability and good electrode-electrolyte contact (Gray 1991 and Scrosati 1993).

In this research work, the influence of PMMA and salt concentration on its conductivity behaviour will be discussed.

Methodology

The gel electrolyte was prepared by mixing the poly (methyl methacrylate) PMMA (mw of 1.2 x 10^5) with the $LiClO_4$ salt dissolved by Propylene carbonate (PC). The temperature was slowly increased up to about 100 ^oC forming a highly transparent gel. The ionic conductivity was measured by sandwiching the gel between stainless steel blocking electrodes by AC impedance method. Infra Red spectroscopy analysis was carried out using FTIR (Nicolet-Model Magna 560) using attenuated total reflectance (ATR) solution cell accessory with a ZnSe internal reflection element.

Results

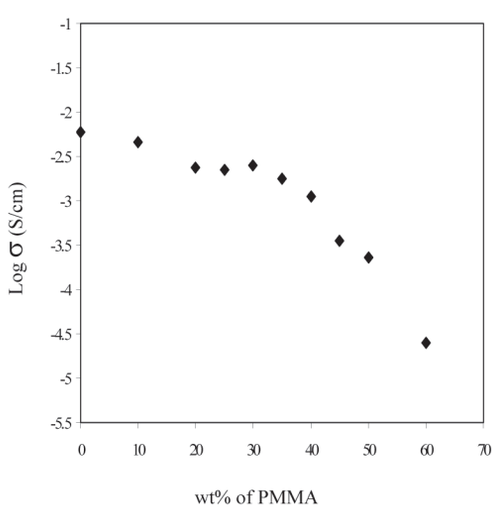


Figure 1. Room temperature conductivity of gel electrolyte with various wt% of PMMA.

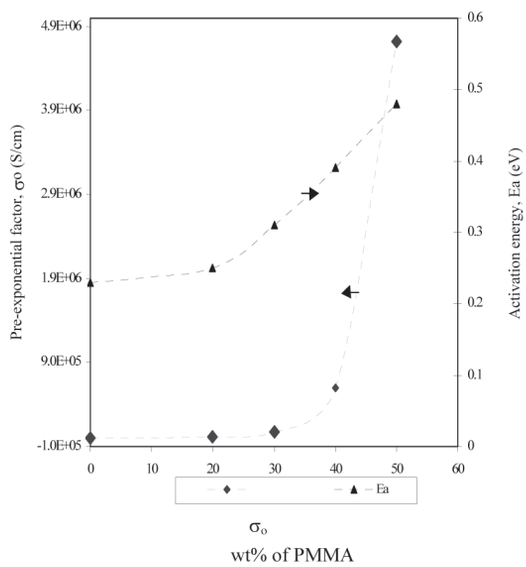


Figure 2. Variation of pre-exponential factor and activation energy of conduction as a function of PMMA

Figure 1 illustrates a drastic decrease in conductivity upon addition of PMMA more than 35 wt%. PMMA concentration above 35 wt% imparts a high viscosity, making the mixture into a rubbery transparent solid, but up to 30 wt% PMMA the gels are highly viscous liquid. Further analysis of the conductivity behaviour as a function of temperature using Arrhenius relationship

$$\sigma T = \sigma_o \exp (E_a/kT) \qquad \text{.....(1)}$$

leads to the determination of pre-exponential factor, σ_o which is related to the number of charge carrier and the activation energy of conduction, E_a. Figure 2 indicates some changes in the conduction of the gels around 30-40 wt% of PMMA that may postulate two conduction path in the system. A very small variation of σ_o and E_a at low PMMA concentration suggests that the number of charge carriers is almost constant, resulting a small variation of conductivity.

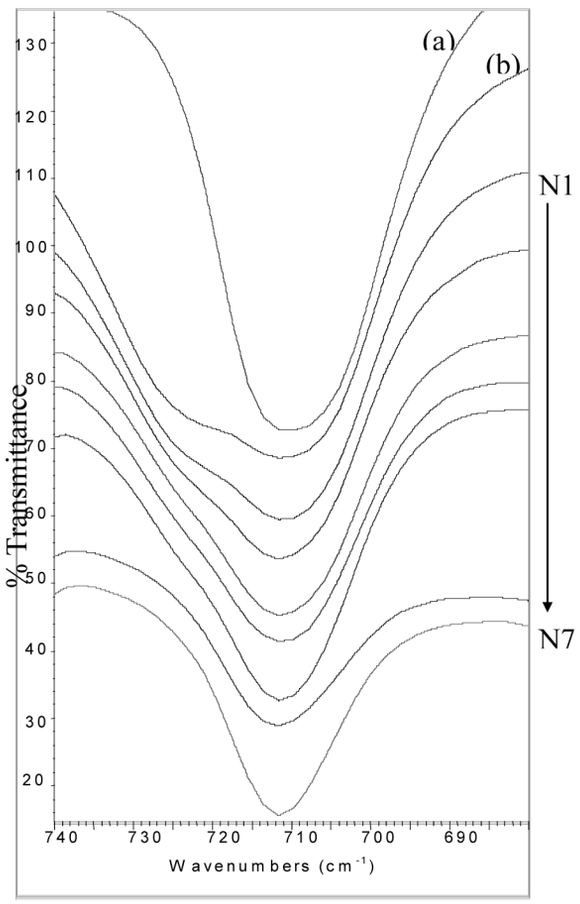


Figure 3. Infrared spectrum in the symmetric ring deformation region of PC in PMMA-PC-$LiClO_4$ system with different PMMA concentration [(a) PC, (b) PC-$LiClO_4$ and N1-N7 are the electrolytes with different PMMA concentration.

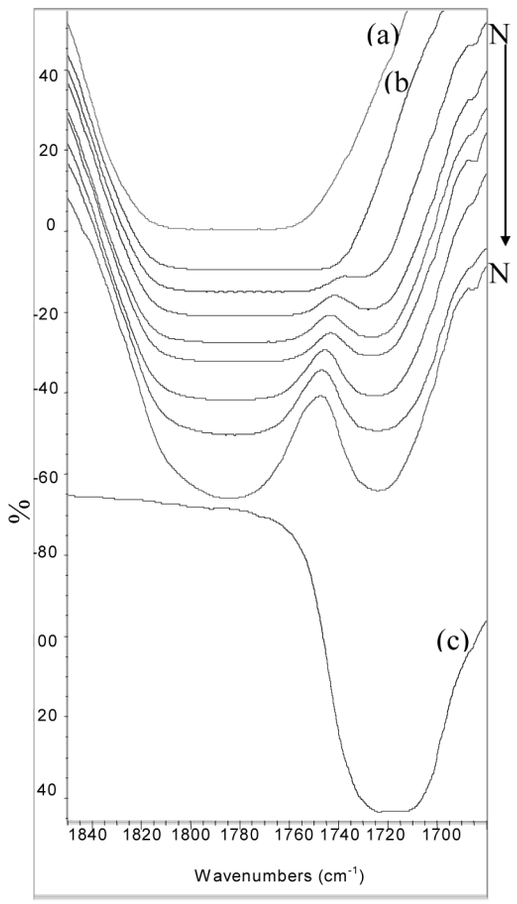


Figure 4. IR spectrum in the C=O stretching of PC in PMMA-PC-$LiClO_4$ system with different PMMA concentration [(a) PC, (b) PC-$LiClO_4$ (c) PMMA and N1-N7 are the electrolytes with different PMMA concentration.

Although the σ_o increases, PMMA and the salt at high PMMA concentration may possibly have some interaction reducing ion pairing that decreases the number of charge carriers, resulting a rapid drop in conductivity. Such interaction is illustrated by the variation of IR spectrum as shown in Figure 3. The appearances of the weak shoulder upon addition of $LiClO_4$ at 710 cm^{-1} of PC mode gives a strong indication of the interaction between Li^+ ion and PC. By PMMA addition, the shoulder becomes weak and slowly disappears when further increase the concentration. It proofs that some interaction has occurred between the

PMMA, which weaken the Li^+-PC interaction. Figure 4 indicates the interaction, as shown by significant splitting observed at high PMMA concentration, to give an additional component at about 1725 cm^{-1} which is referred to the C=O stretching mode of PMMA. The interaction may be due to the solvation of Li^+ ion through the C=O of PMMA.

Figure 5 shows the conductivity behaviour as a function of salt concentration for the gel with 30 wt% PMMA. It shows that the conductivity increases as salt concentration increases before it starts to decrease at high salt concentration. Analysis of the conductivity behaviour as a function of temperature using Arrhenius relationship (Figure 6) illustrates that drastic initial increase is mainly due to the addition of charge carriers. As salt concentration increases, mutual interaction between the ions becomes stronger to promote the cation-anion pairs, which is in equilibrium with the free ions,

$$Li^+ + ClO_4^- \Leftrightarrow [LiClO_4]^o$$

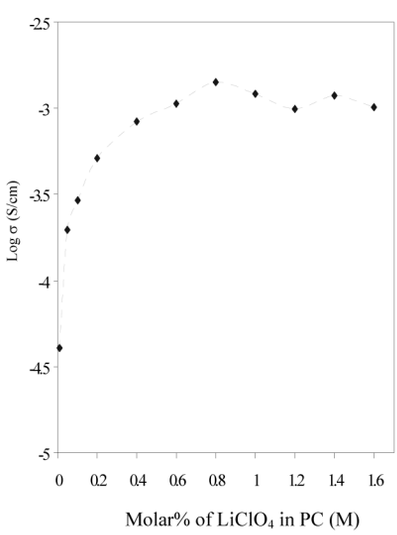


Figure 5. Room temperature conductivity of gel electrolytes with various salt concentrations.

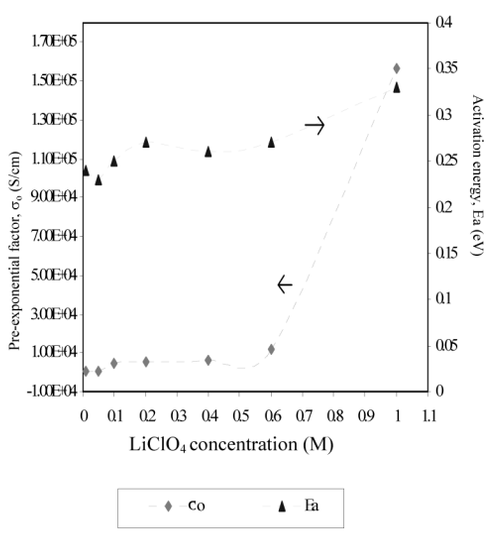


Figure 6. Pre-exponential factor and activation energy of conduction of gel electrolytes as a function of salt concentration.

The interaction between PMMA and salt may occur by solvation of Li^+ ions through the negative charge of PMMA molecule, located at the carbonyl group C=O. Significant broadening of the carbonyl PMMA stretching mode at 1720 cm^{-1} (as shown in Figure 7) at high salt concentration indicates such interaction. Thus the anion becomes very difficult to approach Li^+ ion resulting in drastic increase in the number of charge carriers as shown in Figure 6.

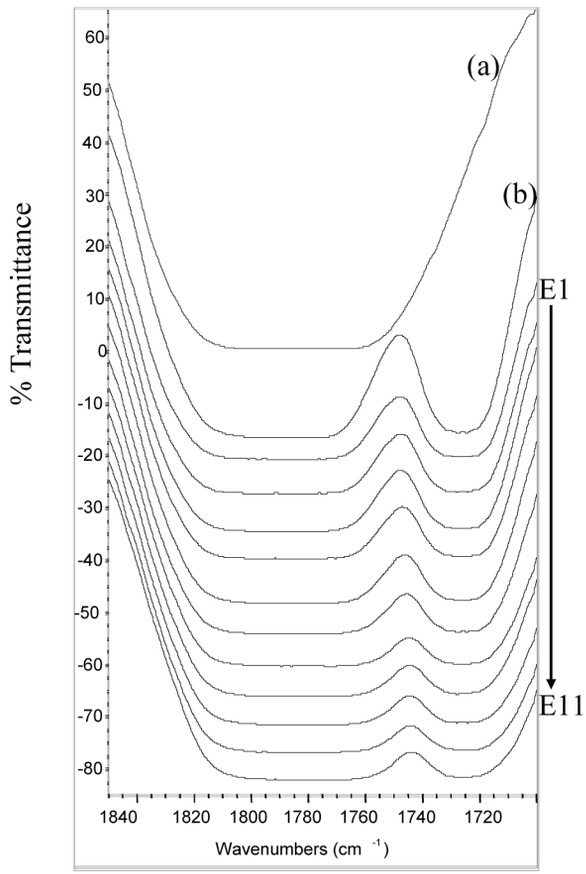


Figure 7: Infrared spectrum in the C=O stretching of PC in PMMA-PC-$LiClO_4$ system with different $LiClO_4$ concentration [(a) PC, (b) PC-PMMA and E1-E11 are the electrolytes with different $LiClO_4$ concentration, 0.01 M-1.6 M]

Unsolved problems and future directions

Further work and analysis need to be done to understand the mechanism of conduction because of its behaviour as a liquid electrolyte at low PMMA concentration and as a solid electrolyte at high concentration. Electrochemical analyses need to be done before recognizing this electrolyte for electrochemical devices can be pursued.

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"A Study on the New Langmuir-Blodgett (LB) Thin Films"
Year 1996 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Langmuir-Blodgett technique has well been established as one of the method to fabricate ultra-thin films on appropriate substrate. One of the main attractions of the LB technique is the ability to deposit organic layers with an ultrafine control of film thickness (multilayer structure) and to incorporate the "functionality" of material. The latter can be achieved by enlisting the assistance of synthetic organic chemist to produce organic materials with tailored properties which have been used in several applications.

Results

The study which has been carried out within the above-mentioned period can be summarized as follows:

(i) Monolayer & Deposition Studies of Mixed/Stearic Acid LB Films
(ii) Structural Characterization of Synthesized Organic Materials For Thin LB Films
(iii) Scanning Electron Microscopy of Aromatic Amines

The hitherto research findings or results have also been presented in the conferences or submitted to the international journals.

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"Construction of Recombinant M. Bovis BCG containing the Erythrocyte Binding Antigen (EBA) of P. *faciparum* – Potential use as a Malarial Vaccine"
Year 1996 MTSF Science & Technology Research Grant Recipient

Introduction

Malaria, caused by the protozoan parasite *Plasmodium*, is one of the major diseases afflicting mankind threatening about 40% of the world's population and killing over one million people annually (1). The advent of drug-resistant parasite strains and insecticide-resistant mosquito strains in many parts of the world including Malaysia has increased the intensity of the search for an effective vaccine against the disease. Of the four malarial species that infect humans, the most important are *Plasmodium falciparum* and *P. vivax* and of these, *P. falciparum* is responsible for much of the acute and often fatal malaria. Not suprisingly, research on vaccine development has focused on *P. falciparum*. Thus far vaccines against malaria were either disappointing in providing adequate protection due to the lack of T cell responses, or like Spf66, requires several boosters before an appreciable level of protection could be observed.

Candidate Blood-Stage Epitopes of P. *falciparum*

The life cycle of *P. falciparum* is very complex. However, one of the stages when the parasites are exposed to factors of the host's immune system and could potentially be destroyed or neutralized is at the asexual blood stage (merozoites). This is the stage of the parasite life cycle that causes the reported mortality and mobidity of malaria.

Many molecules on the merozoite surface are candidates for vaccine construction. One such molecule is the C-terminus of the merozoite surface protein-1 (MSP-1C). This region of the MSP-1 has been shown to be the target of inhibitory antibodies in *P. falciparum* and *P. yoelii* (2)

The other candidate epitope is the erythrocyte binding antigen (EBA). Evidence from a number of studies indicates that this 175kDa protein is a ligand for erythrocyte invasion (eg.3) and that antibodies against a fragment known as the region II on the EBA (RII-EBA), block merozoite invasion of erythrocyte *in vitro* (4).

M. bovis bacille CALMETTE-GUERIN (BCG) – A PROMISING VEHICLE FOR VACCINE DELIVERY

Previous work on malarial vaccine development have been problematic due to the low protection rate and the need for boosters to maintain adequate protection levels. Our research plan has been to deliver putative malarial epitope(s) via *Mycobacterium bovis* BCG, the live vaccine currently used against tuberculosis.

The use of recombinant BCG (rBCG) provides a very promising prospect for malaria vaccine production since BCG as a vaccine vehicle offers several advantages. Firstly, BCG itself, is a stimulator of cellular immune responses, one of the two major arms of the immune system which has been shown to be important in protection against the asexual

blood stages of the parasite (5,6) or could enhance the antibody response to these forms of the parasite. Secondly, BCG is a remarkably safe vaccine, which has been approved for administration at birth for decades. Thirdly, BCG has an immunoadjuvant effect and the use of a live vaccine could provide long term protection hence reducing the need for frequent boosters.

Finally, since BCG is still the most effective vaccine against tuberculosis, the use of rBCG may help in the protection against one other disease affecting the same endemic area for tuberculosis.

Specific Objectives and Milestones

The aim of this project is to construct and assess the expression of recombinant BCG clones expressing the region II of erythrocyte binding antigen (RII-EBA) of *P. falciparum* as a preliminary objective towards the development of a vaccine for malaria.

To achieve this objective, the following initial milestones were planned:

1 Construction of integrative and shuttle recombinant plasmids containing RII-EBA.

2 Assay the expression of recombinant BCG clones.

Results

1. Integrative vector used

The integrative vector obtained from Prof. Jeremy Dale, University of Surrey, United Kingdom, designated pUS937 which contains the *M. leprae* 18kDa gene driven by a mycobacterial hsp60 promoter, and the kanamycin and ampicillin resistance markers was used for cloning.

2. Problems of cloning - codon bias

Preliminary results obtained demonstrated that cloning of malarial epitopes using PCR products generated from genomic *P. falciparum* DNA resulted in low and unstable expression of the recombinant protein. This may be due to the different codon usage between mycobacteria and plasmodia. Mycobacteria is G:C rich (~70%) whereas plasmodia is A:T rich (~70%). This hypothesis was tested as discussed below.

3. Strategy for overcoming codon bias

A technique known as assembly PCR was used to overcome the codon bias as explained above. As a preliminary strategy to assess the success of this approach, the shorter MSP-1C malarial epitope, was cloned into *M. smegmatis* . This was carried out by generating the whole 300bp DNA fragment using a series of oligonucleotides designed in favour of mycobacterium codon usage. Recombinant clones containing this synthetic DNA seemed to increase the expression level of the recombinant MSP-1C by at least 100-fold as compared to those containing the 'native' epitope. The transformation efficiency was also increased by 10 fold when the former clones were used. Furthermore, we were now able to clone this synthetic version of MSP-1C into BCG. These findings were recently published in an International journal.

4. Cloning of the RII-EBA

Using the same approach above, cloning of the RII-EBA was attempted. We also took advantage of the versatility of the assembly PCR technique to include many other DNA fragments necessary to drive and improve the expression of RII-EBA (for example incorporating a strong promoter, signal peptide, 6-histidine tag into the DNA cassette). After several attempts and sequencing runs, we have successfully constructed a synthetic DNA fragment containing the RII-EBA.

5. Planned future work

The construct containing the synthetic RII-EBA will be used to generate the recombinant protein for raising polyclonal antibodies to be tested against the native RII-EBA protein. In addition, the DNA fragment containing the RII-EBA sequence of *P. falciparum* will be transfomed into BCG for subsequent experiments to determine the immunogenicity and efficacy of the putative vaccine.

Benefits

1. Although initially we did not anticipate problems with expression of the RII-EBA epitope in BCG, this caveat has provided us with the opportunity to develop a new technique, assembly PCR, which was utilised for the cloning of two different malarial epitopes, one of which is already cloned into BCG.
2. Furthermore, this technique is now being employed for other cloning work carried out by researchers in this Institution. Researchers from two other Institutions (one from Brazil and the other from Japan) have also approached us regarding this technique.

Publication

Presented at the 10th. International Congress of Immunology, New Delhi, 1-6 November, 1998.

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"Studies on the medicinal properties of Eurycoma Longifolia Jack (Tongkat Ali)"

Year 1996 MTSF Science & Technology Research Grant Recipient

The research was undertaken because in Malaysia, E. longifolia Jack commonly known as Tongkat Ali has already gained notoriety as a male aphrodisiac since it is reputed to increase male virility (Gimlette & Thomson, 1977) when taken as a decoction of the roots in water. However, this claim is largely based on subjective opinion rather than scientific data. Hence, the objective of this study was to evaluate scientifically, both in vivo and in vitro using animal models.

Materials and Methods 1

E. longifolia Jack was investigated for aphrodisiac property on sexually naive male mice using both the modified runway-choice and open field methods after treating them with 500 mg/kg for 10 days.

Outcome : Results showed that oral administration of E. longifolia Jack resulted in a transient increase in the percentage of male mice responding to the right choice after chronic consumption of 500 mg/kg of E. longifolia Jack, more than 50% and 75% of the male mice after given E. longifolia Jack and yohimbine respectively scored right choice after 5 days post-treatment, and more than 65% and 85% of the male mice which consumed E. longifolia Jack and yohimbine respectively scored right choice after 8 days post-treatment (Ang et al 1997).

Materials and Methods 2

Further effects of E. longifolia Jack were studied on the copulatory behaviour of male rats after treating them with the above dosing regiments and were observed for their copulatory behaviour with a receptive female in a copulation cage.

Outcome : Results showed that the mean values of EL (ejaculation latency)-1, EL-2 and EL-3 of the control male rats were 192.80 sec, 167.60 sec and 162.50 sec but were significantly increased ($p < 0.05$) to 249.00-292.60 sec, 242.60-364.40 sec and 210.50-262.00 sec respectively in the chloroform-methanol, methanol-butanol-water and butanol-methanol treated male rats. In addition, it showed that PEI (penile erection index)-1 and PEI-2 of the control group were 139.60 sec and 215.00 sec respectively but were significantly decreased ($p < 0.05$) to 56.20-73.80 sec and 20.00-121.67 sec respectively in the chloroform-methanol-water-butanol and butanol-methanol treated male rats (Ang & Sim 1997).

Materials and Methods 3

Male aphrodisiac property was further investigated on the penile erection index and homosexual mounting in the absence of female rats after treating the male rats with 200, 400 and 800 mg/kg twice daily for 10 days leading up to the test.

Outcome : Results showed that 400 mg/kg of chloroform, methanol, water and n-butanol extracts exhibited respective penile erection indices of 28.32 ± 1.75, 31.32 ± 4.35, 39.25 ± 2.34 and 36.49 ± 3.15 whilst 800 mg/kg produced further

increase to 32.00 ± 1.85, 35.32 ± 2.45, 40.24 ± 1.35 and 45.29 ± 2.42 respectively. However, there were no homosexual mountings in either the treated or control animals during the 1-hour observation period (Ang & Sim 1997a).

Materials and Methods 4

Further work was carried out on evaluating the aphrodisiac activity of E. longifolia Jack by investigating the orientation activities of the sexually experienced male rats towards the receptive females (mounting, licking, anogenital sniffing), environment (exploration, raring, climbing), themself (genital grooming, non-genital grooming) and mobility (unrestricted, restricted) after dosing them with the above dosing regimens.

Outcome : Results showed that E. longifolia Jack modified the orientation activities by displaying more frequent and vigorous mounting, licking and anogenital sniffing towards the receptive females, intensified self orientation as indicated by the increased grooming of the genitals as compared to the controls. However, the treated males possessed a lack of interest in the external environment as indicated by a reduction in exploration, raring and climbing on cage wall (Ang & Sim 1998).

Materials and Methods 5

Further aphrodisiac property of E. longifolia Jack was evaluated by assessing the pendiculation (act of yawning and stretching) activities of male rats after dosing them with the above dosing regimens.

Outcome : Results showed that E. longifolia Jack produced a dose-dependent and also a significant increase in the mean number of yawnings when compared with controls ($p < 0.05$) with 400 mg/kg of chloroform, methanol, water and n-butanol produced 1.20 ± 0.03, 1.21 ± 0.03, 1.23 ± 0.01 and 1.28 ± 0.01 whilst 800 mg/kg further increased to 1.70 ± 0.01, 1.63 ± 0.01, 1.63 ± 0.03 and 1.72 ± 0.03 respectively. Similarly, 400 mg/kg of chloroform, methanol, water and n-butanol exhibited mean number of stretchings of 1.41± 0.31, 1.37 ± 0.21, 1.35 ± 0.31 and 1.39 ± 0.02 whilst 800 mg/kg further enhanced to 1.44 ± 0.02, 1.40 ± 0.19, 1.38 ± 0.14 and 1.40 ± 0.03 respectively during the 1 hour observation period (Ang & Sim 1998a).

Materials and Methods 6

Further aphrodisiac parameters were carried out after treating the male rats with the above dosing regimens for 10 days after which they were observed for MF (mount frequency), IF (intromission frequency) and EF (ejaculation frequency) on the 11th day. The penis for each sexually experienced male rat was exposed by retracting the sheath and 5% xylocaine ointment was applied 20 minutes before starting observation.

Outcome : Results showed that E longifolia Jack produced a dose-dependent increase in mounting frequency of the treated animals with 400 mg/kg of chloroform, methanol, water and n-butanol fractions resulting in mounting frequencies of 5.3 ± 1.2, 4.9 ± 0.7, 4.8± 0.7 and 5.2± 0.2 respectively but there were no erections, intromissions, ejaculations or seminal emissions during the 20-minute observation period (Ang & Sim 1997b).

Materials and Methods 7

E. longifolia Jack was investigated for aphrodisiac property on sexually sluggish old adult rats after treating them with 500 mg/kg daily of various fractions of E. longifolia Jack and were observed for copulatory behavour for 12 consecutive weeks.

Outcome : Results showed that repeated and chronic dosing of E. longifolia Jack elicited mountings in non-copulator male rats, and further, facilitated both intromissions and ejaculations after 4th week observation period with both the intromissions and ejaculations were consistently maintained on a high level during the 9-12th week observation period (Ang & Sim 1998b).

Materials and Methods 8

E. longifolia Jack was evaluated for aphrodisiac property on sexually naive male mice using the electrical copulation cage (0.12mA).

Outcome : Results showed that E. longifolia Jack produced a slow and transient decrease in the hesitation time of the sexually naive male mice as compared to yohimbine and control with E. longifolia Jack produced 700-750, 685-740, 680-740, 670-675, 660-710, 650-700, 540-685, 540-680, 530-640, 500-620 sec whilst yohimbine and control produced 600, 580, 570, 570, 550, 540, 530, 530, 500 sec and 900, 890, 870, 840, 820, 800, 780, 770, 760 and 750 sec respectively throughout the investigation period (Ang & Sim 1998c).

Materials and Methods 9

Further evaluation was carried out on the aphrodisiac property of this plant by using an electric grid (0.12 mA) which was used as an obstruction in the electrical copulation cage in order to determine how much an aversive stimulus the sexually naive male rat for both the treated with E. longifolia Jack and control groups were willing to overcome to reach the estrous receptive femaile in the goal cage.

Outcome : Results showed that repeated and chronic dosing of E. longifolia Jack enabled the treated male rats to crossover the electrical grid to reach the estrous receptive female and subsequently, elicited mountings onto the incentive animals in the goal cage after 4th week observation period. Further results also indicated that E. longifolia Jack continued to enhance and also maintain a high level of both the total number of successful crossovers and mountings during the 9-12th week observation period (Ang & Sim 1998d).

Materials and Method 10

Besides the above structured and efficient protocols in evaluating the aphrodisiac property of E. longifolia Jack, extensive study on the in vitro evaluation was carried out the rabbit corpus cavernosum penis. The corpus cavernosum was excised from the penis of mature rabbits and were dissected free from tunica albuginea. Each corpus cavernosum was dissected into strips and each strip was suspended under 2 gm tension in a 10ml-organ bath containing Krebs-Ringer bicarbonate solution with one end connected via a silk thread to an isometric transducer (Ugo Basille) coupled to a polygraph (Ugo Basille Quartet) and the other to tbe base of a gas inlet steel tubing. The bath was aerated with 95% O_2 and 5% CO_2 and maintained at 37°C. Corpus cavernosum strips were precontracted with 10^{-6} M phenylephrine and later followed by different fractions of E. longifolia Jack and yohimbine.

Outcome : Results showed that chloroform, mathanol, water and n-butanol fractions of E. longifolia Jack dose-dependently, relaxed the phenylephrine-induced contractions in the corpus cavernosum penis by 59-337%, 71-327%, 109-370% and 140-662%, respectively in contrast to yohimbine which induced a relaxation of only 34-251% (Ang & Sim 1997c).

Unresolved problems and future directions

Although the above studies showed that E. longifolia Jack has aphrodisiac qualities in rabbits and rodents, but however, further studies should be conducted to determine if E. longifolia Jack has the similar property in humans.

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"Development of Anti-Tumour Heteroconjugates using Novel Ferrocene/Ferrocenium Compounds"
Year 1996 MTSF Science & Technology Research Grant Recipient

Introduction

To explore the possibility of developing anti-tumor heteroconjugates using novel ferrocene/ferrocenium compounds.

Objectives

1. To synthesize new ferrocenium compounds
2. To determine the cytotoxicity of novel ferrocenium compounds against tumor cells
3. To develop anti-tumor conjugates using ferrocenium compounds

Methodology

1. **Synthesis of novel ferrocenium compounds** : Ferrocenium tin tetrabromide salt prepared by the reaction of ferrocene with $SnBr_4$ in dichloroethane was obtained in the form of crystals. Ferrocenium-bismuth tribromide was prepared in acetone as solvent. Crystals were obtained for X-ray crystallographic analysis.
2. **Anti-tumor activity** : Ferrocenium compounds were tested for *in vitro* cytoxicity against tumor and normal cell lines. The tests were performed according to the method described by Anderson *et al*. (1991).
3. **Preparation of conjugates** : Ferrocenecarboxaldehyde (FCA), ferrocene monocarboxylic acid (FMCA) and bis ferrocenium bis (tetrachloroantimonate) trichloroantimony (Bis-Fc) were conjugated to bovine serum albumin (BSA) or transferrin (Tf). FCA was conjugated to protein according to the method described by Suzawa *et al.* (1985), and FMCA according to the method of Degani and Heller (1987). The amounts of ferrocene in the conjugates were determined by the ferrozine assay described by Badia *et al.* (1992).
4. **Determination of *in vitro* mutagenic activity** : *In vitro* mutagenic activity was performed using 4 *Salmonella typhimurium* strains (TA97, 98, 100 and 102) according to method of Ames *et al*. (1973).
5. **Determination of cytotoxic mechanism** : The TUNEL assay was employed to determine if cell death occurred via apoptosis. Cells were cultured for 48 hr in the presence of LC_{50} concentrations of FMCA or FCA.

Results

1. **Synthesis of novel ferrocenium compounds** : Several novel compounds were synthesized and tested against tumor cells.
2. **Anti-tumor activity** : Twelve ferrocenium compounds were tested against 2 normal cell lines, ie.g MRC-5 and BHK. In general the results indicated that these compounds varied in their toxicities depending on the cells used. The LC_{50} values of the compounds ranged from very toxic to non-toxic.

3. **Preparation of conjugates** : Attempts were made to conjugate novel ionic ferrocenium compounds to protein but were unsuccessful. However, the non-ionic forms i.e. FMCA and FCA, were successfully conjugated to albumin and transferrin and tested against tumor cells.
4. **Determination of in vitro mutagenic activity** : Nine ferrocenium compounds were tested against four *Salmonella typhimurium* strains. These results indicate only Fc-SbF_5, Fc-$TiCI_4$, Fc-$SbCI_5$ and $FcFeCI_4$ possess low mutagenic activities.
5. **Determination of cytotoxic mechanism** : After incubation in FMCa or FCA the percentage of apoptotic cells observed were 28.4% and 4.17% respectively. These compounds thus did not appear to kill their target via apoptosis.

Unresolved problems and future directions

Ferrocenium compounds, in their ionic form, failed to form conjugates to proteins. Thus, conjugates could only be developed using FMCA and FCA. Further studies are required to determine mechanism of death besides via apoptosis.

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"Effects of L-Carnitine on the Metabolisms and Detoxification of Aflatoxin B1 in freshly isolated Hepatocytes"
Year 1997 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Aflatoxin B_1(AFB_1) is a well known potent hepatotoxic and hepatocarcinogenic mycotoxin. It has been frequently found to contaminate many foods such as corn, barley, rice, peanuts, and peanut butter. Once AFB_1 is consumed, it then undergoes several biotransformational pathways that produce the reactive AFB_1-8,9-epoxide or the less toxic hydroxylated metabolites.

AFB_1-epoxide is the ultimate carcinogen that covalently binds to cellular macromolecules, namely protein, DNA, and RNA. The covalent bindings are the means by which AFB_1 exerts its toxic and carcinogenic effects. AFB_1-epoxide may also undergo detoxification by conjugating to reduced glutathione (GSH). This conjugation is the principal detoxification pathway of AFB_1-epoxide in many mammals.

L-carnitine, 3-hydroxy-4-N-trimethylamino butyric acid, is an important biomolecule for the intramitochondrial translocation of long chain fatty acids and many other emerging roles in health and disease. L-carnitine has been found to inhibit the binding of aflatoxin B_1 (AFB_1) to rats liver DNA. This L-carnitine action is important to reduce the risk of AFB_1-induced liver cancer formation.

This research was conducted to elucidate the mode of action(s) of L-carnitine in reducing AFB_1-DNA adduct formation. The specific objectives of the study were to determine the effect of L-carnitine on the entry/uptake and detoxification of AFB_1 in freshly isolate hepatocytes.

Briefly the entry of AFB_1 into the hepatocytes experiment was conducted by incubating different concentrations of L-carnitine and AFB1. After incubation, the cells were isolated, homogenised, centrifuged at different speeds to get different organelle fractions. These fractions were then measured for AFB_1 contents. For the detoxification experiment, the effects of L-carnitine on the activity of glutathione-S transferease (GST) and total glutathione concentrations were carried out.

This study found that L-carnitine has no effect on the entry of AFB1 into the hepatocytes, nuclei or the post-nuclear supernatent. Also, L-carnitine has no significant effect on GST activity. However, L-carnitine was found to increase the amount total glutatione concentration. Glutathione is important in the detoxification of AFB_1 activated metabolite. This result is important since availability of glutathione is essential for the GST-catalysed activity of AFB1-epoxide conjugating to glutathione, and subsequently preventing the epoxide binding to DNA. In conclusion , the suggested

mechanism for the reduction in the AFB_1-DNA adduct formation by carnitine is via the increase in the detoxification enzyme cofactor.

The novel finding of the present study is the ability of carnitine to prevent the decrease in total glutathione content due to AFB_1 in the hepatocytes. This observation is important since the availability of GSH is essential for GST to promote AFB_1-epoxide binding to GSH and therefore spare binding to macromolecules. An increase in total glutathione concentration may be indicative of reduced amounts of activated AFB_1 formation in the presence of carnitine. If carnitine decreased the amount of AFB_1-epoxide, there would be a decreased need of GSH for detoxification of the epoxide. It is also possible that carnitine induces the biosynthesis of glutathione. Additionally, carnitine itself may conjugate to the activated AFB_1 and therefore spare the glutathione. The data to support these hypotheses remain to be determined by additional experiments.

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"Synthesis of Magnetic Oxide Nanoparticles in water-in-oil Microemulsions for High Density Magnetic Recording Devices"
Year 1997 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Magnetic nanoparticles were synthesized using a novel method called microemulsion processing. In this process, the aqueous cores (typically 5-25nm in diameter) of water/cetyltrimethylammoniun bromide (CTAB)/n-butanol/octane microemulsions were used as constrained microreactors for the precipitation of magnetic particles directly at room temperature. The typical size of the material was in the range of 3-10 nm. The particle size and size distribution were determined by TEM. The particles were then collected and washed. Phase analysis using X-ray diffraction (XRD) showed broad diffraction peaks corresponding to either magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3). The crystallite size estimated from the XRD is roughly 3.66 nm and is similar to the physical size of the particles probed by TEM indicating that they were largely monocrystals. Magnetization curve showed an absence of hysterisis indicating that the particles were superparamagnetic.

Introduction

Synthesis of particles with nanometer size dimensions is of increasing scientific and technical interest. Materials with single phase nanoparticles in the size range of 10 to 100 A exhibit novel electronic, optical, magnetic, and chemical properties due to their extremely small dimension (Andres *et al*. 1989). It is however difficult to obtain ultrafine and monodispersed nanoparticles by classical methods. In this respect, the aqueous cores of water-in-oil microemulsions have been shown to be ideal reaction media for this purpose (Boutonnet *et al*. 1982).

A microemulsion is defined as a thermodynamically stable isotropic dispersion of two immiscible liquids consisting of microdomains of one or both liquids stabilized by an interfacial film of surface active molecules (Leung *et al*.1981). In water-in-oil microemulsions, the aqueous phase is dispersed as nano-size droplets (typically 5-25 nm in size) surrounded by a monolayer of surfactant and co-surfactant molecules in the continuous hydrocarbon phase. If a water-soluble metal salt is incorporated in the aqueous phase of the microemulsions, it will reside within the aqueous droplets surrounded by oil (continuous phase). These aqueous droplets continuously collide, coalesce and de-coalesce, resulting in a continuous exchange of solute content (Eicke *et al.* 1976 and Fletcher *et al.* 1987). Conceptually, if two reactants A and B are controlled by the rate of coalescence of droplets and inter-droplet exchange (Fletcher *et al*. 1987 and Fendler 1987).

Microemulsions have been used as microreactors to produce ultrafine particles since Boutonnet *et al*. (1982) first obtained ultrafine monodispersed metal particles of Pt, Pd, Rh and Ir by reducing corresponding salts in the aqueous droplets of water-in-oil microemulsions with hydrazine or hydrogen gas. Waer-in-oil microemulsions have also been used to synthesize nanoparticles of metal bordies (Ayyub *et al*. 1990), silver halides (Leung *et al*. 1981), barium carbonate,

and oxalate precursors for $YBa_2Cu_3O_{7-x}$ superconductors. In this paper, we report the synthesis of nanoparticles of magnetite (Fe_3O_4) at room temperature in water-in-oil microemulsions.

Experimental Procedure

All materials used – $FeC1_2$, $4H_2O$, NaOH, CTAB and octane – were certified reagent grade while n-butanol was HPLC grade. The water used was pretreated using Elgastat UHQ system until its resistivity reached 18 MΩcm.

We selected a microemulsion system with cetyltrimethyl ammonium bromide (CTAB) as the surfactant; n-butanol as the co-surfactant; n-octane as the continuous oil phase; and a salt solution as the dispersed aqueous phase. The system solubilizes a relatively large volume of aqueous phase in well defined nano-size droplets of stable, single phase water-in-oil microemulsions (Ayyub *et al*. 1990).

Microemulsions were prepared by solubilizing different salt solutions into CTAB/n-butanol/n-octane system. We took two microemulsions (ME 1 and ME 2) with identical compositions but different aqueous phase. The aqueous phase in ME 1 was ferrous chloride while the aqueous phase in ME 2 was sodium hydroxide. In this case, we used excess amount of NaOH to ensure a complete reaction and to maintain the high pH reaction. These two microemulsions were then mixed under constant stirring. Due to frequent collisions of the aqueous cores of the microemulsions, the reacting species in ME 1 and 2 came in contact with each other. The exchange of content caused a chemical reaction and led to the precipitation of ferrous hydroxide. Under an appropriate condition, ferrous hydroxide transformed into the desired magnetite phase.

Since the two microemulsions were of identical compositions, differing only in the nature of the aqueous phase, the microemulsions did not get destablized upon mixing. The aqueous droplets acted as constrained nano-sized reactors for the precipitation reaction, as the surfactant monolayer provided a barrier restricting the growth of the product particles. The surfactant monolayer also hindered coagulation of particles.

The product particles were separated using a high-speed centrifuge. The precipitate was then washed in a 1:1 mixture of methanol and chloroform, followed by pure methanol to remove any oil and surfactant from the particles. The precipitate was then dried at 100°C. The temperature was maintained low to prevent sintering that would cause grain growth.

Transmission electron microscopy (TEM) was used to study the size and size distribution of the product particles. A few drops of the particles were ultrasonically dispersed in methanol. A drop of the suspension was then deposited on a carbon coated TEM copper grid and visualized using 100kV accelerating voltage. A Philips CM12 transmission electron microscope was used for the studies.

Phase analysis on the powder was performed using X-ray diffraction on a Philips MPD Powder Diffractometer at room temperature using Cu Kα radiation.

Magnetization measurement was performed using an Alternating Gradient Magnetometer (AGM) (Princeton Measurement Corporation). A small sample of the dried material was subjected to an alternating field gradient that exerted an alternating force, which was proportional to the gradient field and the magnetic moment of the sample.

The product particles were then sintered at temperatures of 800, 900, 1000, 1100, 1200 and 1300°C to induce particle growth and coarseness. The magnetic properties of the particles of the particles were then measured again.

Results and Discussion

The XRD pattern of the powder shows broad but well define peaks. They correspond to the peaks of either magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3). An indication of the crystallite size can be performed by measuring the full-width-at-half-maximum (FWHM) of the XRD peaks. The average cystallite size estimated using Scherrer's equation is 3.66nm. Since larger particles scatter the x-ray more strongly, this simple analysis often overestimates the 'average' size.

A comparison is made to show the extent of peak broadening of the product particles. We extract γ-Fe_2O_3 particles (roughly 2μm in diameter) from TDK D60 audiotape and subject them to XRD analysis under the same condition. It can be seen clearly that the position of the peaks is similar but the peak for nanomaterials sample in a lot broader.

Conclusion

In this paper we have presented a new process for the synthesis of ultrafine nanoparticles of magnetite or maghemite. Aqueous droplets of water-in-oil microemulsions have been used as nano-sized reactors to precipitate magnetic oxide nanoparticles at room temperature using ferrous chloride and sodium hydroxide as reactants.

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"Integration of Ultrastructural and Molecular approach in Delineating selected Marine Fungal Phylogenies"
Year 1997 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Taxonomy employs a hierarchical system of classification and molecular biology provides powerful tools for systematic. Classification of living as well as extinct organism is vital for biologists to communicate to each other about these organisms. Importantly and ideally, the classification should be based on the evolutionary history of life, meaningful and not arbitrary. Classification is part of the phylogenetic systematic study, while systematic is an attempt to understand the evolutionary interrelationships of living things, trying to interpret the way in which life has diversified as well as it changes over time.

While classification is primarily the creation of names for groups, systematic goes beyond this to elucidate new theories of the mechanisms of evolution. To date systematic (the study of organismal diversity) always been associated with naming and classifying organisms. However, more recently researchers are focusing more on the origin of that diversify. Therefore, the phylogeny studies becoming more as a critical part of our understanding of the history of a group of organisms. This knowledge forms the core of the science of modern systematic and can be called "Phylogeny" or "Organismal Diversity" rather than "Classification".

The phylogeny study, the evolution of group of organisms through time, which is based traditionally on comparative morphology, ultrastructure and molecular study, has been carried out from June 1998 to December 2001. Few subproject had been identified and undertook under this grant :

1. Studies on marine fungi distribution of selected mangrove trees in Morib mangrove stand
2. Vertical distribution of marine fungi on *Rhizophora apiculata* Blume at Morib mangrove stand, Selangor
3. Occurrence of marine fungi (arenicolous and lignicolous) along East coast of Peninsular Malaysia
4. Ecological studies on marine fungi at Remis, Kuala Selangor
5. Studies on manglicolous and lignicolous marine fungi at Pantai Port Dickson, Negeri Sembilan Darul Khusus
6. Biodiversity of mycoflora on the *Nypa* palm of Malaysia
7. Ultrastrucutre studies of selected species in the Halosphaeriales
8. PCR analysis of Lignicola laevis using the ITS regions

Materials and Methods

i) Collection of natural substrata for diversity study of marine fungi

Various collections have been carried out at selected field sites along the coastal area of the Peninsular Malaysia to collect natural substrata. The natural substrata include arenicolous, manglicolous and lignicolous materials. Asc.

Prof. Kevin Hyde, Director of Fungal Diversity Group, Department of Biodiversity, University of Hong Kong was invited to work on Biodiversity of mycoflora on the *Nypa* palm of Malaysia from 14-21 July 1997.

ii) Ultrastructure studies of selected member in the Halosphaeriaceae family

Selected Halosphaeriales species have been ultrastructurally examined using scanning electron microscopy studies.

iii) PCR analysis of selected Lignincola laevis using the ITS region

Results and Discussion

i. Biodiversity of marine fungi natural substrata

A total of 384 species were identified on 1180 collected samples. The total number of species and their composition in each sampling method was tested by the Jaccard and Sorenson indices to estimate fungal diversity. Common fungi were *Lignincola laevis, Periconia prolifica, Verruculina enalia, Lulworthia sp., L. grandispora* and *Trematosphaeria malaysiana* on twigs with bark, with *Lignincola laevis, Periconia prolifica and Verruculina enalia* common on debarked twigs. The similarity index showed that differenct species composition were observed on debarked and barked materials at all stages of colonization, and especially at the intermediate stage.

Jones (2000) reviewed some of the factors influencing fungal diversity in the marine environment : availability of substrata for colonization, geographical distribution and temperature, salinity, inhibition competition, tidal amplitude, oxygen availability, abundance of propagules in the water and substrate specificity. While some of these have been examined under laboratory conditions e.g. effect of salinity (Jennings, 1983; Clipson and Hooley, 1995), the alkalinity of sea water (Davies, Brownlee and Jennings, 19901b), temperature (Panebianco, 1990), a few of these effects have been studies in the field.

The last decade has seen extensive studies on fungal colonization of mangrove wood (Hyde, 1991; Leong, Tan and Jones, 1991; Prasannarai and Sridhar, 1997; Alias and Jones, 2000), with over 340 fungi recorded (Ascomycetes 151, Mitosporic species 37, Basidiomycetes --) from 55 mangrove tree species (Alias, 1996). However, little evidence of host specificity has been reported (Hyde and Jones, 1988; Hyde, 1990; Hyde and Lee, 1995; Alias and Jones, 1996 2000), e.g. *Leptosphaeria avicenniae* and *Tremarosphaeria mangrovis* only from *Aviennia* and *Rhizophora* species respectively. Hyde (1990) investigated the fungal distribution on host species and compared the mycota of five intertidal mangrove trees. *Caryospora mangrovei* was restricted to *Xylocarpus granatum*, and *Aigialus mangrovei* to *Avicennia* wood. However, the latter species has also been reported on other mangrove trees (Borse 1988; Volkmann-Kohlmeyer and Kohlmeyer, 1993). From the current studies, *Nypa fruticans*, a mangrove palm species, does support a distinctive mycota and this may be accounted for by the nature of the fronds and rachis of the plant and the salinity range under which the palm grows, usually low salinity (Hyde and Nakagiri, 1989. Hyde, 1993; Hyde and Alias, 2000).

ii. PCR analysis of selected Lignincola laevis using the ITS region

DNA extraction and amplification of internal transcribed spacer region of marine fungi *Lignincola laevis* has been done. The extraction of genomic DNA was done by using DNAzol reagent. The results showed that the DNA yield ranging from 3.75 – 6.00 µg/mg mycelia, while purity analysis by UV absorbance spectrophotometry showed that the DNA ranging from 1.5 – 2.0. Amplification of L. laevis using ITS1 and ITS4 primers gave PCR product with single band at 650 bp after separation by agarose gel electrophoresis and detection by staining with ethidium bromide.

Future Work

1. Since fungi have not been among the most important contributors to our knowledge of DNA and how it works (Hendrick, 1992), further intensive work need to be done to understand its function and its replication as well as the concept of "gene cloning".

2. The techniques of molecular biology have not only given us a great deal of detailed information about the genetic material, but also permit the movement of genetic material movement from one species to another. The origin of marine mycota is still at a debating phase, either they are originally from the terrestrial counterpart or the ocean itself.

3. Even though from the past and this study have yielded extensive data of diversity of marine fungi in Malaysia, further collection work need to be done at Sabah and Sarawak coastal area. However, to obtain permission to work and to collect samples from these areas is the main problem currently.

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"Marine chemical Ecology of associated Fauna and Effects of Environmental Stressors on the Associations"
Year 1997 MTSF Science & Technology Research Grant Recipient

Experiments/Training :

1. Anti-feeding and ichtyotoxicity tests were carried out in the field (P. Payer) and in the lab (USM).
2. A series of UV-irradiation experiments on the same genus of *Clavelina* tunicates were performed at Alfred-Wegener Institute for Marine and Polar Research (Germany) with main funding from a German foundation i.e. Carl Duisberg Gesellschaft (CDG).
3. Chemical Analytical Methods (HPLC, etc) training at Max Planck Institute (Jena, Germany) with main funding from CDG also.

Total Experiments Completed:

EXECUTED EXPERIMENTS	CONTROL TUNICATES	THERMAL-STRESSED TUNICATES	UV-STRESSED TUNICATES
* RESPIRATION	YES	YES	YES
* MORTALITY	-	-	YES
* DISSOCIATION & PREFERENCE	YES	YES	-
* ANTI-FOULING	YES	YES	-
* ANTI-PREDATION	YES	YES	-
* ICHTYOTOXICITY	YES	YES	-
* MISCELLANEOUS E.G. Taxonomy	YES	YES	YES

Conclusion

The study has yielded exciting results on this particular tunicate genus, *Clavelina*. The new additional study on the effects of UV irradiation on the colonial tunicates is also a first (scarce studies done only on single tunicates).

Publications

1. Effects of Thermal Stress on Defence Capability of the Tunicate *Clavelina fecunda*. (orgal presentation). 9th International Coral Reef Symposium, co-organised by International Society for Reef Studies, AUS-AID, AIMS, CRC, US-NOAA, US-AID, US-NSF, ICRI, CORDIO, IUCN, UNEP, IOC, etc. Bali, 23-27 October 2000. Partial travel grant from The David and Lucille Packard Foundation (US).

2. Thermal-Induced Dissociations Prior to Coral Bleachings : An Early Warning System ? (poster presentation). 9th International Coral Reef Symposium, co-organised by International Society for Reef Studies, AUS-AID, AIMS, CRC, US-NOAA, US-AID, US-NSF, ICRI, CORDIO, IUCN, UNEP, IOC, etc. Bali, 23-27 October 2000. Partial travel grant from The David and Lucille Packard Foundation (US).
3. Effects of Thermal Stress on the Tunicate *Clavelina fecunda* (Ascidiacea) of Pulau Payar Marine Park (Kedah, Malaysia). (oral presentation). National Symposium on Pulau Payar Marine Park, organized by Marine Park (Malaysia) and Institute of Fisheries Research, 21-22 Nov. 2000.
4. N. Syed Ibrahim, 2001. Anti-fouling Properties of the Tunicate *Clavelina fecunda*. 2nd Asia Pacific Conference on Chemical Ecology. August 2001.
5. N. Syed Ibrahim 2001. Apoptosis in Tunicates due to Environmental Stress? Straits of Malacca Conference II., October 2001.

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"Development and in vivo evaluation of a Buccal Bioadhesive Drug delivery system"
Year 1997 MTSF Science & Technology Research Grant Recipient

I. An In Vitro Method for Buccal Adhesion Studies

Satisfactory bioadhesion is essential for the successful application of a buccal bioadhesive drug delivery system. It implies the strength of attachment of the dosage form to the biological tissue. Presently, there is still no universal test method for bioadhesion measurement and some results of bioadhesion reported in the literature appeared to be contradictory. Hence, we developed a method to evaluate the adhesive properties of polymers using texture analyzer equipment with chicken pouch as the model tissue under simulated buccal conditions. The instrumental variables such as contact force, contact time and the speed of withdrawal of probe from the tissue, which could affect the bioadhesion were studied using two polymers, namely, polyacrylic acid (CP 974P) which has well characterized bioadhesive properties and hydroxyprophylmethyl cellulose (HPMC K4M), a weaker bioadhesive polymer. This method was then applied to evaluate the bioahesive properties of a range of polymers.

From the results obtained, the bioadhesive measurements of polymers could be influenced by instrumental variables such as contact force, contact time and speed of probe removal from the tissue. Therefore, a test system should be adequately assessed to optimize the conditions for conducting the measurements. It was found that longer contact time and higher probe speed, not only gave better reproductibility of results, but also produced higher measurement values, thus giving better sensitivity. On the other hand, a certain level of contact force was also required for affecting the bioadhesion, but beyond which did not contribute further to the process. Also, both the work of adhesion and peak detachment force appeared to be suitable for evaluating the bioadhesiveness of the polymers.

II. Polymeric Films as vehicle for Buccal Delivery

Buccal drug delivery has lately become an important route of drug administration. Various bioadhesive mucosal dosage forms have been developed, which included adhesive tablets, gel, ointment, patches, and more recently film. The use of polymeric films for buccal delivery has not yet been widely investigated, although they have been extensively employed in pharmaceutical tablet coating formulations to protect tablet cores from environmental extremes, improve appearance, mask undesirable taste, and control the drug release. Buccal film may be preferred over adhesive tablet in terms of flexibility and comfort. In addition, they can circumvent the relatively short residence time of oral gel on the mucosa, which is easily washed away and removed by saliva. Moreover, the buccal film is able to protect the wound surface, thus reduce pain and also could treat oral diseases more effectively. An ideal buccal film should be flexible, elastic and soft yet adequately strong to withstand breakage due to stress from mouth activities. Moreover, it must also possess good bioadhesive strength so that it can be retained in the mouth for a desired duration. Swelling of film, if exists should not

be too extensive to prevent discomfort. As such, the mechanical, bioadhesive, and swelling properties of buccal film are critical and essential to be evaluated. The objective of the present study was to investigate the suitability of sodium carboxymenthyl cellulose/polyethylene glycol 400/Carbopol 934P (SCMC/PEG400/CP) and hydroxypropylmethyl cellulose/polyethylene glycol 400/Carbopol 934P (HPMC/PEG400/CP) films as drug vehicle for buccal delivery. The films were evaluated in terms of mechanical, bioadhesive, and swelling properties. In addition, in-vivo bioadhesion of the films was evaluated by measuring the film residence time on human buccal mucosa of human volunteers.

The mechanical and in-vitro bioadhesive strength properties of the films were investigated using texture analyzer equipment, while swelling behavior was studied in different media, namely, distilled water and simulated saliva solution. In addition, the in-vivo bioadhesion of the film was studied by estimating the film residence time on buccal mucosa of human volunteers. Increase in CP content was found to increase the elasticity, softness and bioadhesive strength but decrease the strength and degree of swelling of both SCMC and HPMC films. SCMC films swelled more extensively in distilled water while HPMC films in simulated saliva solution. HPMC films exhibited greater in-vivo bioadhesion although the in-vitro bioadhesive strength was lower than SCMC films. Correlation existed between the in-vivo and in-vitro bioadhesion data within the polymer, but no rank correlation was observed between the two polymers. HPMC/PEG400/CP films may be preferred over SCMC/PEG400/CP as drug vehicle for buccal delivery as the former was tougher, more elastic, more bioadhesive in-vivo and swelled in a more tolerable manner in the oral cavity than the latter.

III. Formulation and Evaluation of controlled release Eudragit Buccal patches containing Metoprolol

A suitable buccal drug delivery system should be flexible and possess good bioadhesive properties, so that it can be retained in the oral cavity for the desired duration. In addition, it should release the drug in a controlled and predictable manner to elicit the required therapeutic response. In the present study, a flexible buccal patch for the controlled delivery of metoprolol as a model drug was developed using a water insoluble polymer, Eudragit NE40D as a base matrix. Several polymers with known bioadhesive properties (Methocel K4M, Methocel K15M, SCMC400, Cekol 700, Cekol 10000, CP934P, CP971P and CP974P) were incorporated into the Eudragit patches to provide the patches with bioadhesive properties and to modify the rate of drug release. The in-vitro release characteristic of the patch systems were evaluated using a modified design closely similar to the USP 23 dissolution test apparatus 5. On the other hand, the adhesive measurements were conducted using texture analyzer equipment with chicken pouch as a model mucosa.

Insoluble, flexible, organic solvent-free, controlled release patches could be fabricated using Eudragit NE 40D as a base matrix. The drug release as well as the adhesive properties of the patches could be modified by the incorporation of bioadhesive polymers. Cekol 700 appeared to be the most suitable since it provided both satisfactory bioadhesion and predictable rate of drug release.

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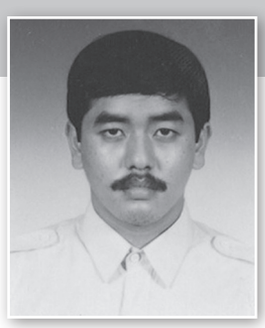

"Molecular Studies of Genetic changes in the Tumorigenesis of Meningiomas and Gliomas in the East Coast of West Malaysia"
Year 1997 MTSF Science & Technology Research Grant Recipient

Introduction

Progression of human cancer results from unique combination of genetic alteration in proto-oncogenes and tumor suppressor genes. The study was concentrated on the p53 tumor suppressor gene, which most frequently involved in the tumorigenesis process of human cancers. The p53 tumor suppressor gene has been termed "the Guardian of the cell". This gene acts as transcriptional activator, controlling the expression of variety genes important in cell cycle regulation and apoptosis. Loss of p53 function is thought to contribute to more than half of human cancers including gliomas. Molecular genetic analysis has shown that p53 gene mutations may be considered as a marker for malignant transformation in gliomas.

Objective

The aim of this study was to investigate whether genetic alterations/mutations of the tumor suppressor gene, p53 is involved in development of gliomas from a group of Malaysian patients.

Methodology

We have investigated mutation in exons 5-8 of the p53 gene by single strand conformation polymorphism (SSCP) analysis of PCR products amplified from tumor samples of 33 individual gliomas. The mutational patterns observed by PCR-SSCP were subsequently confirmed by direct DNA sequencing.

Results

Eleven (33%) mutations of 33 gliomas were identified by this studied method. The mutations were observed in two cases of exon 6, five cases of exon 7 and four cases of exon 8. No mutations were found in exon 5. Seven (63.6%) of 11 mutations were single nucleotide point mutations which including 5 missense mutations, 1 nonsense mutation and 1 silent mutation. Three (27.3%) showed insertion mutation and 1 (9.1%) showed deletion mutation. Of the point mutations, 57% were transitions and 42.9% were transversions.

Discussion

This is the first investigation performed to detect the presence of the p53 mutation in Malaysian patients with gliomas. Most of the p53 gene mutatons found in our study could change the protein structure and function, and thus may cause an inactivation of the p53 gene, strongly suggesting that p53 gene mutations play an important role in the progression of gliomas in Malaysian populations. The loss of normal p53 function plays the potential role in multistep tumorigenesis of this disease and might be useful as prognostic markers or for the selection of better treatments for brain cancer patients in Malaysia.

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"Prevalence and Molecular characterization of Enterohemorrhagic Escherichia Coli O157:H7"
Year 1997 MTSF Science & Technology Research Grant Recipient

Foodborne diseases due primarily to bacteria are an important cause of morbidity and mortality worldwide. Foodborne bacterial infections with diarrhea symptoms are usually self limiting in a normal individual, however the vulnerable groups with diminished immunity such as the young children, pregnant women, elderly and infants may succumb to death in case of systemic infection. An increase in the emergence of multidrug-resistant bacteria in recent years is worrying the world population. Though we have an array of potent antibiotics available to combat antibiotic resistance, the growing problems with antimicrobial drug resistance are beginning to erode our antibiotic armamentarium.

Food contaminated with antibiotic resistant bacteria could pose a major threat to public health due to the possibility that antibiotic-resistance genes that are carried on plasmids or mobile genetic elements may be transferred to other bacteria of human clinical significance. Like in many developing countries in the world, raw food hygiene and antimicrobial resistance epidemiology is at its infancy in Malaysia due to lack of stringent controls and abuse of antimicrobial usage in human health an animal productions systems. Diarrhoeagenic *E. coli* strains are categorized into specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes and distinct O:H serotypes. Amongst these *E. coli* O157:H7, contaminations of raw meats and poultry represent the most commonly reported food safety problem in the developed countries. However data on this pathogen is rudimentary in beef retailed in Malaysia. This study was conducted to address some of these issues and to provide a current baseline profile on the prevalence and to infer the antimicrobial patterns of *E. coli* O157:H7 isolated from frozen beef sold in the market in Selangor, Malaysia. The strategy was to compare *E. coli* O157:H7 susceptibility to selected antibiotics and their prevalence in the samples analyzed.

Imported frozen beef meat and fresh local beef meat samples were randomly purchased from local supermarkets in Selangor in the year 2007 and 2008 for the isolation and identification of *E. coli* O157:H7. The *E. coli* O157:H7 isolates obtained were randomly selected for antibiotic susceptibility test to selected antibiotics by the disk diffusion method on Mueller-Hinton agar plates, and were fingerprinted by using the randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) and pulsed field gel electrophoresis (PFGE).

The results obtained showed that the fresh local beef and imported frozen beef meat a developed country were found to be negative for *E. coli* O157:H7. However, more than 50% of the imported frozen beef meats from a developing country were found to be contaminated with *E. coli* O157:H7. The results of the antibiotic susceptibility test demonstrate the high individual and multiple resistances to antibiotics in the *E. coli* O157:H7. Genotyping of the *E. coli* O157:H7 isolates using the RAPD-PCR and PFGE techniques shows that no predominant clone exists; the bacterial population is rather diverse despite being isolated from imported frozen beef meat samples from a single exporting country of origin.

To the best of our knowledge, this is the first report on the incidence of *E. coli* O157:H7 in the imported beef marketed in Malaysia. Hence, the importance of this study is the finding that *E. coli* O157:H7 in the frozen imported beef marketed into Malaysia are significant reservoirs of antibiotic resistance genes. In the light of this finding, it endorses the need for more rigorous surveillance and improved stringent quality control requirements to reduce or eliminate the risk from antibiotic resistance and pathogenic bacteria originating from imported frozen beef.

Based on the findings in this study, there is a greater need for a system that can identify food safety hazards due to *E. coli* O157:H7 in the early stage so that these hazards can be tackled in time, before developing into real risks. Though the exporting country may employed the Hazard Analysis Critical Control Points (HACCP) approach to assess the risk and control *E. coli* O157:H7, such measures are not effective as can be seen by the high prevalence of the pathogens in the imported frozen beef samples examined. Nowadays the internationally harmonized system used by the national government to protect the consumers is the scientific risk analyses which comprise of a quantitative microbiological risk assessment component and is define as a scientific process which consists of determining the likelihood and severity of an adverse health effect in a population exposed to a certain pathogen/food combination.

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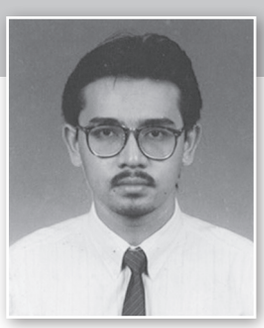
Dr. Wan Kiew Lian
Professor Mohd Sanusi Jangi

Universiti Kebangsaan Malaysia

"Expressed Sequence Tags : An approach to Gene discovery in Eimeria tenella"
Year 1997 MTSF Science & Technology Research Grant Recipient

Eimeria tenella is a protozoan parasite and one of the seven species that are the cause of coccidiosis in the domestic fowl. As an approach to gain a better understanding of the parasite, a study of 491 expressed sequence tags (ESTs) from the merozoite lifecycle stage was initiated. Of the ESTs, 47.7% had significant matches with entries in existing public databases, including ribosomal proteins, metobolic enzymes and proteins with other functions, of which 14.3% represented previously known *E. tenella* genes. Thus over 50% of the ESTs had no significant database matches. In order to complement these data, a total of 556 ESTs were derived from the sporozoite lifecycle stage of the parasite. Comparison of sequences from the two EST dataset identified approximately 128 genes that demonstrated different levels of expression. Of these 51 genes exhibited higher expression level in the sporozoite lifecycle stage, while the other 77 genes showed a more dominant expression profile in the merozoite lifecycle stage. Further analysis also revealed 49 genes that are possibly expressed specifically in the sporozoites and 73 genes that are possibly expressed exclusively in the merozoites. This study underpins the potential value of the EST strategy for discovery of novel genes and may allow for a more rapid increase in the knowledge and understanding of gene expression in the merozoite and sporozoite lifecycle stages of *E. tenella*.

Dr. Zamri Zainal



Universiti Kebangsaan Malaysia

"Molecular Biology of Chilli *(Capsicum Annum)* Fruit ripening"

Year 1997 MTSF Science & Technology Research Grant Recipient

The aim of this research project was to generate a cDNA library from ripening chilli fruit and to identify novel clones from mRNA involved in chilli fruit ripening. The library was screened differentially to identify cDNAs whose sequences showed increased expression in fruit of ripening. Thus, it was predicted that this research would identify cDNA clones for mRNA involved in these processes.

The genus *Capsicum* is a member of the Solanaceae family that includes tomato, potato, tobacco and petunia. The genus *Capsicum* consists of approximately 22 wild species and five domesticated species (Bosland 1994) : *C. annuum, C. baccatum, C. chinense, C. frutescens* and *C. pubescens*. *Capsicum* is endemic to the western hemisphere and the pre-Columbian distribution extended from the southern most border of the United States to the temperate zone of southern South America (Heiser 1976). It is a perennial small shrub in suitable climatic conditions, living for a decade or more in tropical, South and Central America. *Capsicum* probably evolved from an ancestral form in the Bolivia/ Peru area (Heiser 1976). Chilli fruits are considered vegetables, but are berries botanically. Chilli types usually are classified by fruit characteristics, i.e. pungency, color, shape, flavor, size, and their use (Smith et al. 1987; Bosland 1992). Despite their vast trait differences most chilli cultivars commercially cultivated in the world belong to the species, *C. annuum* is one of the most economically important vegetable fruit crops.

The ripening of chilli fruit is a complex development process that involves many changes in gene expression, physiological and biochemical of the fruit. The biochemical events that occur during the ripening of chilli result in the importance changes affecting the quality. These include the loss of cell wall structure causing softening, the accumulation of yellow/orange carotenoid pigments and the production of volatile flavour compounds. These biochemical events are regulated at gene level. Several mRNA, declined and others reported increased during ripening (Rattanapanone, 1978). In tomato, this has been shown to involve the production of many different messenger RNA species, which are specifically synthesized during ripening (Gray et al., 1992). Beside, there are evidences show that the tomato is active in protein synthesized throughout the ripening and there are a number of ripening related changes in pattern of protein synthesis were observed (Grierson et al., 1985). Using biochemical methods, it is often difficult to isolate ripening related enzyme due to high background of similar enzymes that are already present in the cell or perhaps the proteins are produced at low level.

A useful approach to investigate such changes is through the construction of the cDNA library. This molecular technique will enable the cloning of a large range of cDNA clones representing genes that show expression during ripening. Using differential screening or differential display techniques it will facilitate the isolation of the cDNA clones encoding proteins associated with the ripening from the library. Understanding of the fruit ripening process is an utmost importance in improving fruit quality and storage potential. In this paper, we report the construction of a cDNA library from mango mesocarp tissues obtained from different stages of mango fruit ripening.

Materials and Methods - Plant material

Mature green chile fruits cultivar MC11 were obtained from MARDI Jalan Kebun. Based on color intensities, the

fruits were divided into five different development stages. The fruits were surfaces sterilized with detergent and rinsed twice with sterilized water. The fruits were cut into two, while the seed and placenta were then removed. The pericarp were placed into liquid nitrogen and then stored in –80°C prior to RNA isolation.

Extration of total RNA from mango mesocarp

The method used for RNA extraction was a slight modification of that López-Gómez and Gómez-Lim (1992). 30g frozen pericarp tissue was ground to a fine powder using a coffee grinder and transferred to a chilled mortar containing 50 ml extraction buffer (2% w/v SDS, 1% v/v mercapthoethanol, 5% v/v phenol, 50mM EDTA and 150 mM trisborate pH 7.5). The mixture was homogenized and 0.25 volumes of absolute ethanol and 0.11 volumes of 5 M potassium acetate were added. The homogenate was then extracted once with chloroform/isoamyl alcohol (49:1 v/v), once with phenol/chloroform (1:1 v/v) and a second time with chloroform/isoamyl alcohol (49:1 v/v). The RNA was then precipitated from the aqueous phase with LiC1 (3 M final concentration) at –20°C overnight and collected by centrifugation at 13,000 rpm for 90 min at 4°C. The pellet was washed with 3 M LiC1 and resuspended in water and reprecipitated by the addition of potassium acetate (0.3M final concentration) and 2.5 volumes of absolute ethanol. Following incubation overnight at –20°C the RNA was pelleted for 10 min in a micro centrifuge at 13,000 rpm, washed with 70% ethanol, dried in a vacuum desiccator and resuspended in sterile distilled water. Poly (A^+) rich RNA was prepared with the Poly A Tract mRNA isolation system (Promega) as described by the manufacturer.

Construction of chilli cDNA library

The cDNA library was constructed in bacteriophage λ ZAP (ZAP-cDNA synthesis kit, Strategene USA). cDNA was synthesized from 5 µg of a pooled sample of Poly (A^+) mRNA isolated from pericarp tissue of ripening chilli (25%, 50%, 75% and 100%). Approximately 100 ng of the double stranded cDNA was directionally cloned into λ ZAP arms, packaged in gigapack II gold extract (Stratagene) and used to infect *E. coli* XLI-Blue MRF'.

Differential screening of the library

The cDNA library was screened by differential screening method. Approximately 180,000 pfu (recombinant clones) were screened in primary screening using radiolabeled. Probes were prepared by labeling sscDNA (single stranded cDNA) synthesized from mRNA isolated from ripe and unripe pericarp tissues in the presence of α-^{32}PdCTP. One of the membranes was hybridized to radiolabeled sscDNA from ripe fruit and the duplicate was hybridized to radiolabeled sscDNA prepared from unripe fruit. Hybridization were at 65°C using Denhart solution, 10% SDS, 20X SSPE and salmon sperm DNA as blocking agent.

Plaques that showed appreciable differences in intensify of the autoradiograph signal between the two probes used were identified as positives and subsequently isolated and purified. *In vivo* excission of pBluescript from Uni-ZAP XR vector was performed according to manufacturer's instruction (Stratagene). Purified plasmid DNA of the resqued clone was digested with *Eco* RI and *Xho* I to determine the insert size. Selected clones were then sequenced by automated sequencer using T3 and T7 primers.

Sequece Analysis

Initially BLASTX and BLASTP programs developed by Genetics Computer Group, Madison were used to identify the sequences from other plant species.

Results and Discussion

RNA extraction from chilli fruit

The used of a method developed by López-Gómez and Gómez-Lim (1992) was successful. A homogenizing buffer containing high concentration of Tris-borate was able to eliminate the polyphenol compounds. For precipitation of RNA, LiCL with the final concentration of 3M was used. This step removed carbohydrate as well as DNA and protein from the preparation. The amount of RNA isolated depends on the development stages. For mature green fruit about 79.33 ug/g tissue, while at 50% ripening only 30.0 ug/g was isolated. The differences might be due to the carbohydrate and other contamination, which were present in the preparation. The carbohydrate will co-precipitate with nucleic acid during ethanol purification step and lead to yield decrease. For a pure sample the ratio for OD 260:280 is 1.8 – 2.0. From these data, it seems likely that very little carbohydrate or polyphenol was present in the preparations. About 10 µg of total RNA from different stages of ripening was resolved on a 1% (w/v) agarose to determine the quality and integrity of the isolated RNA.

CDNA library construction

The poly (A^+) RNA samples isolated from pericarp tissues from four ripening stages of chilli fruit (25%, 50%, 75% and 100%) was pooled. The purpose of using the pooled RNA was to ensure that the library is well represented with ripening specific transcripts. First strand cDNA was generated from 5µg of poly (A^+) RNA and this was used to make double stranded cDNA. The quality of the first and second strands cDNA were checked on 1% agarose gel.

Titreing of the cDNA library

The library was titred in order to estimate the number of recombinant phage by incubating 1 µL of the final packaging reaction (phage stock containing cDNA) with 200 µL of XLI-Blue MRF'. The reactions were plated on NZY agar plate containing IPTG and X-gal to differentiate between recombinant and non-recombinant plaques. The plaques without inserts gave blue colonies while recombinant plaques gave a white colour. From the titre, it was calculated that the total clone in the library was approximately 1.9×10^6, while the percentage of blue (non-recombinant) is about 3%. Thus, the number of recombinant clones was approximately 1.84×10^6. Based on the size of the library, we think that the library is well represented.

Differential screening of a ripening chilli library

Differential screening of approximately 180,000 recombinant plaques were performed using labeled sscDNA as described in materials and methods. From primary screening about 80 clones were obtained. These clones were subjected to secondary screening in order to obtain a single positive clone. Following the secondary screening about 12 ripening-related cDNA clones were isolated and partially sequenced. Homology search using BLAST showed these cDNAs have homology with those genes deposited in database. The isolated cDNA clones were designated as CUKM series.

CUKM2 and CUKM3 were partially sequence from 5' end of the cDNAs. These two clones are identical when we compared. CUKM2 & 3 mRNA was shown to be highly expressed in ripening fruit. The transcript was first detected at 25% sample and increased toward 100% ripening. Database searches revealed homology with Capsanthin capsorubin synthase (CCS). CCS is a bi-functional enzyme involved in carotenoid biosynthesis, which catalyzed conversion of antheraxanthin and violaxhanthin to capsanthin and capsorubin respectively. The accumulation of these pigments,

result red color in ripening chilli fruit. Alignment of putative amino acids sequence derived from DNA sequence with CCS homolog in SwissProt Database revealed that CUKM2 and 3 were not full-length. There are missing of about 600 nucleotides at 5' end of the coding region. Clone CUKM4 with a size of 0.5 kb encodes enzyme Gluthathione-S transferase (GST). GSTs are abundant proteins encoded by a highly divergent gene family. Soluble GSTs form dimmers, each sub unit of which contains active sites that bind gluthathione and hydrophobic ligands. Plant GSTs attach gluthathione to electrophilic xenobiotics, which tags them for vacuolar sequestration. Itzhaki & Woodson (1993) isolated cDNA clone namely pSR8 from cDNA library of senescing petal. It is suggested that GST play a diverse functions in cellular metabolism but its actual role during ripening is unknown. CUKM6 with a size of 2.3 kb. The cDNA was partially sequence from 5' end. Database searches showed no significance homology with other sequences. Further sequence of this clone might give some clues about the putative function.

The partial sequence of the CUKM10 has significant degree of homology (60% identity) to homeobox leucine zipper from *Arabidopsis thaliana*. Homeobox leucine zipper genes encode homeodomain proteins with a tightly linked leucine zipper motif (Schena et al., 1994), which play an important regulatory function in DNA binding. Several evidences have also demonstrated that homeobox-leucine zipper gene mediates some development control in plant (Schena et al., 1994). The importance of this gene in ripening fruits could be elucidated by further characterization of this ripening-related cDNA clone. Clone CUKM12 has been identified to be a putative translation factor (EF-1α), a multifunctional protein isolated from Vicia faba. EF-1α is thought to control plant development through the regulation of protein synthesis (Durso et al., 1994). In addition, the expression of EF-1α may also indicate the stimulation of transcription (Ursin et al., 1991). This finding might suggest that the presence of putative EF-1α is to regulate transcription in ripening chilli.

Meanwhile clone CUKM11 encodes a polypeptide with similarity to ornithine decarboxylase of *Datura stramonium*. Ornithine decarboxylase is a catalyst for the biosynthesis of putrescine from ornithine during the early stage of fruit development (cohen et al., 1982). Rastogi et a., (1989) suggested that the enhanced level of putrescine in fruit might be responsible for ripening and storage as reported in tomato. Several clones were selected for Northern analysis in order to elucidate the expression pattern. From Northern Blot experiments clones CUKM2, 4 and 10 which encoded capsanthin capsorubin synthase, gluthathione s-transferase and homeo leucine zipper shows up regulated pattern during ripening. From this finding, it is suggested that those clones are ripening-related and might play importance roles during fruit ripening.

Conclusions

- A cDNA library from ripening chilli fruit was successfully constructed from chilli with a size of 1.84×10^6
- Thirteen novel ripening-related cDNAs from ripening chilli library have been identified by differential screening with probe synthesized from mRNA of ripe and unripe fruit. Database homology searches suggest possible functions for isolated cDNA clones.
- Further sequence and Northern analysis might give a better understanding of the genes function during ripening.

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Universiti Kebangsaan Malaysia

"Characterization of Putative Fusion Protein involved in Mammalian Gamete Fusion"

Year 1998 MTSF Science & Technology Research Grant Recipient

(Funded by Toray Science Foundation, Japan)

M1 antigen is a fusion protein involved in hamster (*Mesocricetus auratus*) sperm-egg fusion and located at the equatorial segment (ES) region of hamster sperm. This research was carried out to

(i) investigate the importance of Ca2 ion and the acrosome reaction to presentation of M1 fusion protein on ES region

(ii) biochemical characterization of M1 fusion protein

(iii) study the potential of M1 protein/antigen as an immunocontraceptive agent.

The effect of calcium ionophore on the acrosome reaction and M1 fusion protein were analysed by Giemsa and indirect immunofluorescent staining respectively. SDS-PAGE together with Western blotting technique were conducted to characterized biochemically M1 fusion protein. To study the potential of M1 antigen as an immunocontraceptive agent, six male hamsters were given active immunization and the number of implantation sites were counted after the hamsters mated. Changes of body weight were observed and comparison of the reproductive organ weight (testis and epididymis) between the control group and immunized group were undertaken. Histological study was carried out on the reproductive organ to observe any pathological changes after the hamster immunized with M1 antigen.

The results showed the presentation of M1 protein on ES of hamster sperm is Ca2+ and AR dependent. Western blot analysis showed disulfide bond is not present in M1 antigen and is an integral membrane protein. The results also suggest that the maturation process in epididymis affect M1 antigen molecular weight. Progesterone did not change biochemical property of M1 antigen as detected by immunoblot, however calcium ionophore alter the molecular weight of M1 antigen from 34 & 37 kDa to 77 kDa. Immunizaton of hamster with M1 antigen showed contraceptive effect. No significant changes ($p>0.05$) in body and organ weight of immunized hamster except for the testis and epididymis ($p>0.05$). During the period of the experiment no pathological changes were detected on the immunized hamsters organ (reproductive and non-reproductive) except on testis and epididymis. It can be concluded that M1 protein involved in fertilization process and has potential as immunocontraceptive agent.

Benefits of the Project

1. An understanding on the role of antigen in hamster fertilization. Several factors such as Ca2+ and AR are important in M1 presentation.
2. Knowledge on the immunocontraceptive ability of M1 antigen. This output can be used to develop immnocontraceptive agent in rodent.
3. Human resource training : 10 undergraduates and two postgraduates
4. Publication of results

Miss Ling Shui Nyuk
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Universiti Malaya
"Molecular characterization of a multiple Antibiotic Resistance Transposon from *Salmonella typhi*"
Year 1998 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Salmonella typhi, a member of Enterobacteriaceae, is the causative agent of typhoid fever. Typhoid fever is distressingly endemic in development countries of Central America, Africa and Asia. The annual global incidence of this disease has been estimated to be 21 million cases, with more than 700,000 deaths (24).

Chloramphenicol is an excellent drug for treatment of typhoid fever. Other antibiotics such as ampicillin, amoxycillin, and cotrimoxazole are also used as alternative drugs (2,8).

Multiple drug resistant (MDR) *S. typhi* strains were first discovered in 1950 in England (4). The first major outbreaks of typhoid fever caused by MDR strains occurred in 1972 in Mexico City (15) and in South India (17). Since then, MDR *S. typhi* strains were reported in other countries from all over the world such as Vietnam (3), Thailand (10), Indonesia (20), Bangladesh (7), Indian subcontinent (1), Latin America, Nigeria, Korea, Africa and the Philippines (16). In Malaysia, chloramphenicol resistant *S. typhi* strains were reported in 1980 (9). These strains are resistant to chloramphenicol, ampicillin, kanamycin, neomycin, streptomycin and sulphadiazine.

Generally, *S. typhi* does not carry plasmids. However, a notable feature of all chloramphenicol resistant strains of *S. typhi* from outbreaks in Mexico, the Indian subcontinent and South-east Asia is that although the strains belong to different Vi phage types, resistance to chloramphenicol is encoded by a plasmid of the IncHI incompatibility complex group, often in combination with resistance to streptomycin, sulphonamides, ampicillin, trimethoprim and tetracyclines (12,23).

Plasmids of the IncHI group are greater than 150 kb, which has made them rather difficult to be analyzed physically and genetically.

Although many multiple antibiotic resistance transposons from other members of the Enterobacteriaceae have been characterized (14), little is known about multiple antibiotic resistance transposons from *S. typhi*. To the best of my knowledge, only two reports have been published on the genetic confirmation of trimethoprim and tetracycline resistance transposons in *S. typhi*, indistinguishable from Tn*7* (18) and Tn*10*(11), respectively. The main objective of this research was to investigate and understand the entire structure of the multiple antibiotic resistance transposon from *S. typhi* S5 by determining the antibiotic resistance genes and the transposable elements involved.

Results and Discussion

S. typhi strain S5 was isolated from a patient at the Kuala Lumpur Hospital during an outbreak of typhoid fever in 1990

in Malaysia. S5 is resistant to ampicillin, chloramphenicol, cotrimoxazole, streptomycin, and tetracycline, and this multiple antibiotic resistance trait has been found to be mediated by a multiple antibiotic resistance transposon, Tn*S5*, located on a large conjugative plasmid, pS5 (Lim M.E. and Koh C.L., personal communication). After transposition of Tn*S5* to a recipient replicon, pUB307, in a *recA Escherichia coli* strain, a recombinant plasmid pUB307::Tn*S5*-3 was obtained.

The genetic organization of transposon Tn*S5* was accomplished through a series of cloning, subcloning, DNA sequencing, restriction pattern analysis, and Southern hybridization of pUB307::Tn*S5-3*. Various antibiotic resistance genes and transposable elements in Tn*S5-3* were identified by comparing the nucleotide sequences obtained in this study with those in GenBank and EMBL databases.

The Tn*S5-3* contained two copies of Tn*21*-like transposon with similar content of genes but in different orientations: these two Tn*21* backbone transposons were named Tn*S5-3*ΔI and Tn*S5-3*Δ*II*. Both Tn*S5-*Δ*I* and Tn*S5-*Δ*II* contained the mer operon of Tn*21* of plasmid R100 (Accession number : AP000342), the 5-CS of In2 including IR_i and *intI1* (22), and part of the 3'CS of In2 including *qacE*Δ*1*, *sulI*, part of *tniA*, and IR_t, *dhfrVII* gene [similar to that of Tn*5086* (22)] as cassette in the integron, transposition module of Tn*21* (*tnpM*, *tnpR*, and *tnpA*), and IR_{tnp} and IR_{mer} (Accession number : AP000342) of Tn*21* at both ends of the two transposons. A composite transposon comprising direct repeats of IS*26* (13) at both ends and an intervening IS*26* in inverted orientation was found inserted downstream of *sulI* gene of both Tn*S5-3*Δ*I* and Tn*S5-3*Δ*II*. This is a new composite transposon and is designated Tn*26*. It harboured partial *repA*, *repC*, *strA*, *strB,* and *sulII* similar to those of RSF1010 (21) and partial *tnpR* and intact *bla* identical to that of Tn*2* (6,5). Tn*S5-3*Δ*II* harboured an extra copy of IS*26* compared with Tn*S5-3*Δ*I*. The integron of Tn*S5-3*, including the region bounded by IR_i and IR_t, is a new integron and is designated InS5.

From analysis of the adjacent sequences at both sides of the target sequences of IS*26* in this study, and its relatives from other published papers, IS*26* was found to preferentially insert next or into a region of short inverted repeats (5 to 9 bp).

A type I chloramphenicol acetyltransferase gene, *cat*, and a copy of IS*1*, *ybjA*, *trhN* (Accession number : AP000342), and an unknown gene of plasmid R27 (19) were located in between Tn*S5-3*Δ*I* and Tn*S5-3*Δ*II*. This intermediate region is designated Tn*S5-3*Δ*III*.

The original structure of Tn*S5* and pS5 was postulated to possess only Tn*S5-3*Δ*I* and the *ybjA*, *catI* and IS*1* region, into which the Tn*S5* had undergone replicative transposition before it transposed into pUB307. Later, Tn*S5* underwent a replicative invesion after transposition into pUB307 to generate pUB307::Tn*S5-3*. The evolution steps from the common ancestor of Tn*21* family transposons to Tn*S5* and then from Tn*S5* to Tn*S5-3* in pUB307::Tn*S5-3* were proposed.

The structure of the transposon Tn*S5* revealed the picture of how the transposons had picked up the antibiotic resistance genes from the gene pool and also how these transposons getting into contact with each other to form the complicated and huge DNA segment in plasmid *S.typhi*.

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"Epitope Mapping of the Tropomysosin Allergen of House Dust Mites *Dermatophagoides spp."*
Year 1998 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Patients with allergy-related diseases, namely allergic rhinitis and asthmatic, normal healthy subjects, and asthmatic children were studied for sensitisation to house dust mite. This study was aimed at obtaining antibody profile of subjects affected by house dirt mites in general and to find out the allergenic frequency of components in the repertoire of allergens in house dust mites. The characterisation of allergencity to one of the components, mite tropomyosin (Group 10) was emphasized.

The skin prick tests (SPT) involving 291 allergic rhinitis and 298 asthmatic patients from the University of Malaya Medical Centre, Kuala Lumpur revealed that 80% of asthmatic and 76% of the allergic rhinitis patients reacted to either or both *Dermatophagoides pteronyssinus* and *D. farinae*. Other aerollergens affecting atopic subjects included cockroach, pollen and fungal spores. Their sensitivity to these 3 species of mites suggested a slight cross-reactive nature of their exposure to them. Interestingly, specific IgE to *D. farinae* was found more frequently in these young asthmatic compared to the adults and *Blomia tropocalis*, a newly reported mite species was indeed found to significantly affect the Malaysian population.

The *Escherichia coli* and the yeast *Pichia pastoris* expression systems were employed and compared to obtain mite tropomyosin Der p 10. The purification was then facilitated with the presence of glutathione-S-transferase (GST)-tag while the *Pichia*-produced recombinant Der p 10 was histidine-tagged. These recombinant proteins were found to be antigenic but failed to show the high ability of the native Der p 10. Both recombinant mite tropomyosins in this study could only be recognized by 20-29% of the allergic patients compared to the 80% allergencity in atopic individuals initially reported by Aki *et al*. 1995. Through DNA sequencing, the *E. coli*-produced Der p 10 was found to have >95% homology with the native protein while *Pichia*-produced Der p 10 showed significant immunogenicity in mice; thereby producing antiserum that was able to recognize both E. coli- and *Pichia*-produced Der p 10 as well. The subsequent testing of these recombinant proteins complemented the study of component-resolved allergens in both *D. pteronyssinus* albeit not the complete repertoire. This part of the study was able to produce a component-resolved allergenicity profile of the mite, *D. pteronyssinus* and *B. tropicalis* among Malaysian patients.

Based on the sequence of house dust mite tropomyosin, Der f 10, both linear and conformational peptides were synthesised as non-cleavable peptides on pins using the Multipin Peptide Synthesis technique. These synthetic peptides were then used in a modified ELISA to screen for binding to human IgE antibodies of the sera of allergy patients. These pepscan experiments showed IgE binding to prominent regions : Chimeric peptides, bearing conformational structures

of the possible epitopes on the complete Der p 10 molecule were similarly tested for significant IgE-binding at regions. Using individual patient's serum, 4 linear immunodominant epitopes were identified in this allergen; at EVRAL, LQKEV, DVRLE and EDELV showing over 75% IgE-binding reactivity. In the conformational study of chimeric peptides EKSEEEVRALQKKIQQ, DGLENQLKEARMMAED and QKLQKEVDRLEVELVH were significantly more reactive than other 16-mer synthetic peptides of mite tropomyosin in the conformational pepscan. Subsequently, computer-aided programmes were used to analyse these regions.

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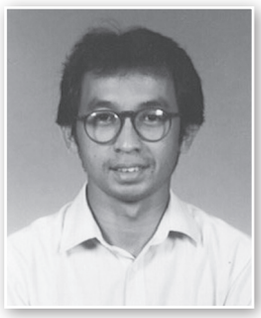

"Development of Microwave Borehole Tomography (MBT) System for Geophysical Applications"
Year 1998 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Objective

The project was aimed primarily to developing a new and advance type reconstruction algorithm for microwave tomography.

Background

Translated literally from the Greek, 'tomography' means 'section writing' and this succinctly describes the reconstruction of a two-dimensional image of a section taken through a three-dimensional object. Tomography was first made available as a practical medical imaging tool in 1972 by the work of Nobel prize winner Geoffrey Hounsfield at EMI Ltd. All Hounsfield's work involved the use of X-rays and now the term CT is conventionally associated with X-ray computed tomography. Most people gained first-hand experience of CT probably during unplanned visit to a hospital. The success of CT for medical imaging has resulted in the proliferation of this technique not only for medical diagnosis but also for scientific and industrial applications. To-date, the techniques used cover almost all the energy spectra ranging from high frequencies to low frequencies, and thus the images produced, ranged from high resolution to low resolution. Three major tomography techniques can be identified as follows :

Transmission Tomography [1,2]

This technique involved the use of high penetrative sources such as X-rays, gamma rays and other ionising sources. The rays travel in straight line manner and hence the images produced by them are also referred to as the hard-ray tomogram. The reconstruction algorithm developed for this technique originated from one-dimensional Fourier transform and its variations. These have extensively been documented on interested readers are referred to references listed.

Electrical Tomography[3]

Unlike transmission tomography, the electrical method utilises non-ionising sources such as low frequencies electrical currents as a mode of exploration. It is well known fact that electrical signal does not travel in straight line, rather through a least resistive path. Therefore, image reconstruction algorithms described for straight-ray tomography are not applicable. A diversity of algorithm, both single-step and iterative have been established. Most prominent among them is the qualitative backprojection[4] and the quantitative Newton-Raphson method[5].

Diffraction Tomography[6]

Rays and waves interact with materials quite differently depending on frequency of the sources which produced them. Unlike X-rays, for example microwaves have wave-length comparable to the size of objects thus reflection and diffraction that lead to 'ray' bending cannot be neglected. Thus, reconstruction algorithms described for transmission tomography systems become inapplicable. Similar to electrical tomography, diffraction tomography system also

exhibits soft-field properties. The different between these two cases is the latter uses sources the frequencies of which range from hundreds of megahertz to a few tens of gigahertz. Hence, the governing equations resulting from the high frequency electromagnetic propagation are quite different from its low frequency counterpart. Frequently, the reconstruction algorithm required to solve these high frequency propagation problems are both mathematically complicated and computationally intensive, despite the fact that they produced images of improved quality. Compared to the above two imaging modalities, this technique is the latest tomography invention, having been proved feasible in mid '90s.

Results

Diffraction tomography is relatively recently invented tomographic technique for scientific applications. The person first responsible in putting up this idea into a right mathematical perspective was J.H. Richmond in 1965[7]. He developed scattering model based on dielectric cylinder with arbitrary cross section shape. Since then many researchers have extended Richmond work, shaping and engineering into what it is today known as microwave tomography. Two research groups appear to be at the forefront in this research. The first one is led by Jean-Charles Bolomey, a French group of the d'Electramagnétisme/Laboratoire des Signaux et Systémes, Ecole Supérieure d'Electricité, France[8,9,10]. The second one is the USA's Carolinas Medical Center and headed by Prof. P. Tatsis[11,12]. These two groups focussed their research to developing and applying microwave tomography for medical oriented applications, such as control and detection of hyperthermia of a newborn baby. Much of the results, particularly the reconstruction algorithms, produced from this project were based on Bolomey and Tatsis's findings. However, the methods and techniques proposed by them are not optimized for applications other than the medical ones. Besides undertaking basic research, the project also included algorithm optimisation so that the tools and system developed are suitable for industrial oriented application. The one tested here was the mapping of complex dielectric profiles of food container nondestructively. The availability of such information is very critical since it enable food engineers and technologists to design better storage and helps in food development.

Reconstruction algorithms

Three types of image reconstruction algorithms have emerged from this study. They are the Moore-Penrose (MP), the Newton Kantorovitch (NK) and the backprojection. The first two methods enable tomographic images be reconstructed quantitatively. In contrast the backprojection method only enable image reconstruction be performed qualitatively. Refer to final year project dissertations by Kwong. F.K. and Ooi. Y.J. for details description of this algorithm.

Thus far the MP and NK methods have been demonstrated feasible by computer simulation. These algorithms require both phase and amplitude measurement for use in image reconstruction. Hence, a specialised equipment is needed to do this. Alternatively, the present facility has to be modified and upgraded to enable such a reconstruction. Until this has been affected, the characteristics and performance of MP and NK algorithms can only be studied using computer generated data. The backprojection method developed in this research was based on straight ray propagation. This algorithm only requires time of flight data which can easily be measured in a laboratory.

Food imaging

In order to validate the methods investigated, a container having dimension of 123X76X60 cm was constructed using fibre glass material. The tank was filled with rice (relative permittivity approximately 4.5) constituting the background material. A crosshole measurement of multiple offset type were performed using dipole antenna transmitting at 1 GHz. Reconstructions were attempted under two different cases : the homogeneous and the inhomogeneous cases. The

first one comprised of the homogeneous rice corresponding to the object to be reconstructed. In the second one, the inhomogeneous body was simulated by placing an air filled cylinder of 20.32 cm in diameter at the center of the tank. Images reconstructed via a backprojection algorithm. These images serve an example of the inherent blurring artifact which is resulted from straight ray assumption of this algorithm. Further work incorporating ray-bending algorithm is clearly needed to solve this problem.

Conclusion

In this research, we have demonstrated for the first time the successful application of microwave tomography for non-medical application. Retrieving complex permittivity profiles of food process vessel non-destructively was one of the application problem studied. Three different microwave reconstruction algorithms were resulted from this research. They are the quantitative Newton-Kantorovitch and Moore-Penrose methods, and the qualitative backprojection algorithm. The latter has successfully been tested using a laboratory scaled phantom whilst the first two have been investigated feasible using computer simulated models.

Future and present works

The results described in this report are preliminary achievements to some of the research projects which are currently be pursued or will be pursued. Present and active research in progress include the following :

- Validation of the newly developed reconstruction algorithm using experimental data
- Optimisation of the image reconstruction algorithm
- Investigation of SIRT based reconstruction method incorporating ray bending technique
- Applications – retrieving complex dielectric profiles of inhomogeneous object (food and geophysical based applications)
- 3D reconstruction

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"Microwave Magnetic and Dielectric Properties of Ferrite Thermoplastic Natural Rubber (TPNR) Composites"
Year 1998 MTSF Science & Technology Research Grant Recipient

In recent years, the usage of electrical and electronic devices has grown rapidly. Many devices such as AC motors, digital computers, calculators, point-of-sale terminals, printers, modems, electronic typewriters, digital circuitry and cellular phones are capable of emitting radio-frequency energies. There is an ongoing controversy worldwide over the potential health hazards associated with exposure to electromagnetic field. The ability of ferrite materials to absorb energy from electromagnetic waves is well known. Ferrite have been used as absorbers in reducing backscattering from objects or radar targets, electromagnetic interference suppressors, anechoic chambers, ceramic tiles and paints. On the other hand, the use of polymers for housing electronic devices is popular due to their being light weight, flexible and inexpensive. However, polymers are electrical insulating materials and transparent to electromagnetic radiation.

Composites, in the past, have mainly been used for saving weight. Fillers have played an important role for many years as additive materials to produce polymer composites. Thermoplastic natural rubber (TPNR) is a polymer that exhibits intermediate properties between those of natural rubber and thermoplastics. As TPNR polymers are nonmagnetic, it is possible to incorporate a magnetic material into the polymers to produce magnetic polymer composites. Magnetic properties of plastic magnets composed of a polymer matrix and magnetic powders are normally inferior to those of conventionally cast or sintered magnets but they are various advantages and fulfil the above-mentioned requirements. Ferrites are one of the magnetic materials that have been extensively used for this purpose.

The objective of this research is two fold :

i) to study the effect of incorporating the ferrite into TPNR on microwave dielectric, magnetic and absorption properties and
ii) to study the effect of natural rubber content on microwave properties of the magnetic composites.

Through this research, we obtained information about the mechanism of dielectric behavior and influence of ferrite and natural rubber contents of thermoplastic natural rubber magnetic composites.

Results

Three important findings were obtained from this research project :

1. **Mechanism of microwave dielectric behaviour of TPNR Composites**

   The dependence of μ_r' on the frequency is almost the same for the composites. The value of μ_r' seems to increase with decreasing frequency from about 1.3 GHz, but the effect becomes smaller for composites with higher PP content. On average μ_r' is approximately constant from 1.3 GHz upwards. Four factors mainly contribute to the dielectric constant of a material : electronic, ionic, orientational and space-charge polarizations. The space-charge

contribution depends on the inhomogeneities of the material. Its influence is small at low temperature and is more noticeable in the low-frequency region. The dipolar orientational effect can sometimes be observed in some materials even up to 10^{10} Hz. Ionic and electronic polarizations always exist above 10^{13} Hz. The constant value of $\square_r$' is probably due to a domination of the orientational polarization process where the oscillation of the dipole moments is in phase with the microwave frequency [20]. The value of $\square_r$' within this range of frequency increases from approximately 2.5 for the PP80NR20 sample to approximately 4.2 for the PP40NR60 sample. The present results show that the TPNR matrix with different content of NR or PP is the component that influences the dielectric properties of the composites. This is due to the $\square_r$' values for the composites being very similar to the $\square_r$' values for the TPNR. The variation of $\square_r$' in TPNR with NR/PP content is in the vicinity of 3.2 for pure NR and 2.8 for pure PP. The dielectric polarization in both NR and PP is due to the orientational mechanism, where the induced dipoles are due to the existence of C-H polarity as a result of the difference between the electronegativities of the two types of atoms. The dielectric loss ($\square_r$'') for pure NR is higher than those for pure PP and the composites. The increase of $\square_r$'' with decreasing frequency can possibly be explained based on the relation that $\square_r'' = \sigma_{dc}/\omega\varepsilon_o + \varepsilon_{ac}$, where σ_{dc} is the d.c. conductivity, ω is the angular frequency, ε_o is the permittivity of free space and ε_{ac} is the a.c. loss contribution at high frequencies.

2. The effect of natural rubber and polypropylene on the microwave properties of the composites

The magnetic permeability (μ_r') and magnetic loss (μ_r'') is dependence to frequency for all the composites. It can be seen that μ_r' decreases exponentially with increasing frequency up to about 10 GHz before reaching a constant value in the higher frequency regime. At a fixed frequency, μ_r' decreases with increasing NR content in the TPNR matrix. The exponential variation of μ_r'' can be seen for all samples in the lower frequency regime up to about 7 GHz, before showing a decreasing trend to almost the same value for all composition in the higher frequency regime. However, two samples with higher NR contents (50 and 60 weight percent) show slightly higher μ_r'' values at around 3 GHz for a range of about 1 GHz on either side. This is due to the low μ_r'' values around 1 GHz for the two samples. For a fixed frequency, the effect of increasing the NR content in the TPNR matrix is to reduce the value of μ_r''. The magnitudes of dielectric permittivity ($|\square_r^*|$) and magnetic permeability ($|\mu_r^*|$) are respectively dominated by $\square_r$' and μ_r', since $\square_r' >> \square_r''$ and $\mu_r' >> \square_r''$; hence, the profiles of $|\square_r^*|$ and $|\mu_r^*|$ versus frequency are similar to those of $\square_r$' and μ_r' versus frequency for the same samples.

This study has revealed that the composites can be used as electromagnetic wave absorbers. The microwave dielectric properties and minimum reflection loss of these composites are largely dependent on the NR and PP contents. The increase of NR content in the TPNR has improved the power absorption of the composites at both matching frequencies. The fact that the power absorption is not 100% even at $t = n\lambda/4$ (n=1 and 2) is due to the impedance mismatch at the air-material interface.

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"The role of Tyrosine Phosphorylation in Malarial Infection"
Year 1998 MTSF Science & Technology Research Grant Recipient

Plasmodium invasion of its host has been suggested to involve tyrosine kinase signaling mediated by protein tyrosine kinase-protein tyrosine phosphatase (PTKase-PTPase) enzyme systems. An important component of this signaling pathway is the phosphotyrosine-containing proteins; the levels of which are influenced by the PTKase-PTPase activities. The present study was carried out to investigate the involvement of PTKase-PTPase-mediated signaling in *P. berghei* infection by monitoring phosphotyrosine-containing proteins in *P. berghei*-infected mice erythrocyte membrane. Immuno-detection of erythrocyte membrane phosphotyrosine-containing proteins (1o Ab= anti-phosphotyrosine (pAb); 2^{o} Ab=HRP-conjugated anti-rabbit IgG) was carried out after the proteins were separated by SDS-PAGE and transferred onto nitrocellulose membrane by western blotting. ECL detection showed the presence of ten immuno-reactive protein bands (with approximate molecular weights of >207, 192, 66, 58, 30, 28, 15, 7 and <7 kDa) in *P. berghei*-infected mice erythrocyte membrane. Densitometric analysis suggests that the levels of the >207, 192, 66, 53 and 30 kDa phosphotyrosine-containing proteins decreased during infection from day 5 and 9 post inoculation. However, the 58, 28, 15, 7 and <7 kDa phosphotyrosine-containing protein only appeared on day 7 and day 9 after inoculation. Whether the infection-mediated change in phosphotyrosine-containing protein content observed here in mice erythrocyte membrane is directly related to phosphorylation-dephosphorylation activities or differential expression of these proteins remains to be further investigated.

Objective achievement

Several mice erythrocyte membrane phosphotyrosine-containing proteins, a group of protein components important in the tyrosine kinase signaling pathway and the identities of which remain unknown, have been shown to be influenced by *Plasmodium berghei* infection.

Data from the present study suggests either of two possibilities:

a) tyrosine phosphorylation-dephosphorylation activities is important during *P.berghei* infection; or
b) differential expression of these phosphotyrosine-containing proteins during *P.berghei* infection

Objectives not achieved

The origin of these mice erythrocyte membrane phosphotyrosine-containing proteins to be affected by infection as well as the identities of these proteins remain to be further addressed to fully understand their importance in infection.

Inhibitor studies were hampered by the lack of proper facilities to handle the human *Plasmodium* pathogen. Cell culture of the human *Plasmodium* counterpart (i.e. P. *falciparum*) is necessary to confirm the importance of the proteins observed in experimental mice to be influenced by *Plasmodium* infection.

Results

Data from this study indicate that tyrosine kinase signaling (emergent from tyrosine phosphorylation-dephosphorylation activities; or differential expression of specific phosphotyrosine-containing proteins) is important in *Plasmodium berghei* infection.

The identities and origins of these proteins (host or parasite) will help in understanding the mechanism of *Plasmodium* infectivity.

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"Application of Biotechnology in strain selection and development of selected edible Mushrooms for commercial production"
Year 1998 MTSF Science & Technology Research Grant Recipient

Introduction

The mushroom industry is a global expanding industry with world production greater than four million metric tonnes annually. China produces 80% of the world demand followed by United States (7%). The main mushroom species commercially cultivated are *Agaricus bisporus, Lentinula edodes,, Pleurotus* species, *Auricularia* species, *Flammulina velutipes, Volvariella volvaceae, Tremella fuciformis, Hypsizygous marmoreus* and *Grifola frondosa* (Chang, 1999).

In Malaysia, mushrooms have been successfully grown since 1934 (Baker, 1934) and continuing to progress until today. In the year 2001 until 2004, Malaysia is producing around 5.5 tonnes annually which is less than the market demand (Sofian, 2005). One of the major problems that hindered the industrial growth of mushroom industry in Malaysia is the shortage of spawn availability and lack of quality spawn. Good quality spawn is one of the most important decisive factors for the success in getting high yield of mushroom. Research carried out on mushrooms cultivation in Malaysia focuses on screening for suitable substrate for spawn run. Among the substrates screened were rice straw, palm oil residues, rubberwood and sago 'hampas'. No research has been carried out to obtain good quality spawn.

Usually grain spawns are used which had long incubation time and are easily contaminated as grains harbour a lot of fungal spores and bacteria if improperly sterilized. In addition, mushroom growers have no facility to store good quality mushroom cultures for long period of time and periodic transfers of cultures results in reduction of rate of growth and eventually loss of viability, hence, a medium must be developed to easily obtain new cultures for the production of good quality spawn.

Objectives

The objectives of this project are :

i. To formulate suitable media for growth of cultures from mushrooms fruiting bodies
ii. To optimize the growth parameters for the production of liquid inoculum
iii. To produce liquid inoculum in a bioreactor and to assess the fruiting ability of the mushroom

Methodology

1. Formulation of media for growth of edible mushrooms

1.1 The effect of carbon sources on the radial growth rate

Seven mm diameter plug of five to seven days old mycelium of edible mushrooms grown on malt extract agar was centrally inoculated on agar consisting of 2% carbon source in 9-mm diameter Petri dishes. The carbon

sources investigated were glucose, lactose, sucrose, white sugar and brown sugar. The inoculated plates were incubated at 25 °C and the diameter of radial growth was measured at various time intervals.

1.2 The effect of nitrogen sources on the radial growth rate

Seven mm diameter plug of five to seven days old mycelium of edible mushrooms grown on malt extract agar were centrally inoculated on agar consisting of 2% optimum carbon source (as determined in 3.1.1) and 0.4% nitrogen sources in 9-mm diameter Petri dishes. The nitrogen sources investigated were ammonium chloride, yeast extract, malt extract, rice bran and palm kernel cake. The inoculated plates were incubated at 25 °C and the diameter of radial growth was measured at various time intervals.

1.3 The effect of different combinations of nitrogen sources combinations and concentrations on the radial growth rate

Seven mm diameter plug of five to seven days old mycelium of edible mushrooms grown on malt extract agar were inoculated centrally on agar consisting of 2% optimum carbon source and 0.4% optimum nitrogen source (as determined in 3.1.1 and 3.1.2) in 9-mm diameter Petri dishes. Various concentration and combinations of nitrogen sources were investigated. The inoculated plates were incubated at 25 °C and the diameter of radial growth was measured at various time intervals.

2. Tissue culture technique to obtain new mycelial culture from fruiting bodies

Tissue culture of edible mushrooms was carried out by transferring aseptically tissues from the central region of mushroom fruitbodies onto the formulated media above. The rate of success of the tissues to germinate and form mycelial colony was noted.

3. Production of mushrooms liquid inoculum in shake flasks and optimization of growth parameters for the production of *Pleurotus sajor-caju*

Forty-five millilitres of optimized medium (determined in 3.1) consisting of 20g/L of brown sugar, 4g/L of rice bran, 4g/L of malt extract, and 4g/L of yeast extract) in 250ml-Erlenmeyer flask was autoclaved at 121°C and 15psi for 20 minutes. All Erlenmeyer flasks were inoculated with 5 mls of mushroom mycelia suspended in sterile water. Inoculated flasks were incubated in a shaking incubator at 25 ± 1°C rotating at 250rpm for 10 days. Triplicate flasks were prepared. The mycelia pellets formed were collected by centrifugation at 9000 rpm for 10 minutes and the supernatant was discarded. The dry weight of mushroom mycelia was measured after repeated washing with distilled water and left to dry at 70°C until constant weight was achieved.

4. Production of *Pleurotus sajor-caju* liquid inoculum in a bioreactor and to assess the fruiting ability

The fermentation was carried out using a 2L stirred tank bioreactor (Biostat B+ Sartorius, BBI). The cultivation conditions in bioreactor were as follows: cultivation temperature of 28°C, agitation speed of 250rpm, initial pH of 5.5, and oxygen partial pressure of 30–40%. The batch cultivation of *Pleurotus sajor-caju* in an automated bioreactor was carried out for four days in order to get the growth profiles, reducing sugar concentrations, and

for morphological changes observation. The liquid inoculum was transferred into sterile sawdust supplemented with 10% calcium carbonate and 1% rice bran. The bags were incubated until complete spawn run and opened for fruiting. The ability of each bag to produce mushroom sporophores was noted.

Results

Nutrient sources affect the production of high density mycelia used as liquid spawn/inoculum in the cultivation of edible mushrooms. A selection test of nutrient sources for liquid spawn production was carried out. The effect of carbon and nitrogen sources and concentrations on the mycelial growth of edible mushroom species were studied using petridish culture. Brown sugar (2%) was found to be the optimum carbon source compared to white sugar, sucrose, glucose, and lactose for most mushrooms. Organic nitrogen sources (rice bran, yeast extract and malt extract) showed greater mycelia proliferation compared to inorganic nitrogen source i.e. ammonium sulphate. Higher concentration of total nitrogen (6.4%) tested gave lower rate of radial growth compared to a total nitrogen concentration of 1.2% indicating that nitrogen can inhibit mycelial growth.

Hence, brown sugar (2%) and a combination of rice bran (0.4%), yeast extract (0.4%) and malt extract (0.4%) also designated as BRMY supported good mycelial growth. The mycelia morphology showed very dense, whitish, and tenacious mycelia mats compared to other media formulations. In addition, this media formulation was able to support growth of mycelia from sporophore tissue of the mushrooms. This enable pure culture to be obtained regularly from mushrooms fruiting bodies hence, long term storage of culture which is laborious and requires expensive equipment is not necessary.

The production of mushrooms mycelia in liquid BRMY showed the highest dry mycelia weght of $14.64 \pm 2.39 gL^{-1}$ by *S. commune* followed by *P. sajor-caju* ($9.96 \pm 1.14 gL^{-1}$), and *P. sapidus* ($7.56 \pm 2.03 gL^{-1}$). Moderate growth occurred in the cultivation of *P. cystidiosus* ($6.13 \pm 0.37 gL^{-1}$), *P. citrinopileatus* ($6.03 \pm 0.42 gL^{-1}$), *P. florida* ($5.51 \pm 0.79 gL^{-1}$), *Pleurotus* (hungarian) ($5.07 \pm 1.23 gL^{-1}$), *P. ostreatus* ($4.34 \pm 0.49 gL^{-1}$), and *G. tsugae* ($3.40 \pm 0.89\ gL^{-1}$). However, these media did not support growth of *Clitocybe* sp. ($1.90 \pm 0.58 gL^{-1}$) after ten days of fermentation.

The production of *P. sajor-caju* liquid spawn in BRMY medium under controlled conditions in an automated bioreactor was investigated. The highest biomass production was obtained on the third day of fermentation (11.72 ± 5.26g/L dry weight). This was relatively faster compared to shake flasks where maximum dry weight biomass of *P. sajor-caju* was achieved ($9.96 \pm 1.14 gL^{-1}$) after ten days of fermentation. Liquid spawn of *P. sajor-caju* grown in BRMY media in an automated bioreactor was able to produce sporophores when grown in solid medium consisting of sawdust supplemented with rice bran (1%) and calcium carbonate (10%). Fruiting bodies were produced two to three days after completion of spawn run which took two to three weeks.

This work forms a basis for the production of larger scale liquid inoculums for the cultivation of edible mushrooms in Malaysia. In addition to the production of liquid inoculums the medium formulation can also be applied for the production of mushrooms mycelia in the extraction of bioactives for the drug and nutraceutical industries. Mycelial

products are the 'wave of the future' because they ensure standardized quality and year around production.

Unresolved problems and future direction

The application of the liquid inoculum developed can be further optimized for the mushroom industry. Other growth parameters such as pH, aeration, temperature, inoculum age and size during fermentation needs to be optimized for different species of mushrooms. Future work should be to evaluate the storage life and conditions of the liquid spawn before distribution and to design an automated method of inoculation onto the fruiting substrates. The adaptation of liquid culture technology to the production of mycelia of higher fungi offers the possibility of industrial scale application to the mushroom spawn industry.

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"Detection and Characterization of Endonuclease activity of Mycobacterial Inteins"

Year 1998 MTSF Science & Technology Research Grant Recipient

Current research on intein gene in *M. tuberculosis recA* in Malaysia is non-existence but extensively done in other countries. The unique features of my research plan are the information generated from the mycobacterial inteins. Derbyshire et al. (1997) stated that endonuclease-containing inteins are far more common in modern genomes than the endonuclease-free inteins. The endonuclease seems to provide the means for intein to be maintained and, indeed, to spread among different genes, organisms, and perhaps kingdoms.

Furthermore, the inteins have been hypothesized to be evolutionarily related to the self-cleaving hedgehog proteins, which are involved in eukaryotic development pathways (Dalgaard et al., 1997; Koonin, 1997; Lee et al., 1994). It has been suggested that the hedgehog family, which exists in arthropods and all vertebrates from amphibians to mammals, arose from an intein that lost ligation activity (Dalgaard et al., 1997). Regardless, finding such nonmobile, autocatalytic, intein-like molecules with related functions in deeply branching organisms will ultimately help address issues relating to intein ancestry and evolutionary age.

As for the presence of the intein itself, it is proposed to affect the regulation of RecA protein synthesis (McFadden, 1996). Its effect is possibly by altering the overall DNA topology at the recA locus that may affect transcription, or by post-transcriptional effect on mRNA stability, translational efficiency or by conditional splicing of the intein (Davis et al., 1994; Colston and Davis, 1994).

The techniques involved were randomly and site-directed mutagenising the *M. tuberculosis recA* intein. Site-directed mutagenesis was based on polymerase chain reaction (PCR) which was a versatile procedure available to date. In the site-directed mutagenesis, the method utilised three types of oligonucleotide primers and two rounds of PCR. The intein was identified in *recA* gene of *Mycobacterium tuberculosis*. Since there were constrains in this slow growing and pathogenic bacterium, mutants were generated at splice junctions of intein in *galK* gene in *Escherichia coli*.

The methods used in this site-directed mutagenesis used high concentration of template DNA and productions of megaprimers, large cycling numbers and high expand fidelity (HEF) Taq polymerase. The experiments were able to introduce mutations in the N- and C-terminals of the intein. A mutagenic primer was produced at the N-terminal while 5 mutagenic primers were produced at C-terminal. These mutagenized primers differ in the sequence of amino acids from the parent sequence.

Randomly mutagenized the *M. tuberculosis* intein were cloned in a selectable reporter gene and the mutations in the intein were identified by automated sequencing. Specifically mutated combinations of amino acids at the splice junctions were known to be important for protein splicing in *M. tuberculosis* intein. Mutations were incorporated into *LacZ* of *Escherichia coli* and identified by automated sequencing. Spliced or nonspliced products were detected by immunoblot assay.

Achievement

Completed PhD under the supervision of Dr J Colston, Dr at National Institute Medical Research, Mill Hill, England. Field of study was *Mycobacterium tuberculosis* using the microarray technique for genomotyping and gene expression.

Publication

Presentation "M'sian Science & Tech Congress 2006", Kuala Lumpur

Associate Professor Dr. Rusli Daik
Dr. Musa Ahmad

Universiti Kebangsaan Malaysia

"Synthesis of Photoluminescent Polymeric Materials and its Application for Optical Fibre Sensor Development"
Year 1998 MTSF Science & Technology Research Grant Recipient

Introduction

Conjugated polymers were first discovered in early 1970s. Since then tremendous effort has been put forward to explore the possibility of using conjugated polymers as the replacement material for metals in terms of conductivity. This seemed to be possible since some conjugated polymers in certain circumstances exhibit electrical conductivity comparable to that of metals. However, in early 1980s researchers started looking at its pristine characteristics which is semiconducting properties. These include photoluminescence and electroluminescence. In fact, in late 1980s the first publication appeared reporting on the exploitation of conjugated polymer as light emitting layer in LED (light emitting diode).

At the time this grant was applied, the use of conjugated polymer in terms of photoluminescence as a sensing material, particularly in optical fibre sensor had not been reported. Whereas, it was known that conjugated polymers are photoluminescent with high brightness, and the brightness as well as the colour of emission are dependent on its environment. This brought about the idea of exploring the potential of conjugated polymer in optical fibre sensor.

Objectives

- To synthesis a series of photoluminescent organic polymers via transition metal catalysed polycondensation, namely the McMurry reaction
- To explore the possibility of using the photoluminescent polymers in optical fibre sensors

Methodology

In the first stage, aromatic diketone monomers were synthesized via acylation of aromatic acyl chlorides with aromatic compounds. These aromatic diketones were later polymerized via McMurry coupling reaction, in which transition metal compounds were used as the catalyst. The polymers were then used as sensing material in optical fibre sensor.

Results

It has been shown successfully that the polymers synthesized were potentially useful to be applied as sensing material in optical fibre sensor. The novelty of the research carried out has been proven as some of the results have been published in international journals.

Unresolved Problems

Some polymers were suitable as sensing material in only few aspects. The polymer that is suitable in all aspects has yet to be explored.

Publications

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Miss Kim Lian Hua
Associate Professor Peh Suat Cheng

Universiti Malaya

"Cell Death and Proliferation in Hodgkin's Lymphoma and related diseases"
Year 1999 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction & Objectives

The balance of cell growth, survival and programmed death (apoptosis) are central to the development and homeostasis of tissue. Inappropriate regulation of apoptosis is the common theme in the pathogenesis of neoplasm. The neoplastic cell of classical Hodgkin's lymphoma (cHL), the Hodgkin/Reed-Sternberg (H/RS) cell, is vastly outnumbered by a surrounding inflammatory infiltrate. How this neoplastic cell persists and disseminates in this hostile cellular environment are not known. Better knowledge gained in the interplay of factors influencing growth of the HIRS cells can result in the understanding of the mechanisms of tumour survival and immune-escape.

Studies have shown that the H/RS cell and its variants express antigens from members of the tumour necrosis factor receptor (TNFR) superfamily (e.g. CD30, CD40 and *Fas/Apo-l)* which is involved in the initiation and regulation of apoptosis upon interaction with their ligands from the surrounding lymphocytes. Despite the expression, the H/RS cells appear to survive. The prolonged survival and resistance to apoptosis of the H/RS cells may be due to the over-expression of the Bcl oncogene family (Bcl-2, Bcl-xL and Bax), as Bcl-2 and Bcl-xL, have anti-apoptotic ability. Bax, is able to induce death signal. However over expression of Bel-2 and Bcl-xL can inhibit the death signal transduced by Bax. Tumour suppressor genes e.g p53 and Rb are also known to be involved in regulation of cell cycle and apoptosis. All these genes may be dysregulated or mutated in Hodgkin's lymphoma, resulting in induction of proliferation and cytokine secretion.

The association of Eptein-Barr virus (EBV) and cHL is well established. EBV -infected *H/RS* cells typically expressed the viral latent membrane protein-l (LMP-l), which has been shown to have oncogenic properties, particularly the LMP-l gene variant with a 30-base pair (bp) deletion. The LMP-l 30-bp deletion variant has been shown to influence cell growth, differentiation and apoptosis by interacting with the tumour necrosis factor receptor-associated factors (TRAFs) and the NF-KB transcriptional regulators. How the *H/RS* cell 'exploits' the TNFRs, oncogenes and tumour suppressor genes expression as well as interplay of EBV to favour its own survival remains largely unknown.

Methodology

66 cases of cHL were retrieved from the archives of the Department of Pathology, University Malaya. Immunohistochemistry was performed for detection of the expression of TNFRs (CD30, CD40 and CD95), Bcl-2 family members (Bcl-2, Bcl-x, Bcl-xL and Bax), tumour suppressors (p53 and Rb) as well as Ki-67 for assessment of proliferation. In situ hybridisation for the presence of EBV early RNA (EBER) and apoptosis by TUNEL was also carried out. Detection of the 30-bp gene deletion in the viral LMP-l gene was performed by PCR and Southern blot analysis.

Results

Ki-67 proliferation marker was detected in 86.7% of the cHL cases. Expression of the TNFRs (100% CD30, 93.9% CD40, 90.5% CD95) and tumour suppressor proteins (81.5% p53, 89.1% Rb) were frequently detected in the H/RS cells of these cases. Bcl-2 was only expressed in 43.5% of the cases whereas higher level of Bcl-x (87.5%), Bcl-xL (67.2%) and Bax (74.6%) was observed. Presence of EBV was observed in 53% of the cases, with a statistical significant association with the mixedcellularity subtype as compared to the other subtypes (p=0.003). Apoptotic body was rarely observed in the cHL involved tissue. Deleted LMP-l variant was detected in 63.3% (19/30) of the EBV-positive cases, where 30% of these cases harboured dual LMP-1 variant (concurrent presence of deleted and non-deleted variant). Statistical analysis revealed an inverse pattern of Bcl-2 expression with presence of EBV (p=0.003), whereas expression of Bcl-xL showed an inverse pattern with presence of apoptotic H/RS cells.

The results showed that high frequency of cHL express apoptotic-regulating proteins. However, the H/RS cells did not undergo apoptosis as demonstrated by TUNEL, suggesting that these proteins may interact with each other for the survival of H/RS cells. The inverse pattern of Bcl-2 expression and EBV infection suggests that in cases where EBV is negative, Bcl-2 may play an important role in anti-apoptosis; whereas when EBV is presence, the Bcl-2 is replaced by the EBV BHRF-l protein, which is a homologue to Bcl-2, in anti-apoptosis. The inverse relationship between Bcl-xL expression and apoptotic H/RS cells also suggest that Bcl-xL may be a more potent protein in inhibiting apoptosis as compared to Bcl-2. Detection of the deleted LMP-l variant in cHL may further support the pathogenic role of EBV in cHL.

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Miss Nazni Binti Wasi Ahmad
Dr. Lee Han Lim
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Institute for Medical Research

"Detection of Bancroftian Filarial Worm Infection in Urban Culex Quinquefasciatus Adults and the Susceptibility Status of Cx Quin. to Insecticides"
Year 1999 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

More than 1.1 billion people i.e 20% of the world's population live in areas where they are at risk of infection from lymphatic filarial parasites. Bancroftian filariasis occurs in two forms: in the most common form, the microfilarie circulate in the peripheral blood at night, whereas in the second form they occur continuously in the blood but increase in number during the day. The vectors of the first form are *Culex quinquefasciatus* and certain *Anopheles* species (which bite at night). The second form is found in the South Pacific and in some rural areas in Southeast Asia where the main vectors are daytime mosquitoes such as certain *Aedes* species. In rural areas, bancroftian filariasis is mainly transmitted by some *Anopheles* species that are also malaria vectors, and by *Aedes*.

Although *W bancrofti* infection was thought to be present among the early immigrants from India and China, previous study in the early 60's in Malaysia has indicated the presence of *W bancrofti* infection in rural village and in aborigines living in close proximity to Kuala Lumpur. With the increased inflow of migrant workers from Bangladesh and India, both endemic for bancroftian filariasis into Malaysia, the possibility of migration could facilitate the spread of *W. bancrofti* to places where lymphatic filariasis has never existed or from where it has been eradicated especially in the urban areas where *Cx.quinquefasciatus* is found in abundance. Thus, it is necessary to detect the bancroftian filariasis worm in the *Culex quinquefasciatus* populations and implement a successful control programme in line with the WHO objective of the elimination of filariasis as a public health problem globally (GPELF) by 2020. In this study our objectives were (i) to study the bionomics of *Cx. quinquefasciatus* , (ii.) to determine the susceptibility status of the *Cx quinquefasciatus* against insecticides and (iii) to develop a more convenient and sensitive technique to detect the presence of the *Wuchereria bancrofti* larvae in mosquitoes

In the present study we have studied the vector binomics of *Cx quinquefasciatus* from three different sites, that is, urban , a semi-urban and a rural area. The sites were chosen based on the concentration of the migrant workers from endemic countries.

The peak biting period and no. of bites per person per hour obtained from our study is shown in this table below::

Sites	Biting Peak (hr)	Landing Rate per person per hour
Urban	2400 – 0100	5.1/person /h
Sub – urban	2300 – 2400	6.4/person/h
Rural	0100 – 0200	1.2/person/h

All mosquitoes were identified using a dissecting microscope and only the thorax of *Cx quinquefasciatus* were dissected for of filarial parasites. The mosquitoes were further dissected for the parity and dilatation rate. All mosquitoes were identified until species level and the total number were recorded.

The total number of mosquitoes caught was 3,681 mosquitoes of all fauna. Of this, *Cx quinquefasciatus* accounted for the highest with 1,968 (53.5%).

The overall parity rate for *Cx quinquefasciatus* was 56.9% and the nulliparous rate was 43.1%. The para 1 age group was dominant, indicating that most of the mosquitoes collected were those that had laid eggs for the first time and they were considered as young mosquitoes. These findings also indicated that in the study areas, the mosquito larvae were not under control pressure since new mosquitoes were continuing to emerge.

Screening of filarial parasite by mosquito dissection showed that no filarial worm was detected in any single *Cx quinquefasciatus,* indicating for the time Malaysian *Cx quinquefasciatus* from the study sites were free from the *Wuchereria bancrofti* parasite.

The other states in which the study was conducted was in Cameron Highlands (Pahang), Bayan Baru (Penang), Muar (Johor) and Rantau Panjang, Pengkalan Cepa , Kota Bharu (Kelantan). The sites were chosen based on immigrant population concentrations either near by "kongsi houses" or settlement of Bangladeshi workers. From these sites the most abundant mosquitoes collected were *Cx. quinquefasciatus* and dissection of these mosquitoes for filarial worm indicated that all mosquitoes dissected were free from filarial worm, though these mosquitoes were caught near immigrant settlements.

We have further conducted studies in Kuala Lumpur at Ampang Hilir, Bukit Maluri, Damansara, Kampong Pasir and Segambut Dalam. The total number of *Cx. quinquefasciatus* caught were 1799. It was not possible to conduct dissection on single mosquito due to the large number of *Cx. quinquefasciatus* caught. Hence, the mass dissection technique was used to detect the presence of filarial worm. Again, there was not a single mosquito found infected with filarial worm around Kuala Lumpur vicinity.

The susceptibility status of *Cx. quinquefasciatus* to various group of insecticides was also determined. Though, in Malaysia we do not have a specific programme for *Culex* species control, the indiscriminate use of insecticides globally in agricultural sector as well as in the public health sector would have led to the phenomenon of resistance development in *Cx. quinquefasciatus*. The adult from two different localities from Kuala Lumpur were used in this study. The study sites were Pantai Dalam and Ampang Hill. Standard WHO (1981,a,b) adulticidal and larvicidal procedures for testing insecticide susceptibility in mosquitoes were used.

The resistance ratio of the insecticides for the adult mosquitoes in decending order based on the LT50 value for both the Ampang Hill and Pantai Dalam was DDT=malathion > fenitrothion > propoxur > permethrin > cyfluthrin

> lambdacyhalothrin. Both the susceptible and resistant strain had a similar trend of susceptibility to insecticides. Though the pyrethroids are the most effective insecticides, precautionary measures should be undertaken since the resistance ratio for permethrin, cyffluthrin and lamdacyhalothrin were in the range of 1.41 folds - 12.20 folds . The LC50 values of *Cx quinquefasciatus* larvae against the insecticide malathion for both the Ampang Hilir strain and Pantai Dalam strain were !7,988 folds and 14,053 folds respectively. Our study indicated that though the larvae were highly resistant to malathion , its resistance ratio to temephos was 3.02.

In this study we also report findings of the susceptibility status of both susceptible and resistant strain (to malathion) of *Culex quinquefasciatus* f to *W. bancrofti* (Wb). Mosquitoes were fed on the infected blood from a patient from Myanmar with MF count of 4 WB/60ul blood. DNA was extracted from patient blood as well as mosquitoes using the Qiagen DNeasy Kit. Polymerase Chain Reaction (PCR) amplification of *W. bancrofti* Ssp 1 repeat was carried out as described by Ramzy et al.,(1997). The percentage of infective L3 larvae in resistant and susceptible strain of *Cx. quinquefasciatus* detected by PCR was 20% and 33.3%, respectively. McCarroll et.al.,, (2000) stated that insecticide resistant *Cx quinquefasciatus* were less likely to transmit filariasis compared to the susceptible strain. From this brief study it can be concluded that there is a potential for an establishment of urban transmission of *Wuchereria bancrofti* in this country. Hence, the screening of all immigrant workers into this country for filariasis at the entry point is very important to avoid re-emergence of the disease.

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2. Vythilingam I, Tan CH, Nazni WA (2005) Transmission potential of *Wuchereria bancrofti* by *Culex quinquefasciatus* in urban areas of Malaysia Tropical *Biomedicine* 22(1): 83-86.

Dr. Tengku Sifzizul Tengku Muhammad

Universiti Sains Malaysia

"Molecular Cloning and Mapping of the 5' Flanking and Promoter of Regions of Bovine Lipoprotein Lipase"
Year 1999 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

In this research project, a 412 bp PCR product of the 5' end of the full length bovine LPL cDNA was successfully amplified, cloned and sequenced by utilizing a technique known as RNA ligase-mediated rapid amplication of 5' cDNA ends (5' RLM-RACE). The sequence data revealed that the 3' end of the PCR product shared almost 100% identity with the 5' end region of the published bovine cDNA (Accession No. M16966) over 215 bp region, indicating that the 5' end of the full length bovine LPL cDNA was successfully cloned and sequenced. The sequence was then deposited in GenBank and EMBL databases under accession number AY216661. In addition, the transcriptional start site (TSS) for bovine LPL gene was mapped at the nucleotide cytosine (C), 132 bp upstream of the translational start codon (TSC). Subsequently, primers were designed based on the sequence of the cloned 5' end region, and by utilizing a PCR based approach known as Genome Walker, a 990 bp of the promoter region of bovine LPL gene was successfully amplified, cloned and sequenced. The nucleotide sequence alignment of this promoter region with the corresponding human, rat and mouse LPL promoter region revealed 76%, 68% and 68% identity, respectively. A few important potential transcription factor binding sites (TATA box, CCAAT box, PPAR/RXR heterodimer binding site, C/EBP binding site, PRE, ARE, GRE, GC box and Oct-1 binding site) were predicted to be present in the promoter region. The predicted TATA box located 145 bp upstream of TSS was shown to be functional by using electrophoretic mobility shift assay (EMSA).

Dr. Fong Mun Yik
Professor Koh Chong Lek
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Universiti Malaya

"Molecular Approach to determine the target site of the drug atovaquone in Toxoplasma gondii"
Year 1999 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Toxoplasma gondii is an obligate intracellular coccidian protozoan that is widespread in nature. It has been estimated that 15-85% of the world's population is infected and a variety of warm-blooded animals can also serve as hosts. Traditionally, its major disease manifestation has been in the developing foetus where maternal infection during pregnancy can lead to severe neurological disease and even death. Disease in healthy individuals is mild or asymptomatic but in recent years, this parasite has emerged as a major opportunistic pathogen of immunocompromised individuals, especially those infected with HIV. Available treatments for *T. gondii* infection include pyrimidine and sulfonamide antifolates and clindamycin. However, the side effects of these drugs limit their usefulness. Hence, new drugs are needed. Prominent among the newly emerging drugs is hydroxynaphthoquinone 566C80, generically known as atovaquone. This drug, initially developed as an anti-malarial agent, has been shown to have significant activity against *T. gondii*, both *in vitro* and *in vivo*. The mode of action of atovaquone in *T. gondii* is still unclear. The *de novo* pyrimidine synthesis has been postulated to be a possible target site, but biochemical analysis on laboratory derived atovaquone-resistant *T. gondii* mutants suggests that atovaquone affects the mitochondrial *bc*1 complex.

Objectives

The aim of this study therefore was to probe further the role of cytochrome *b* as target site for atovaquone in *T. gondii*. Molecular approach was used as no such approach had been employed to provide insights into the action of atovaquone in *T. gondii*. In order to achieve the aim of this study, the following steps were carried out:

- Chemical mutagenesis to develop atovaquone-resistant *T. gondii* mutants
- Isolation, sequencing and characterisation of the cytochrome *b* gene of the mutants
- Correlation of mutations in the gene to atovaquone resistance

Methodology

(a) Cloning and sequencing of the cytochrome *b* gene

Before the commence of the project, a sequence search revealed that the nucleotide sequence of the mitochondrial cytochrome *b* gene of *T. gondii* had yet to be determined. Thus, a combination of 3' and 5' rapid amplification of cDNA ends (RACE) was used to clone the cytochrome *b* gene. Prior to the RACE steps, RNA of *T. gondii* was isolated from freshly harvested tachyzoites. Resulting 3' and 5' RACE fragments were cloned into a plasmid vector, and subsequently sequenced. The downstream region of the 5' RACE fragment overlapped with the upstream region of the 3' RACE fragment, and hence, the nucleotide sequences of both the fragments were combined to obtain a full-length sequence of the cytochrome *b* gene.

(b) Dose response

The response of *T. gondii* when treated with atovaquone, was measured by performing titration in a 24-well plate. Host Vero cell cultures were infected with 500 fresh tachyzoites, and 3 hours later, various dilutions of atovaquone were added to the cultures. Parasite growth was determined by harvesting and determining the total count of tachyzoites from the each microwell at 2 days post infection. Both intracellular and extracellular tachyzoites were harvested. For determining drug potency, the concentration that resulted in 50% inhibition of parasite growth at day 2 post infection was taken as the IC_{50}.

(c) Generation of *T. gondii* mutants resistant to atovaquone

Intracellular tachyzoite culture was treated with ethylnitrosourea and the mutagenised tachyzoites were released from the host cells by passage through a syringe. The parasites were then grown for 3 days in a fresh monolayer of host cells in the absence of atovaquone. Following this, the parasites were grown using the same conditions, but in the presence of atovaquone (at 50% inhibitory concentration IC_{50}, determined previously). The parasites were passaged several times until mutants emerged several days later. From the heterogeneous population of tachyzoites, two mutants, designated R1 and R2, were cloned by limiting dilution. The resistance to atovaquone of these mutants was compared to the non-resistant parental RH strain. On the basis of cell count per culture per time incubation, the IC_{50} for the mutants was between 20 and 200-fold higher than the parent strain.

(d) Nucleotide sequencing of the cytochrome *b* gene of the atovaquone-resistant mutants

The nucleotide sequences were determined using routine PCR-sequencing approach. Primers for PCR were designed based on the cytochrome *b* gene nucleotide sequence determined previously [section (a) above]. The sequences of the atovaquone-resistant mutants were compared with that of the parental RH strain.

Results

(i) Properties of the *T. gondii* cytochrome *b* gene and its encoded polypeptide

Analysis of the nucleotide sequence revealed a coding region of 1080 base pair in length, which encoded a polypeptide of 359 amino acids. Codon frequency analysis showed a high preference for adenine (A) and thymidine (T) residues in the third position of each codon. The predicted molecular weight and isoelectric point of the polypedtide were 40619 and 9.63, respectively. The polypeptide was highly hydrophobic as 36% of the amino acid residues are of the hydrophobic type. It was observed that the polypeptide had high leucine content (15% of total amino acid residues). Six cysteine residues were noted, and they were possibly for the formation of three disulfide bridges in the tertiary folding of the polypeptide. The polypeptide was 65% similar (54% identical) to the malaria parasite *Plasmodium falciparum*, and 56% similar (44% identical) to human cytochrome *b*. Hydropathy analysis revealed eight hydrophobic transmembrane domains.

(ii) Nucleotide sequences of the cytochrome *b* gene of the atovaquone-resistant mutants

Two changes were identified in the sequences of the mutants, resulting in single amino amino acid changes. The nucleotide change in the gene of R1 occurred at position 385, i.e. ATG to TTG, which resulted in the amino acid substitution from methionine to leucine (position 120 of the polypeptide). For R2, the nucleotide change was

located at position 760, where the codon ATC became CTC, and this resulted in amino acid substitution from isoleucine to leucine (position 254 of the polypeptide). Both mutations occurred within the regions predicted to be the Q_O domain of cytochrome *b* protein. The methionine to leucine change in the gene of R1 occurred within a strongly conserved region in the domain of the protein. The isoleucine to leucine change in R2 was within a conserved region in the domain.

(iii) **Prediction of target site of atovaquone in *T. gondii***

The methione 129 and isoleucine 254 of *T. gondii* cytochrome *b* protein are in the same region of the Q_O domain of cytochrome *b* of several other organisms. The fact that mutations at these sites occurred in the atovaquone-resistant mutants may suggest that atovaquone binds to this domain. Published reports on with the malaria parasite *Plasmodium* cytochrome *b* has shown that the isoleucine residue equivalent to isoleucine 254 is mutated in atovaquone-resistant *P. falciparum*. Likewise, the methionine residue equivalent to methionine 129 in *P. berghei* cytochrome *b* is mutated in atovaquone-resistant mutants of this species. Mutations in other residues within the domain were also reported in atovaquone-resistant strains of *Pneumocystis carinii*.

Therefore, the Q_O domain of cytochrome *b* is very likely the target of the drug atovaquone in *T. gondii*.

Publication

This research has been submitted to the journal *Experimental Parasitology* (2008) for publication.

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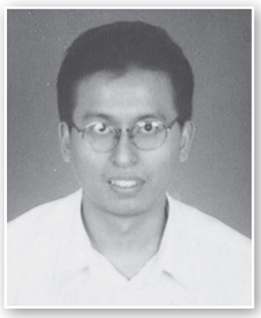

"Mechanism of Toxicity and Apoptosis of Nonsteroidal Anti-Inflammatory Drugs"
Year 1999 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Nonsteroidal anti-inflammatory drug (NSAID) are used for the treatment of a variety of rheumatic and musculoskeletal disorders (Kenny GNC 1992). NSAIDs have been associated with several rare but severe adverse reactions, that include Stevens-Johnson syndrome, pancreatitis, aplastic anemia, thrombocytopenia and hepatotoxicity (Tarazi et al). The pattern of hepatotoxicity in man involves mainly cholestasis and mixed hepatocellular/cholestatic injury. The mechanism/s of NSAID-associated hepatotoxicity remains unknown. However, the report of Tarazi et al. (12) that 66% of the 91 cases of hepatotoxicity had clinical hallmarks of hypersensitivity suggests that immunological idiosyncrasy may be implicated. NSAIDs have produced a variety of distinct renal syndromes: acute ischemic renal insufficiency and acute interstitial nephritis caused by haemodynamic effect, a direct result of COX inhibition (Kenny GNC 1992; Brater DC 1988). The most severe is the analgesic-associated nephropathy where the cause remains unknown (Bennet WM and DeBroe ME 1989; Murray M.D. and Brater, DC 1993). The NSAID sulindac is the first pharmacological agent to cause regression of colonic tumours in familial adenomatous polyposis (FAP) (Waddel WK and Loughry RW, 1983; Giardiello et al. 1993). The mechanism by which NSAIDs mainly sulindac exert the antitumor effect remains unclear. However, sulindac inhibits proliferation of colon cells in vitro (Shiff et al. 1996) and induced apoptosis independent of prostaglandin pathway (Hanif et al. 1996) and does not require p53 induction (Piazza et al. 1997). Maintenance of tissue are achieved by regulated processes that includes cell proliferation, differentiation and apoptosis (Saunders 1966). Cells will undergo apoptosis which partly depends upon the balance between proteins that mediate cell death i.e. BAX (Oltvai et al. 1993) and proteins that promote cell viability i.e. BC1-2 (Jacobson et al. 1993).

Objectives

1. to evaluate the toxicity of NSAID Piroxicam and Mefenamic acid mainly effects on the liver and kidneys.
2. to determine the in vitro toxicity and apoptosis of these two NSAIDs.

Materials and Methods

In vivo studies : Male *Balb/c* mice from Institute of Medical Research (IMR) 30g to 40g in body weight were kept in plastic cages (8 mice/cage) with wood shaving as bedding. They were acclimatized to the vivarium environment for one week prior to the experiment and were fed standard laboratory pellets with tap water *ad libitum***.** The mice received single ip injections of mafenamic acid (at 100 or 200 mg/kg in 10% Dimethyl sulfoxide/Palm oil) or were treated daily with the drug ip (at 50 or 100 mg/kg in 10% Dimethyl sulfoxide/Palm oil) for 14 days. Control animals received equivalent amount of dosed vehicle. Six hours after the final dose, mice were sacrificed by cervical dislocation and their kidneys were removed. A section was fixed in formalin for 24 hr before processing for histological examination. Venous blood samples from mice in the subchronic dosing study (50 µ1) were taken prior to and 14 days post-dosing from the tail vein into heparinized collecting vials (0.3 mL vials containing 15 UmL^{-1} lithium heparin: Sarstedt, UK). Blood samples were centrifuged at 4000 rpm for 15 min and the plasma transferred to 0.5 mL plastic eppendorf tubes, stored at –20^{o}C until analysis. Plasma Alanine aminotransferase (ALT) activity was measured using a commercial kit (Sigma Chemicals, UK).

In vitro studies : Female Sprague Dawley rats (200-250 g in body weight, n=4/group) housed in plastic cages in groups of four with wood shavings as bedding. The rats were fed on rat pellets and tap water ad libitum. The animals were cared for in accordance with the guidelines of the University Ethical Committee. In experiments with phenobarbital induction, rats were dosed intraperitoneally at 75 mg/kg in 0.9% (w/v) sodium chloride daily for 4 consecutive days. The animals were anaesthetized using pentobarbitone sodium at 60mg/kg ip 24 hr after receiving the last dose of Phenobarbital for liver perfusion. Hepatocytes from control (n=4) or Phenobarbital induced rats (n=4) were isolated by a two-step collagenase perfusion technique as previously described 19,20. Viability of freshly isolated rat hepatocytes was determined by trypan blue exclusion 21. After isolation, hepatocyte suspensions were incubated at a density of 1 x 106 viable cells/mL in L15 incomplete medium. Itraconazole or fluconazole (0.0001 to 1.0mM) were added in DMSO (final DMSO concentration of 1.0% v/v). Control hepatocyte suspensions were incubated with an equivalent amount of DMSO. The flasks were sealed in 95% O2/5% CO2 and placed in a shaking water bath at 37oC. Samples were taken from these flasks at time points of 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5 and 6 h. Viable cells were determined by lactate dehydrogenase (LDH) activity in medium and in the lysed cells at each time point. LDH activity was assessed spectrophotometrically as described by Marshall and Caldwell (1992)22. The cytotoxicity effects of mefanamic acid and Piroxicam on MCF-7wt breast cancer cells, Caco-2 Cells (Colon cancer cells) and Hep G2 cells (Liver cancer cells). MTT assay was performed to determine the effects of mefenamic acid and piroxicam on MCF-7wt, Hep G2 and Caco2 cell lines. Data was expressed as mean ± sd from four separate hepatocyte isolations and analysed using student's t test or analysis of a variance (ANOVA). Values of $p \leq 0.05$ is considered significant. For significant treatment means obtained by ANOVA, they were subjected to Duncan multiple posttest.

Results

a) In vitro Studies (Cancer Cells)

Piroxicam showed statistical reduction in the Caco-2 cell line. With the reference of control, cell survival of Caco-2 cells at concentration of 0.01µM, 0.1µM, 1µM, 10µM, and 100µM reduced approximately 5%, 8%, 14%, 21% and 35% respectively. MCF-7wt and Hep G2 cells revealed reduction in viability at 10 and 100µM (approximately 10 to 20%). Mefenamic acid caused cytotoxicity in all cell lines but was not statistically significant. With reference to the control, cell survival of MCF-7wt cells at the concentration of 0.01µM, 0.1µM and 1µM was higher. However, at 10µM, and 100µcell survival was reduced about 12% and 15% respectively. In the Hep G2 cells, the cell survival was not significantly reduced in all the drug concentration. In the Caco-2 cells, only at 100µM mefenamic acid concentration revealed 17% reduction in cell survival.

b) In vitro Studies (Normal Cells)

The effect of piroxicam in hepatocytes suspension was evaluated. From dose dependent data, 30 min incubation of the 0.1mM and 30 min and 3 hour of the 1.0 mM were significantly different ($p \leq 0.05$). The rest of the data were not significantly different. From time dependent data, there were revealed that piroxicam treated rat hepatocytes dependently from time 0 until time 4. Mefenamic acid revealed less potent to kill normal liver hepatocytes when compared to piroxicam.

c) In vivo Studies

Hepatocyte degeneration was detected in the periportal and midzonal regions of livers from the chronic 50 mg/kg mefenamic acid treated mice. Abundant of pyknotic nuclei were observed. Liver of the mice treated with high dose of mefenamic acid (100 mg/kg for 14 days) showed massive degeneration involving periportal, midzonal and centrilobular region. Scattered areas of necrosis were noted with some focal inflammatory reactions. There were abundant hepatocytes with pyknotic nuclei. Liver of the mice treated with single 100 mg/kg mefenamic acid showed mild focal degenerative changes at periportal region. Midzonal degeneration with pkynosis of hepatocytes was observed

in the liver resulting from high dose mefenamic acid treatment (single 200 mg/kg). Liver sections of the control mice showed the normal histology at periportal, centrilobular and midzonal. As in the histological findings of single doses and repeated doses of mefenamic acid showed a dose-dependent insult to mice's liver. The injuries progressed from mild reversible degeneration to irreversible cellular necrosis. Repeated doses of mefenamic acid causes more profound hepatocyte lesions compared to single doses treatment. The effect of piroxicam in liver. Histologically, there were mild degeneration at pericapsular and fibrin organization was observed in the liver of mice treated with piroxicam. Portal triad region degeneration were observed in the liver of mice treated with 200 mg/kg. No histological changes were observed in centralobular region. Haemorrhage and congestion was detected in piroxicam treated rat kidneys dose dependently. Control mice livers and kidneys revealed normal histology.

Conclusion

1. Piroxicam is able to exert a dose-dependent cytotoxic effect in the cancer cell lines (Caco-2 cells) but not in the MCF-7wt and Hep G2 cells. Mefanamic is not effective to exert cytotoxic effects against all MCF-7wt, Hep G2 and Caco-2 cell lines. These results show the potential use of piroxicam in colon cancer patients.
2. It was revealed that piroxicam induced hepatocytes death and was expressed time and dose dependently which was more toxic than mefenamic acid.
3. Interestingly, in vivo studies revealed that piroxicam was less toxic to the livers than mefenamic acid. It was proven by histologically and the activities of liver enzymes in the serum.

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Miss Tey Wan Chee

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"Molecular Characterization of a leuβ-Complementing DNA from *Thermus ruber* in *Escherichia coli*"

Year 1999 MTSF Science & Technology Research Grant Recipient

The *leuB* gene of a moderate thermophile, *Meiothermus ruber* (previously *Thermus ruber*), (Nobre *et al*., 1996) encoding the enzyme β-isopropylmalate dehydrogenase (β-IPMDH; EC 1.1.1.85) of leucine biosynthetic pathway was subcloned, sequenced, and expressed in *Escherichia coli*, and the recombinant β-IPMDH was partially characterized.

Previously, by using the genetic complementation method and a shotgun cloning strategy, Chan (1995) succeeded in isolating a 13 kb *Pst*I *leuB*-complementing DNA from *M. ruber* in *E. coli* HB101, a *leuB* auxotroph. Subsequently, Hee (1997) subcloned a 2.9 kb *Eco*RI-fragment that still retains the *leuB*-complementing activity from the 13 kb *Pst*I DNA.

The complete nucleotide sequence of the 2.9 kb *Eco*RI *leuB*-complementing DNA from *M. rube*r was determined. It comprised 2,652 nucleotide pairs and contained the *leuB* gene of *M. ruber* of 1,053 bp encoding 350 amino acid residues. The molecular weight of the deduced protein product of the gene was calculated to be 38,889. The GC content of the *leuB* gene of this moderate thermophile was 67.62%, lower than the GC contents of extreme thermophiles but higher than the GC contents of mesophiles.

The deduced amino acid sequence of *M. ruber* β-IPMDH was compared with other similar amino acid sequences from thermophilic and mesophilic bacteria. It showed the highest similarity (77%) with β-IPMDH of *Thermus thermophilus* HB8.

Two genes, *leuD* and *ilvD,* were found upstream and downstream of *leuB*, respectively. The complete nucleotide sequences of these two genes were obtained by sequencing the 5.110 kb *Pst*I fragment, previously trimmed from the 13 kb *Pst*I *leuB*-complementing DNA by Hee (1997). The organization of the two leucine biosynthetic genes, *leuDB*, in *M. ruber* is different from that in *Thermus aquaticus* (thermophile) and mesophiles. Neither *leuA* nor l*euC* was detected. Hence, the organization of *leuABCD* in *M. ruber* remains unknown.

The *leuB* was amplified by the polymerase chain reaction with two designed primers for direct cloning into a pUC-derived expression vector, pRSETA, which has T7 promoter and six histidine sequences. The recombinant clone, *MrleuB*/pRSETA, obtained was maintained into *E. coli* K-12 strain, TOP10F', but was transformed in *E. coli* B strain BL21(DE3)pLysS for protein expression and HB101 to confirm its *leuB*-complementing activity. The expressed fusion protein was shown by SDS-polyacrylamide gel electrophoresis (SDSPAGE) to be about 41 kDa, inclusive of the 3 kDa of the N-terminal 6xHis-tagged peptide from the expression vector, pRSETA. The expressed β-IPMDH was purified and confirmed by Western blot analysis.

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"Red Blood Cell Cytoskeletal Proteins in β-Thalassemia Trait associated resistance to Malarial Infection"
Year 1999 MTSF Science & Technology Research Grant Recipient

Introduction

In a malarial endemic area, the high mortality form of malaria infection has drastically modified the human genome by the selection of genetic variable that reduces the risk of dying from malaria. Such genetic abnormalities presumed to be protective are the presence of abnormal hemoglobin and thalassaemia. The population of this area slowly acquires the natural immunity that allows them to tolerate the parasite load without developing severe symptoms. Thalassaemia (α and β), an inherited disease of hemoglobin synthesis can be divided into major, intermediate and minor (trait) forms. A trait or carrier is asymptotic. In Malaysia the commonest abnormal hemoglobin and thalassaemia among population in malaria endemic area is HbE and β-thalassaemia trait. It has been suggested that resistance to malarial parasite infection in these population is caused by changes of red blood cell morphology from normal discoid shape to ovalocytes. Ovalocytosis red cells are much less deformable in comparison to normal red cells suggesting that resistance to parasite invasion is the results of genetic mutation that causes increased membrane rigidity. The aim of this research is to determine the changes in red blood cell cytoskeletal protein components occurred in β-thalassemia trait individuals and to understand how these changes affect malaria resistance.

Objectives

i. To investigate the presence of abnormal hemoglobin such as HbA_2, HbF and HbE and the frequency of β-thalassemia in a selected population
ii. To investigate cytoskeletal proteins of red blood cells such as α-spectrin, β-spectrin, ankyrin, band 3 and band 4.2.
iii. To determine the erythrocyte membrane fragility
iv. To measure the IgG levels of β-thalassemia individuals in a selected malaria endemic area.

Methodology

Orang Asli population (200 subjects, age 7 – 61 years) in Post Piah, Sungai Siput Perak has been selected as samples from a malaria endemic area in this research. Blood were collected in EDTA tubes and analyses were performed in Biochemistry Laboratory, School of Biosciences and Biotechnology, Faculty of Science and Technology, UKM. The presence of hemoglobinopathies was based on percentage of Hb A_2, Hb F, hemoglobin instability and Hb S solubility test. The same parameter were also analysed on Biochemistry students (BK samples) for comparison to a non malaria endemic area population. The components of red blood cell cytoskeleton protein were investigated on both Orang Asli of Post Piah and UKM Biochemistry students. Pyrimidine 5'-nucleotidase screening test and thin blood film examination were performed and red blood cell membrane proteins were electrophoresed on SDS-PAGE

Results

Based on the findings of the various parameters studied, it is fervently believed that hemoglobinopathies are present in Post Piah population. In Post Piah children, 49.5% of the subjects are found to have abnormal Hb A_2 levels whilst 83.8% of this group shows abnormal percentage of Hb F. Hemoglobin instability test on the children an adult subjects revealed that 50% and 53.4% of the respective groups have abnormal hemoglobin stability. However, non of the Post Piah samples were tested positive for the presence of Hb S with the Hb S stability test. The intensity of Band 1 protein in Post Piah samples was 6.93% lower than BK samples but the intensity of band 3 protein in Post Piah samples was 2.37% higher than the BK samples. These results suggest that the component of spectrin in red blood cell membrane was decreased in Post Piah samples and this could be correlated with the presence of abnormal hemobglobin and red blood cell morphology in the malaria endemic area population.

Unresolved problems and future direction

The aim of this research is to investigate how changes in red blood cell cytoskeletal protein components occurred in β-thalassemia trait individuals. There are many other techniques and analysis that should be done in order to understand how these changes affect malaria resistance. This research should also be extended to many other malaria endemic areas especially in Sabah and Sarawak.

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"Laboratory and field studies of Sand flies in relation to Bionomic and infection of *Leishmania* Parasites"
Year 1999 MTSF Science & Technology Research Grant Recipient

Leishmaniasis is a disease caused by a protozoan called *Leishmania* spp. Visceral leishmaniasis or kala-azar (black sickness) is cause by *L. donovani*. Kala-azar is a common disease in certain region of Bangladesh, Brazil, India and Sudan. The influx of migrant workers to Malaysia has brought with them the risk of disease transmission. In Malaysia the sand flies are indigenous but no local leishmaniasis case was reported before.

Sand flies of *Phlebotomus* and *Lutzomyia* are the only proven genera that are reported as vectors of leishmaniasis. Studies on sand flies existence, distribution and breeding habitats in Malaysia were carried out in year 1999 to 2003. Focus was given to the limestone areas, such as Gua Kelam in PERLIS, Gunung Senyum in PAHANG, Gua Kota Gelanggi in PAHANG and Batu Caves in SELANGOR, due to reported presence of sand flies.

CDC-IMR modified light-trap method was found to be useful for sand flies collection compared to other methods such as sticky trap and resting catches. Most of the flies were found outdoors but for cave, a lot of sand flies can be found both out- and inside the caves.

The *Phlebotomus* species that were identified are *P. argentipes*, *P. stantoni*, *P. major, P. asperulus, Phlebotomus* sp. and four unknown species. Under *Sergentomyia* genera, 19 species were identified with 10 unknown species.

Biting activity of sand flies at limestone areas were also observed at several places. Sand flies in certain areas *viz*. in Batu Caves were observed not to bite human i.e. no antrophophily activity even though the sand fly population was very high. However, in some other areas *viz*. Gunung Senyum, Gua Kota Gelanggi and Gua Musang the sand flies do bite human. The sand flies activity at Gunung Senyum was observed to start at 2100 hours, peak at 0100 hours and stop at 0700 hours.

A comparative study was conducted in cattle shed and residential areas. The flies were found near residential area and cattle sheds. However, the number of sand flies collected was low, between 1-9 flies per light trap. In residential area, two species of *Phlebotomus* were recorded *viz*. *Phlebotomus stantoni* and *P. asperulus*. In cattle sheds two species of *Phlebotomus* were also recorded *viz*. *Phlebotomus stantoni* and *P. major*. No sand fly was found in selected residential areas and at the high land area, Cameron Highlands.

Random screening for promastigote-liked parasite in 30% of sand fly from each collection of each area was negative. A complete life cycle of sand flies was found to vary between 40 to 45 days.

Publication

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Dr. Aileen Tan Shau Hwai

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"The development and research into the production of fast growing Triploid Oysters for Malaysian Food Industry"
Year 1999 MTSF Science & Technology Research Grant Recipient

Introduction

Malaysian oyster industry is still in the cottage-operation stage and this is due to the limited spat supply (Zulfigar, 1997). More than 97 % of the spat supply for this operation comes from wild spat collection (Tan, 1997) and the availability of spats is affected by the vagaries of the seasons (Tan, 1992; Gunarto, 1994). High mortality of the spats occurred during prolonged period of low salinity (rainy season). Diploid oysters take too long to mature to marketable size with an increased risk of failure.

Therefore, triploid is proposed as a genetic manupulation of potential value in aquaculture. It has been thought that triploid oysters, like polyploid plants, would grow larger simply because they have larger cells. The primary benefits of triploidy derive indirectly from the fact that triploids have poorly developed gonads. Therefore, triploidy can be beneficial to aquaculture when reproductive output of the animals causes a decline in product quality, causes mortality, or impedes growth. For the present cottage-industry to grow into viable fisheries industry in Malaysia, production of triploid spats is necessary.

From a commercial point of view, sterility is desirable for three main reasons. First, energy usually diverted to gamete production is available for somatic growth in sterile triploid individuals. Therefore, adult triploids should grow faster. Second, in shellfish such as oysters, the gonad ramifies throughout the somatic tissue often rendering ripe animals unmarketable. Depleted glycogen stores in the body also affect flavour. Triploid oysters overcome this problem and have enabled year-round production of *Crassostrea gigas* (Allen, 1988). Third, the potential to produce sterile triploids of non-native species enables their use as aquaculture organisms in areas which might be sensitive to the accidental introduction of competitor species.

Objectives

The specific objectives of the project are :

i. To produce triploid oysters through the manipulation of the polar body
ii. To study the biology and growth of triploid larvae
iii. To formulate a system for intensive larval culture'
iv. To optimize this system for high production for food industry

Materials and Methods

The oyster species studied in this project is *Crassostrea belcheri*, which is presently the commercially important species, and can be found from the west coast of Peninsular Malaysia.

For each experiments, eggs from at least ten females and sperm from several males were collected by strip-spawning the gonads. The fertilized eggs were treated with different triploidy induction as listed below:

i. Cold shock 5.0 – 15.0 ^{o}C) – these are the temperatures at the lower tolerance range of the adult oysters (Davenport *et al*., 1992).
ii. Heat shock (30 – 35 ^{o}C) – these are the temperatures at the higher tolerance range of the adult oysters (Davenport *et al*., 1992).
iii. Cytochalasin B (0.1 – 1.0 mg/L) – this is a commercially used hormone to induce triploidy in *Crassostrea gigas* which is a temperate species (Downing, 1987).
iv. Caffeine (5 – 20 mM) – this is the most economical chemical used to induce triploidy in *C. gigas* practiced in Japan (Kusaka, 1995).

D-hinge larvae produced from each triploidy induction were reared and fed with *Isochrysis galbana* strain T-Iso. A few environment experiments have been conducted to determine the following :

i. The optimal larval density for the culture of triploid oyster
ii. Type of food
iii. Concentration of feed and frequency of feeding

Results

In general, caffeine and cytochalasin B have proved to be the reliable chemical agent for inducing "chromosome-doubling" during the maturation divisions in *C. belcheri*. Thermal shock can also be used but, generally, with a lower success rate. This is probably because the timing of physical shocks is more critical; eggs have only a small window of vulnerability compared to chemical treatment.

Besides yielding the highest percentage of triploidy D-hinged larvae, induction using caffeine and cytochalasin B also showed the highest percentage survival when the larvae produced from these treatments were cultured normally until settlement. Based on the high survival of larvae as well as the high percentages of triploidy formed, inducing triploidy using chemical inductions like caffeine and cytochalasin B is considered feasible for commercial purposes.

This project has found that the best triploid larvae density is 10 larvae/ml, although larval density at 0.5, 1.0 and 5.0 larvae/ml showed similar results with larval density at 10.0 larvae/ml. Culturing the triploidy larvae at 10.0 larvae/ml is considered the best in terms of high percentages of survival as well as more economical compared to culturing larvae at lower densities for commercial purposes.

Isochrysis galbana was recorded as the best food type for rearing *C.belcheri* triploid larvae especially during the early stages of the larval development. *Chaetoceros calcitrans*, *Tetraselmis* sp., *Sprirulina* sp. and *Chlorella* sp. were not suitable as food for the larval due to its cell size, which is too big to be consumed by the larvae at the early stage. However, when the larvae have reached a certain size (more than 150 µm in shell height), the larvae are able to

consume these phytoplanktons. Therefore, maybe it is practical to mixed the food during the later stage of the larval development in order for the larvae to have a mixed diet.

The best feeding concentration recorded in this project is 10,000 cells/ml daily. The larvae that were fed with 1,000 cells/ml showed good growth and survival during the early development stage but showed high mortality after day 5. This is due to the insufficient food supply as the larvae grew older and larger in size. The larvae that were fed with 50,000 cells/ml and 100,000 cells/ml showed high mortality throughout the culture period. This is due to the build-up of bacteria concentrations in the culture vessels due to the excess phytoplankton been fed to the larvae. The excess phytoplankton sank to the bottom of the culture vessel and formed a breeding ground for bacteria, which later infested the larvae and caused high mortality.

The triploid larvae of *C. belcheri* were also found to be able to survive successfully when fed at a higher frequency per day, compared to feeding just once a day. Increasing the feeding frequency with the same total amount of food will enable all the food provided at any time be consumed totally without leaving any dead cells or plankton which may form a breeding ground for bacteria and viruses.

Future Direction

To be commercially successful, the induction process must be reproducible, safe and cost effective. The project has shown that the induction procedure is reproducible at least on the research level. Poor egg quality will increase the expense of large-scale treatments because it leads to reduced larval survival and requires the maintenance of a larger broodstock population. Establishing a good broodstock management programme focusing on conditioning to increase egg quality will be an essential ingredient for commercial production.

With this information, triploid oysters can be produced commercially to support the oyster demand in Malaysia as well as our neighbouring countries like Singapore and Thailand. Triploid oysters will ensure that the condition of the meat is in high quality throughout the year, unlike the diploids where they will become watery and skinny after the spawning season. Triploid oysters use far less of their glycogen reserves than diploids during the reproductive period, triploids in that season constitute a more nutritious product, higher in protein and glycogen and lower in lipid. For the same reason, triploid oysters are also a firmer, tastier product.

Further research should be carried out to determine the survival and growth rate of the triploid oysters cultured in the natural environment, from the size of a few millimeters to the marketable size.

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"Establishment of CdNA Library for Sex determination of Calamus manan"
Year 2000 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Rattan manau or its scientific name Calamus manan is climbing palm belonging to the family of Palmae and subfamily of Calamoideae. It has a wide distribution in Southeast Asia, and grows well on slopes of hill dipterocarp forest at altitude of 600-1000m. As other rattan species, rattan manau is a dioecious plant. The male and female inflorescences are found on different plants. Basically the morphologies of male and female plants are alike except their inflorescences. Female inflorescence contains fertile female flowers but sterile male flowers whereas the male inflorescence has only male flowers. The branching pattern of the male and female inflorescences is obviously distinct which can be observed using a pair of binoculars in the field. The fruits of rattan manau with a size of 2-3cm can only be found on female plants and are covered by reflexed scales.

The sexes of rattan manau can only be determined when the plants start flowering. The rattan plants normally take 5-6 years of growing before flowers appear. Five to seven inflorescences per plant appear in one flowering season. However, this seems to be too long for the establishment of nursery for supplying planting materials. Optimum ratio of male and female plants in nursery is vital to maximize the generation of planting materials from seeds. Therefore, the application of molecular markers, particularly DNA markers, for sex identification in early stage is very much desired. To achieve this objective, molecular approach through the construction of cDNA library derived from floral tissues is attempted. It is expected that the floral mRNA of male inflorescence may differ to that of the female inflorescence of which may be used in identifying the sexes of rattan manau.

Objectives

i) to construct cDNA library of floral tissues in Calamus manna
ii) to generate EST sequences of floral tissue of Calamus manna

Methodology

To achieve the sex identification at early stage of growing, molecular approach through the construction of cDNA library derived from floral tissues was attempted. It is expected that the floral mRNA of male inflorescence may differ to that of the female inflorescence of which may be used in the development of molecular markers for sex identification. Rattan manau plants used in the study were located at F41, Bukit Lagong, Forest Research Institute Malaysia, Kepong. The rattan plants at F41 were planted in 1978. Most of the plants have reached a height of 10-15m. Therefore, scaffoldings were erected next to the selected plants for the purposes of close phenological observation and

sampling of floral tissues for cDNA library construction. Floral tissues of different developmental stages of male and female plants were collected for total RNA extraction and cDNA library construction. cDNA libraries from different developmental stages of inflorescences were constructed. The inserts of the clones were examined for quality by means of direct PCR amplification from bacterial colonies. Selected clones were sequenced to generate EST sequences.

Results

Eight cDNA libraries (4 male and 4 female) from different developmental stages of inflorescences were constructed. The size of the libraries ranged from 1100 to 18000 clones. The insert sizes of the analyzed cDNA clones ranged between 500 to 2500 bp with an average insert size of approximately 1000 bp. Over 1500 EST sequences were generated from the male and female inflorescence tissues of Calamus manan. Eight floral genes were identified from the EST database. These floral genes could be further characterized for development of molecular markers to identify sexes in Calamus manan and other related rattan species.

Future Direction

The floral genes identified from the EST database are potential candidates for the development of molecular markers in the identification of sexes in Calamus manan and other related rattan species. The expression and characterization of these floral genes need to be investigated. Full length cDNA and genomic DNA sequences of the floral genes will be generated for more comprehensive gene characterization. On the other hand, the expression of these floral genes will be approached through southern hybridization and microarray.

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"Surface modification of the Aramid Fibre to improve Adhesion to Natural Rubber and Thermoplastic Natural Rubber (TPNR) Matrix composites"
Year 2000 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Despite the studies on the fibre surface treatment have been done widely abroad, the use of natural rubber as the matrix is still insignificant. The real situation how to determine the adhesion strength between the fibre and the rubber is not well studied. This study is expected to improve the adhesion between the aramid fibre and natural rubber composites as well as for thermoplastic natural rubber (TPNR) matrix composites. Hopefully this project will provide an understanding the nature of the fibre-matrix adhesion and increase the usage of the fibre in rubber composite.

Objectives

Research activities are focused on the effect of fibre surface treatment on Twaron fibre in natural rubber matrix using the microbond pull-out test in model composite.. Resorcinol formaldehyde (RF) resin was used as an aqueous adhesive incorporated into latex because of its superior adhesion and ease of processing. After that Twaron fiber reinforced thermoplastic natural rubber composites are prepared and evaluated. Thus the objectives of the research project are as follows:

a) To study the interfacial failure in aramid fibre/natural rubber composites.
b) To measure the interfacial micromechanical parameters of aramid fibre/natural rubber matrix composites.
c) To study the effect of fiber content on mechanical and dynamic mechanical properties of aramid reinforced thermoplastic natural rubber (TPNR).

Methodology

Interface bond strength measurement

Twaron aramid yarn for interfacial micromechanical studies was supplied by Acordis, Arnhem, The Netherlands. One basic yarn type 2200 was selected, which was studied in two different forms: untreated fibre (HM) and with an epoxy-based adhesion activation treatment (HMA). Natural rubber (NR) and ENR25 (Epoxyprene 25) were supplied by the Rubber Research Institute of Malaysia (RRIM). Linear low density polyethylene (LLDPE) obtained from Dowlex with a density of 0.926 g/cm^3.

The RF latex adhesives were prepared using natural rubber latex. For the RFL treatment, aramid fibres were surface-modified by dipping the fibres in the solution. The treated fibres were then dried at 80^{o}C in vacuum oven for 60 minutes followed by post-treatment at 150^{o}C for 5 minutes. After the treatment, the samples were washed using distilled water and dried. Surface analysis was carried out using XPS, performed using a VG Escalab MKII instrument with a standard dual anode (AlKα radiation). The analyser was operated in constant analyser energy (CAE) mode with pass energy of 50 eV for elemental quantification purposes and 20 eV for C1s peak shape for comparison purposes. Data processing was performed with VG Eclips software.

The specimens were prepared by embedding a single fibre into a drop of latexes. A self-made microbond pull-out apparatus has been used which allows high precision fibre displacement and force measurements. The pull-out force was carried out using tensile testing machine. The experimental relationship between σ_a and L_e was used to calculate the interfacial shear strength (IFSS), σ_s, by extrapolating σ_a values to zero interfacial area (zero embedded length). The IFSS characterizes the bond strength between the fiber and the matrix.

Effect of twaron fiber in TPNR Composites

Composites were prepared in two steps of processing using an internal mixer and extrusion compounding. 60/40 NR/LLDPE matrixes were used in preparing the composites with various composition of Twaron. Tensile tests were performed on sets of 10 identical specimens of each composite according to ASTM D412, using Testometric instrument at crosshead speed of 50 mm/min. Thickness were measured using digital calipers. Izod impact testing was conducted accordance to ASTM D256-88.

Results

A combination of the microbond pull-out test, tensile test and impact analysis together with scanning electron microscope were be used to study the effect of the surface treatment on the aramid fibers upon their interfacial properties in NR matrix. The results also suggested that the addition of aramid fibres significantly improve the physical and mechanical properties of TPNR matrix. The knowledge obtained from the project is useful in the area of surface modification of fibres, polymer composites and polymer processing. The results from the project have been published in many journals and presented in seminars and conferences.

Future Direction

Different types of fibres especially natural fibres with different treatments will be used with thermoplastic natural rubber as a matrix to study the adhesion strength and fibre-matrix interaction.

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"Sedimentary record of Paleoproductivity in the Mangrove Forest Sediments"
Year 2000 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Mangroves are woody, seed-bearing, highly specialized plants and are found along sheltered intertidal coastlines of estuaries and lagoons. Recently, reclamation of mangrove ecosystems for industrial, urban and other forms of development has been increasing and it causes irreversible damage in coastal regions throughout the tropics. Along with the destruction of the mangroves come the anthropogenic effects associated with the new developments. Examination of the profiles of chemical tracers in sediment cores, dated using radionuclide, can provide information of historical land-use changes. The use of dated sediment core profiles of contaminants has proven successful in plotting history of human impact on marine environment. In Malaysia, studies relating to mangroves are not well documented and only little information is known concerning the sedimentation of the mangrove forest (Kamaruzzaman, 1994). In view of the importance of the mangrove to various aspects of the environment, research on the concentration of heavy metals as well as their distribution pattern in sediment was carried out.

Materials and Methods

Sampling was done at mangrove forests region at Pulau Che Wan Dagang and Pulau Sekepeng, Kemaman (Terengganu). For the the determination of the sedimentation rate, radionuclides methods by using the thorium (^{230}Th) were applied. While metals determination was done by using Teflon bomb digestion which was established by Kamaruzzaman (1999) and Noriki (1980).

Results and Discussion

Based on the previous studies (Kamaruzzaman, 1994), sediments from the mangrove forests of Kemaman seem to be well mixed, and the sediment supply homogeneous. In this study, it was noted that the vertical profiles of Ba, U, Cd and Mn show a distinct contrast in the upper and lower parts of the sediments. Although some of these profiles show an increase in concentration toward the surface layer, this is not necessarily an indication for anthropogenic input. It is more likely that early digenetic processes are responsible for this phenomenon (Ridway, et al., 1987 and Macdonald, et al., 1991). In this study, Ba, U, Cd and Mn concentration ranging from 231 to 760 ppm, 11.5 to 21.1 ppm, 0.11 to 0.35 ppm and 108 to 159 ppm, respectively. The distribution of metals in the both core sample showed different patterns and this may be due to the low productivity during that period and/or probably due to the dilution of the biogenic matter with very high amounts of terrigenous material transported by the river nearby. For the estimation of the sedimentation rate, both concentration of $^{230}Th_{excess}$ and the $^{230}Th_{excess}/^{232}Th$ ratio were used and a average sedimentation rate of 0.67 $cmyr^{-1}$ were obtained for both areas. The sedimentation rate obtained is consistent with the result obtained

by Shahbuddin *et al*. (2000) near Pulau Sekeping, with the average of 0.66 $cmyr^{-1}$. Even though, our value is somewhat higher, it is comparable to sedimentation rates reported at other intertidal areas. Our higher value can be explained by the geographical position of our study area, which is located close to the mouth of the estuary and where the 2 main rivers meets, providing it with 2 sediment sources, fluvial and tidal. If these sedimentation rate values are accurate, the mangrove forest at the study areas is probably in an immature stage and estimated to start growing at about 150 years ago.Enrichment factor values were used to evaluate the dominant source of the sediments and as indicators for pollution effects. Enrichment factors close to 1 point to a crustal origin, while those with a factor more than 10 are considered to have a non-crustal sources. In this study, all elements studied have values significantly about unity and are considered to be dominantly terigenous in origin.

Conclusion

It is very difficult to describe its proper physical role in the environment. A detailed study on mangroves concerning all aspects of sedimentation, sediment dynamics and growth models should be very important. This finding suggests that the mangrove forests are not just passive colonizers of mud banks, but actively capture mud to create their own environments. Mangroves are thus an important sink for the fine sediment from rivers and coastal waters.

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"Identification of Markers involved in histological progression and transformation of follicular lymphoma"
Year 2000 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation)

Histological transformation and progression of follicular lymphoma (FL) into a diffuse large cell lymphoma have been reported to occur in 25-30% of the cases. Histological conversion and progression of Fl is frequently associated with poor survival, and no clinical predictive factors of transformation had been identified. A wide variety of genetic and biological alterations are associated with histological progression. One of the most important implicated pathways involves tumor suppressor genes such as p53 and p16. Mutations of p53 had been reported in at least 25-30% of the transformed cases.

This study aimed to investigate the possibility of p53 and p16 alterations playing a role in a series that the mobility shift in the gel band from the PCR product and the change in the seven FL cases with sequential biopsies. Mutational status of the p53 gene was studied using immunohistochemistry followed by PCR-SSCP for exons 5-8. In order to rule DNA sequence observed in cases studied was not due to p53 gene polymorphism, we studied fifty cases of blood sample from normal population to conduct p53 polymorphism study on exons 5-8. Alteration involving p16 gene was determined by loss of protein expression, and deletional status of the gene by FISH. Four out of the seven cases showed over expression of the p53 protein in the subsequent biopsies with transformed histomorphology, and were associated with altered mobility shift in PCR-SSCP. Three of these four cases had confirmed mutation of p53 gene in the sequencing reaction. Three of these cases presented with loss of p16 protein expression and two of them were confirmed having deletion in the p16 loci using FISH analysis. One case demonstrated homozygous deletion and the other showed hemizygous deletion. Our results support the notion that alterations of both p53 and p16 genes are involved in the progression and transformation of FL. We also demonstrated the feasibility of PCR-SSCP for the screening of the p53 gene mutations in archival tissue samples, and the reliability of p16 immunohistochemical stain to demonstrate protein loss due to gene deletion.

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"Cloning of Movement Protein (MP) from Malaysian strains of Cucumber Mosaic Virus (CMV) and its transformation into Tobacco Plants in the Antisense Orientation"
Year 2000 MTSF Science & Technology Research Grant Recipient

Introduction

In this study, we constructed mutant clones containing mutations in CMV RNA 3 encoding the movement protein, which are necessary for cell-to-cell movement of CMV. From previous researches, it was found that the mutant MP of tobacco mosaic virus (a virus that is very similar to CMV), once transformed into a plant, is able to increase the plant's resistance towards the virus. The mutated movement protein may act by disrupting the balance of virus disassembly/ reassembly that exists in the cell or by interacting with an unknown 'receptor' molecule present in the plant that is necessary for virus infection and/or spread. Here in our work, we presume the same resistance may be created in native host of CMV by mutating the movement protein gene of CMV that has been shown to play a role in the proteins assimilation and movement. An interesting and potentially useful attribute of movement protein–mediated resistance is the promise of broad-spectrum efficacy of the resistance (Ding et al, 1995; Lapidot et al. 1993).

The objective of the study is to produce mutant clones through site directed mutagenesis and then, subclone the cDNAs into plant transformation vector and later, to transform the construct into tobacco and other plant systems. Here we report our findings concerning the mutation efforts on three sites on CMV MP gene sequence which were selected as they were conserved and have been shown to be involved in MP gene function.

Materials and Methods

Plasmid DNA from the clone, pGEM-MP CMV, was extracted using the alkaline lysis method of Birnboim and Doly (1979). The DNA was then used in the site directed mutagenesis studies. The PCR reaction for site directed mutagenesis was conducted using the M13 primer together in combination with twenty-five different mutagenic primers in four codons that have been identified to play an important role in movement protein function. The codons that were identified were the codon 70, 90, 125 and 140. Mutagenic primers were constructed to change the amino acid coded by the codons to those that will result in change of protein activity and not protein structure. The mutagenic product was transformed into BMH71-18*mut*S, a repair minus (*mut*S) *E.coli* strain, and subsequent transformed into *E. coli* JM109 (Andrew & Lesley 1997).

Results & Discussion

Four codons 70, 90, 135 and 220, were identified within the conserved regions of the movement protein gene. Twelve different 3a mutants have been generated for codons 70, 90 and 220 respectively. All mutations were conducted to produce amino acids that do not result in comformational changes such as lysine or serine. These mutants will have similar conformation or structure with the native movement protein but it would be altered in its functionality.

When the protein function is altered, the movement protein is still able to bind to the plant cell wall but is unable to migrate from cell to cell. In case of an infection by the virus, there would be competitive binding between the native and mutant protein for the site on the plant cell wall and thus there will be. All 12 of these 3a mutant gene clones were subcloned into the plant transformation vector pCambia1301 and transformed into tobacco via *Agrobacterium* mediated transformation. The presence of the mutant gene within the regenerants produced have been determined for 68.18% from the primary screening on antibiotic selection plates (Kanamycin and carbenicilin) and 67.04% of the putative positive transformants from the primary screen showed positive GUS assay results.

The T1 generation plants that have been assayed for transgene presence via Southern PCR Our examination on the transgenic plantlets generated for disease susceptibility shows that some (5.4% [23/420plantlets]) of them were resistant. We have started growing out T2 generation to determine with certainty that these 5.4% are truly resistant. The constructs that make up for this 5.4% resistance are pCAMBIA1301:*mp70*-101 and pCAMBIA1301:*mp135*-67.

In conclusion we hope that as a spin-off of this study we will be able to produce transgenic plants that will hopefully be resistant to viral infections. These plants can be further developed into new varieties that will be of interest to farmers.

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"A study on the DNA Sequence variation of the Epstein-Barr Virus (EBV) oncogene, LMP-1, in relation to Nasopharyngeal Carcinoma (NPC) in Malaysia"
Year 2000 MTSF Science & Technology Research Grant Recipient

Introduction & Objectives

Epstein-Barr virus (EBV) is known to be the most important aetiologic agent of nasopharyngeal carcinoma (NPC) as it is commonly detected in malignant tissues. However, EBV infection of the post-nasal epithelium is known to precede malignant transformation and may occur during the benign stage when tissue dysplasia is the prominent feature. EBV infection of the dysplastic tissue may contribute to cellular transformation leading to the formation of malignant tumours. The EBV latent membrane protein 1 (LMP1), also known as the viral oncogene, plays major role in the viral transforming ability. This study supposed that by its antigenic nature, LMP1 is subjected to the immune selective pressure and thus it was hypothesised that the LMP1 derived from pre-malignant and malignant stages would differ in gene sequence as well as in biological properties. Such findings would be an imperative in understanding the precise roles of EBV LMP1 in the carcinogenesis of NPC which may open new avenues for EBV LMP1 targeted therapy.

Methodology

EBV LMP1 was isolated from benign dysplastic and malignant NPC tissues following RNA extraction and RT-PCR. The LMP1 cDNA was cloned into pYes2.1 *Saccharomyces cerevisiae* expression vector and pcDNA3.1 mammalian expression vector. The pYes-LMP1 genes were sequenced and their DNA sequences were compared. Hydropathy probing was conducted to access the locations of epitopes. The pcDNA-LMP1 genes were transfected into the EBV-negative NPC cell, TW01. LMP1-transfected TW01 clones were assessed for their biological properties which include cell migration, growth in soft agarose gel, resistance to stimulus-induced apoptosis, ability to suppress E-cadherin expression, ability to invade Matrigel-coated membrane and ability to form tumour in nude mice.

Results

The LMP1 genes derived from the benign dysplastic tissue (NORLMP1) and malignant tumour (NPCLMP1) showed differences in DNA sequence and biological properties. While both LMP1 genes displayed the 30-bp deletion and loss of *Xho*1 restriction site, unique base substitutions in the CTAR-region were noted in the NPCLMP1 that is responsible for the intracellular signalling of the LMP1 gene. As compared to NPCLMP1, NORLMP1 showed greater potential for growth in soft agar, conferred greater ability for membrane invasion and was more suppressive towards the expression of E-cadherin in TW01 cells. Interestingly, NPCLMP1 conferred greater migration rate and greater resistance toward stimulus-induced apoptosis in TW01 cells than NORLMP1. However, NPCLMP1 was shown to be less antigenic than NORLMP1 and displayed the same tumour-initiating potential in nude mice as NORLMP1.

Future Direction

The present and future studies investigate the signalling pathways affected by different LMP1 variants aiming to elude the mechanisms by which EBV contributes to NPC cell invasion and extravasation from the primary tumour.

Publications

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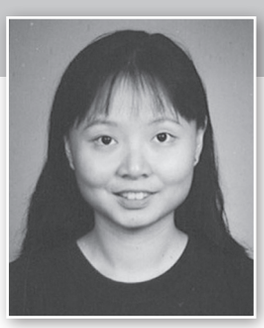
Dr. Chan Yee Peng



Universiti Malaya

"Determination and analysis of Nucleotide sequences of Nipah Virus isolates"

Year 2000 MTSF Science & Technology Research Grant Recipient

Results

1. **Search for mirror-image antigen pairs for differentiation of HeV and NiV antibodies**

   Knowledge of the genomic sequences of NiV and HeV and identification of HeV- ad NiV- specific amino acid sequences were used to differentiate between these two viruses. Two regions in the P gene between NiV and HeV with low homology in amino acid sequence were identified. These regions were located in the N terminal and middle section of NiV and HeV P protein. These regions were then cloned into *Escherichia coli* separately for expression. The expressed recombinant proteins were then purified and used as antigen in western blot to differentiate between NiV and HeV rabbit antisera.

   Figure 1 shows that rabbit and NiV reacts with NiV P fragments and weak with HeV Pn whereas rabbit anti HeV reacts with HeV P fragments and NiV Pms. Rabbit anti NiV did not react with HeV Pms and rabbit anti HeV did not react or reacts very weakly with NiV Pn. Hence, NiV Pn and HeV Pms may be used to differentiate between NiV and HeV antisera.

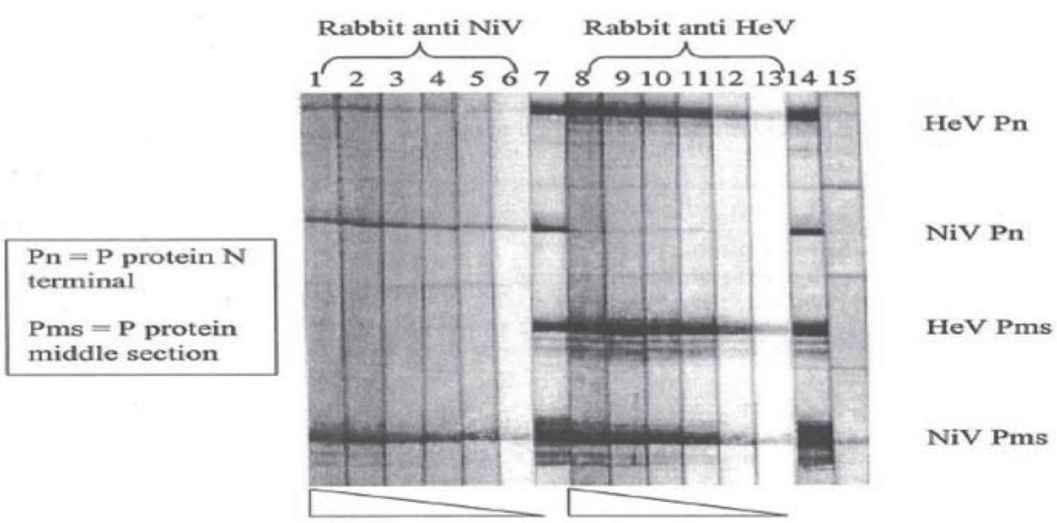
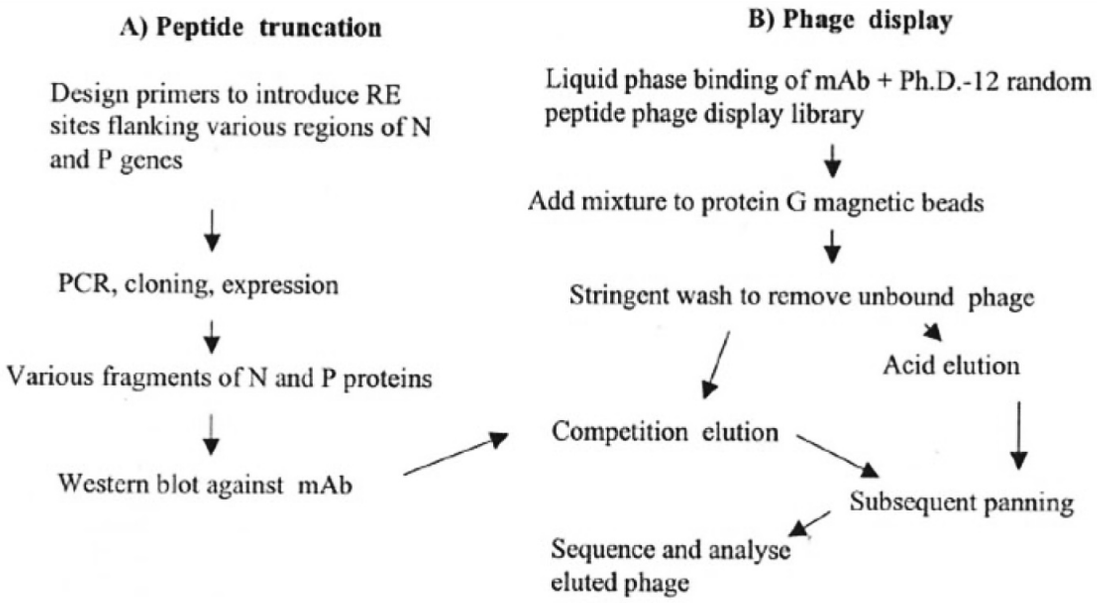


Figure 1: Multi antigen western blot analysis of NiV and HeV P protein variable regions against rabbit antiserum. Lanes 7 and 14 indicate the reactivity of the P fragments with anti Xpress antibody that recognized a common epitope fused with the N terminal of the P fragments. Lanes 1-4 indicate the reactivity of the P fragments with rabbit anti NiV in serial dilutions. Lanes 8-13 indicate the reactivity of the P fragments with rabbit anti HeV in serial dilutions. Lane 15 shows the reactivity of the P fragments against negative rabbit serum.

2. **Mapping epitopes that are common in HeV and NiV by using HeV-N and P mAb**.

Phage display and peptide truncation were carried out to map the common epitopes of HeV and NiV.

Figure 2 : General outline of the methods involved in epitope mapping of the N and P mAbs.



There are two monoclonal antibodies (mAb) against HeV-P and one against HeV-N that cross-react with NiV provided by CSIRO, AAHL. These mAb are useful for mapping the epitopes encoded by the P and N genes of NiV. Expression of several truncated peptides of the N and P genes can determine the binding sites of the mAb in certain region of the gene. Phase display can help to fine map the exact binding amino acid sequence of these mAb. A commercially available random peptide phage display library (Ph.D.-12™ Phage display peptide library kit, NEB) was used to fine map the epitopes. The mAb and the phage library were mixed for liquid phase binding, and then the mixture was bound to protein G magnetic beads for the washing procedures. The unbound phage was washed away. The bound phage were eluted by acid (pH2.2) elution or compete off from the mAb by the expressed truncated peptide that binds specifically to the mAb. The eluted phage was then amplified by growing in liquid culture inoculated with its host cells. The amplified phage will be used as library for subsequent rounds of selection. The selected phage was analyzed by direct sequencing, ELISA, and Western blot.

Results : Both NiV and HeV encode a nucleocapsid (N) protein of 532 amino acids (aa) and a phosphoprotein of 709 aa and 707 aa respectively. This N-mAb was found to react with both NiV and HeV N protein. Deletion analysis of the N protein showed that the mAb was able to react with a 29 aa protein fragment, which represents aa 468 to 496 of the N protein. To further characterize the epitope, the mAb was used to screen a random dodecapeptide phage display library. DNA sequencing of the selected phages revealed a motif of GTRLT representing aa 469 to 473 of the N protein of NiV and HeV. Deletion of the GTRLT residues within aa 468 to 496 reduced the mAb

activity in Western blot. Deletion of aa 483 to 496 completely abolished the mAb activity. These observations showed that the GTRLT residues are critical for complete activity of the mAb. Other residues within aa 483 to 496 are also essential for binding with the mAb. The P-mAb were firstly observed to bind both HeV and NiV viral proteins in Western blot, indicating that the epitopes should reside within the conserved regions of the P protein of both henipaviruses. The same protein truncation approach as described for mapping the epitope of N-mAb was employed to map the epitopes recognized by the P-mAbs. Both P-mAb were then observed to bind to a 208 aa P protein fragment (aa 407 to 614) of HeV.

3. **Investigation of Protein-protein interaction in NiV**

Members of the family Paramyxoviridae contain a lipid bilayer envelope and within the envelope lies a nucleocapsid core containing the RNA genome encapsidated with the nucleocapsid (N) proteins, to which the phospho-(P) and large (L) proteins are attached. This complex initiates intracellular virus replication. Recombinant NiV and HeV-N and P proteins were cloned and expressed separately in *Escherichia coli*. The NiV-N protein was then immobilized on PVDF membrane strips and each strip incubated with NiV-P or HeV-P protein. After several stringent washes, NiV-P and HeV-P proteins were detected on the strips by using a specific monoclonal antibody against the two P proteins. Similarly, when NiV-P protein was immobilized on PVDF membrane strips and each strip incubated with NiV-N or HeV-N protein, followed by several stringent washes, NiV-N and HeV-N proteins were detected on the strips by using a specific monoclonal antibody against both the N proteins. The recombinant N and P proteins of NiV and HeV were able to associate with each other forming a complex on Western blots. The N and P proteins of these two viruses were interchangeable during the formation of the N-P complex and this is most likely due to the high sequence homology between the two viruses. Various truncated NiV N and P proteins were cloned and expressed to map the binding domains between N and P proteins.

Results : Deletion analysis of the N and P revealed that there were at least two independent N-binding sites present in P, which reside at the N-and C-terminus, respectively. Similarly, more than one P-binding sites were observed for N, and one of them was mapped to a 29-amino acid C-terminal region. This C-terminal region alone was sufficient to interact with the extreme C-terminal 165-amino acid region of P.

4. **Development of a novel diagnostic method based on competition ELISA of mAb against NiV anti sera using recombinant NiV antigen**

Recombinant NiV N protein has been successfully expressed in a baculovirus system at high level by Yu Meng (CSIRO, AAHL). This recombinant peptide can be used for Blocking ELISA to detect NiV antibodies. The NiV N protein is immobilized on ELISA plates followed by binding of NiV antisera or control negative sera. Then the anti N mAb is added to compete with the sera. Anti NiV antibodies in the testing sera will inhibit the binding of the mAb and detection of mAb by anti mouse antibody will show a decrease of the mAb binding activity. A strong inhibition indicates the presence of NiV antibodies in the testing serum. Whereas a strong binding of the mAb with low or no inhibition indicates the absence of NiV antibodies in the testing serum. The results showed that all NiV rabbit, pig and horse Ab were able to give higher inhibition values compared to their respective control Ab. In

addition, inhibition as low as 6% was observed for the control Ab. Unfortunately, the NiV pig Ab showed a much lower inhibition compared to that of NiV rabbit Ab.

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"Development of Diagnostic Enzyme Immunoassay Screening Kit for the detection of Chemical Residues in Livestock products"

Year 2000 MTSF Science & Technology Research Grant Recipient

Introduction

This research was undertaken to support the overall research program at Livestock Research Centre, MARDI on development of safe and healthy chicken which was conducted at the MARDI poultry farm and Meat Microbiology Laboratory. The production of safe and healthy poultry products is important to protect consumers from drug/chemical residues especially antibiotic in edible animal tissue and egg. Therefore, the development of this research project will assist in the monitoring of antibiotic residues in the production of the wholesome poultry.

Objective

The objective of this research project is to develop a suitable immunoassay technique for the detection and quantification of several important antibiotic residues in poultry product.

Methodology

Antibiotic streptomycin was selected for this project

- An antibiotic molecule was conjugated with suitable carrier protein such as BSA (Bovine Serum Albumin and Oval-albumin) and was mixed with F'reund Adjuvant to become an antigenic antigen.
- The conjugate or antigen (antibiotic-protein conjugate) was injected into rabbits for a certain time period (2 months) for stimulating the polyclonal antibody.
- After 2 months immunization in rabbit, the blood was taken and the antiserum was further purified using DEAE-cellulose and Protein A affinity chromatography column to get the pure IgG against streptomycin antibiotic.
- Pure antibody anti-streptomycin was successfully used in development of immunoassay technique (Figure 1) for streptomycin antibiotic. The detection limit for streptomycin was found in the range of 0-100ppb. Screening an antibiotic using this developed method could determine antibiotic residues in various samples tested. The results of screening were comparable and cheaper then the imported ELISA kit and HPLC method.

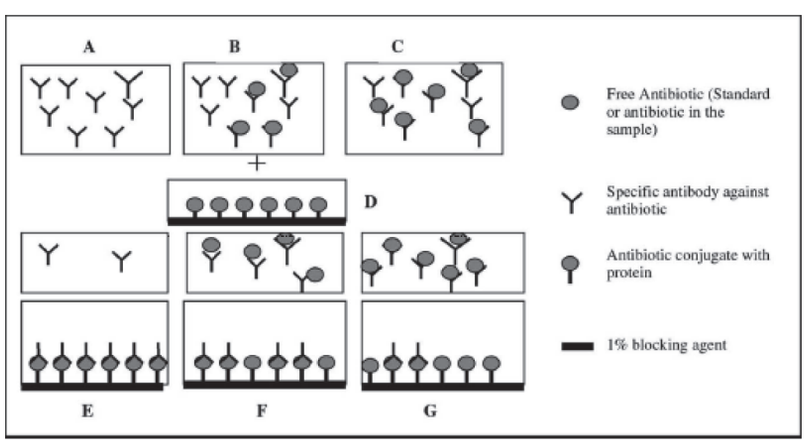

Figure 1. Schematic diagram of the direct competitive ELISA

Outcome of research – achievement and impact of research findings, new discoveries, significant breakthroughs

a. Bioassays / Bio-indicator system specific for antibiotic residues - Potential beneficiaries from these outputs are the enforcement agency in the agriculture department and veterinary drug board. Towards achieving healthy society in the future, Malaysia should have the own safety local agriculture products. Developed ELISA kit/immunosensor, which is cheaper than the imported ELISA kit/HPLC should be, used as rapid and early monitoring system for antibiotic or veterinary drug residues in poultry meat and livestock product.

b. Commercially viable portable and disposable ELISA kit for monitoring of the safety of meat and poultry products - The data obtain from the rapid analysis of the developed ELISA kit/immunosensor could be used as a guide for our farmer or to educate them the right procedure to use antibiotic or veterinary drugs. High antibiotic residues in poultry meat and poultry products from particular plot or farm can be a indicator for action to be taken. Thus a fast and efficient method needs to be implemented to check these products for safe to Malaysian consumer. So, this ELISA kit/immunosensor could be used for screen the antibiotic content for the imported poultry products

c. Organization Outcomes - The expertise built within the institutions involved (MARDI and JPH) to develop this ELISA kit/immunosensor technology will be used for other purpose such as in medical sector. With this expertise, MARDI and JPH can develop other product of ELISA kit/immunosensor. The product derived from this project will be used in government sector as well as in private sector.

d. National Impacts - disposable ELISA kit/immunosensor technology will become one of the promising technologies in the future in the area of detection and diagnosis for screening purposes. This kit will minimize the mishandling of antibiotic by our farmers and also could ensure that our agriculture products safe for consumer. Beside that we can make our agricultural industry more competitive by produce a good product with low risk of high antibiotic content.

Unresolved problem and future direction

The problem of commercialization is the company needs more than one type of antibiotic for the ELISA kit/ immunosensor.

The future direction of this research is to develop the other type of antibiotic or drugs used in poultry industry. This research project was grown up with other antibiotic/drugs used in poultry industry such as tetracycline, chloramfenicol, β-aganist and nitrofuran. To compliance with the industry need, this research project was continued to develop the disposable immunosensor kit based on ELISA principle for fastest and cheapest detection of antibiotic in livestock industry.

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"Regulation of Nitrogen Fixation (nif) Genes from *Paenibacillus azotofixans*"
Year 2000 MTSF Science & Technology Research Grant Recipient

Paenibacillus azotofixans is a Gram positive, facultative anaerobe that falls into a broad cluster of diazotrophs in rRNA group 3, which also include *P. macerans* and *P. polymyxa*. Extensive research in nitrogen fixation thus far had been concentrated on the Gram negative diazotrophs. There had not been much information on Gram positive *nif* gene sequences, structural organizations and its regulation. Our main research objectives include deciphering *P. azotofixans* nitrogen fixation genes, characterizing and determining its gene organization, elucidating the transcriptional start site of *nif* genes, characterizing promoter sequences as well as identifying possible regulatory regions.

Three *Paenibacillus azotofixans* DNA regions containing nitrogen fixation genes were cloned, sequenced and characterized. Sequence analyses of a 0.6 kb *HindIII*-*SacI* and its adjacent 4.6 kb *SacI* DNA fragments revealed a linkage of *nifB1* upstream of the structural *nif* genes. These genes were designated as *nifB1H1D1K1*. The *nifB2H2* and *nifH3* genes were contained in a 6.35 kb *HindIII* and a 2.84 kb *EcoRI*-digested DNA fragment, respectively.

Total RNA was isolated and the 5'-mRNA ends of nitrogen fixation genes were determined using a modified RLM-RACE methodology. PCR amplification, restriction enzymes analysis, subcloning and other standard molecular techniques were also performed. Recombinant plasmids containing *nifH* and *nifB* cDNAs were purified and sequenced.

Inspection of DNA regions upstream from *nifH1* 5' end did not reveal the typical SigN-dependent *nif* promoter in the –24/-12 region with the characteristic structure of 5'-TGGCAC-N_5-TTGCA/T-3'. Instead, the sequence in the –24 region (TATTAATA) showing similarity with promoter regions from *Archaea* was found. The TTGAAA sequence in the –35 region also closely resembles the TTGACA of the –35 region consensus sequence of gram positive organisms. The absence of a typical promoter element could also be due to an alternative mechanism involving RNA processing. The probable *nifB1* 5' end-point was mapped to a T-residue with a distance of 46 bp preceding the translation start site. The –12/-24 DNA region has sequences of TTCCGA and AATGAT, which demonstrated a weak homology to the typical SigN-dependent *nif* promoter. The search for potential transcriptional signal structure in the upstream untranslated region revealed a consensus sequence TGT-N_{10}-ACA in two locations approximately 70 and 120 bases from the putative 5' end of *nifB1*. This sequence, known as NifA box, has been shown to be specifically recognized by the NifA protein in various proteobacterial diazotrophs.

Putative promoter regions upstream from the *nifB2H2* gene cluster revealed two putative promoters that did not show close homology to known SigN-dependent *nif* promoter. RACE-PCR results mapped the *nifB2* 5' ends 67 bp to a T-residue, and 21 bp to an A-residue preceding the translation start site, respectively. Inspection of the promoter region at the -35^a and -10^a regions revealed sequences of TAAGATG and CATACGAA, respectively. These sequences showed

homology with consensus sequence of SigE-regulated region of *B. subtilis*. The second *nifB2* putative promoter region is located downstream from the first, and has sequences of TGGAGG, TG and TCTTTT at the -35[b], -15[b] and –10[b] regions. Only the –15[b] region matches the promoter regions of gram positive in TG residues, whereas the –35[b] and –10[b] region showed less prevalent sequences. Thus, the *nifB2H2* mRNA may be transcribed from one of the promoters under certain condition, and that an activator is possibly involved in transcription under other as yet unknown physiological condition.

The RACE-PCR amplification utilizing oligonucleotides to specify *nifH1* and *nifH2* ends was also able to amplify the 5' end of a fourth *nifH* gene fragment (*nifH4*). Sequencing analyses of the RACE-PCR products consistently mapped *nifH4* start sites to three different 5' cDNA ends: (a) 67 bp to a G-residue (tss1), (b) 40 bp to an A-residue (tss2) and, (c) 28 bp to an A-residue (tss3), preceding the ATG translation start site. Analysis of the –35 and –10 sequences upstream from tss3 revealed that the sequences (TTGCAT and AATAAT, respectively) closely resemble the consensus sequence of gram positive organisms.

Previously, other researchers had reported the presence of multiple *nifH* genes in *P. azotofixans* based on Southern analysis. With the sequences of multiple *nifH* genes characterized, we have shown that *P. azotofixans* contains at least four *nifH* genes. The information presented in this research met the main objectives of characterizing nitrogen fixation genes from the Gram positive *P. azotofixans* and probable mechanisms underlying their regulation. The data generated from transcriptional start sites and cDNA 5' ends experiments will help establish the operon nature of *P. azotofixans nif* gene clusters. This, in turn, will form the basis and foundation for future research that will enable elucidation of mechanisms that are involved in their regulation.

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"Effects of selective logging on Genetic Diversity of Shorea curtisii and Shorea singkawang in Tropical Forests"
Year 2000 MTSF Science & Technology Research Grant Recipient

Effects of logging on forest structure and genetic diversity of *Shorea curtisii* Dyer ex King were determined for different age cohorts after different time periods of logging, viz. immediately after logging, after 2 ½ years of logging and after about 50 years of logging. Three forest reserves in Peninsular Malaysia, i.e. Ulu Sedili Forest Reserve in Johor, Serting Tambahan Forest Reserve in Negeri Sembilan and Panti Forest Reserve in Johor were selected for the study. The effect of logging on forest structure was carried out based on enumeration of all trees ≥ 1 cm diameter at breast height (dbh). The total reduction in mean basal area for trees ≥ 1 cm dbh for both logged stands was significantly different ($p < 0.05$), i.e. 50.9% in Compartment 118 and 51.8% in Compartment 48 under Selective Management System (SMS). In Compartment 118 and 48, all trees more than 60 cm and 75 cm dbh, respectively, were felled in a single selective cutting under SMS. The mean basal area and tree density for seedling and sapling class (< 5 cm dbh) were reduced to one-half of the original stand in Compartment 118 after logging, meanwhile an increment of 24.3% was observed in Compartment 48 after 2 ½ years of logging. This indicates that the logging operation favoured the growth of seedlings and saplings. The net loss in trees was offset by incremental growth in surviving trees. In addition, good regeneration was observed in the regenerated stand of Compartment 69 (RS-C69) with 28.5% and 20.5% relatively more, respectively in basal area and tree density for seedling and sapling class (< 5 cm dbh) compared to Compartment 118 before logging. The high negative correlation of basal area with relative disturbance index (RDI) was observed in Compartment 118 and 48 based on botanical name or native name, indicating that the degree of disturbance was affected by the type and magnitude of disturbances in each of the localities in the compartment. This further implies that a logging operation in the forest is not a homogeneous activity.

Genetic diversity of *S. curtisii* from different age cohorts, i.e. seedlings, saplings and adult trees were determined using six SSR loci. Standard and non-traditional genetic diversity measures were used to define genetic diversity changes caused by logging. In general, the reduction of genetic diversity measures was in the following sequence: saplings < seedlings < adults. The genetic diversity levels of seedlings and saplings after a single logging event under SMS and the Malayan Uniform System (MUS) are still considerably high or equivalent with the genetic diversity levels detected in the unlogged stand. The hypothetical gametic diversity (V_{gam}) and latent genetic adaptive potential (*LP*) increased after logging in seedlings and saplings, suggesting that seedlings and saplings have the potential to produce genetically diverse gametes when mature with the capability for colonisation or adaptation to environmental changes in the long-term. However, logging caused loss in genetic diversity and long-term evolutionary potential of adult trees in the logged stand. There was a significant ($p < 0.05$) loss of alleles (50.0%) detected in adult trees immediately after logging in Compartment 118. Low levels of V_{gam} and *LP* in adult trees suggest that the ability of this gene pool to adapt to changing environmental conditions may have been compromised. Nevertheless, the losses in genetic diversity of adult trees may be compensated for by an existing good seed or seedling bank in the forest management unit (FMU)

or by migration from nearby undisturbed forest areas. Hence, it is crucial to have adequate buffer zones whilst at the same time leaving behind sufficient undamaged good quality adolescent or bigger trees to ensure good regeneration in the residual stands.

The current harvesting system, i.e. SMS in Peninsular Malaysia based on a general cutting limit need to be refined in order to maintain genetic diversity of the residual stands for long-term evolutionary potential in changing environments. The system should consider the abundance of young regenerants (seedling and sapling) and the genetic quality of the residuals left behind in the postharvest stands. The current mechanized logging techniques also need to be improved by adopting the reduced impact logging (RIL) techniques, so as to promote regeneration of desired commercial timber species and as a consequence, sustainability of the forests.

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"Molecular characterization of Human Promoter Elements of Peroxisome Proliferator Activated Receptor-Gamma (PPARγ)"
Year 2001 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Peroxisome proliferator activated receptor gamma (PPARγ) is a member of the PPAR subfamily of nuclear hormone receptors. Two isoforms of PPARγ, γ1 and γ2, are produced by the use of alternative promoters and differential splicing of a single gene. PPARγ forms heterodimer with retinoic X receptor α. This heterodimer regulate transcription by binding to peroxisome proliferator response element in the regulatory domain of target gene. PPARγ plays an essential role in the differentiation and development of adipose tissue, which in turn, is the major depot for the storage of energy in human. It executes this role through the expression of enzymes that synthesis and hydrolyze triglycerides.

Currently, studies of PPARγ and its role in adipogenesis are becoming more important in light of its role in the development of pathophysiological diseases such as obesity, non-insulin dependent diabetes, cardiovascular disease, atherosclerosis and certain cancers. For example: (1) PPARγ gene expression has been shown to be sharply increased during the formation of foam cells that leads to the development of atherosclerosis and subsequently, cardiovascular diseases; (2) premature or ectopic expression of PPARγ in differentiating preadipocyte cells results in the cessation of mitotic growth and promotion of the differentiation programme; (3) increase level of PPARγ gene expression results in the accumulation of fat that leads to obesity; (4) abnormalities of PPARγ has been found to be involved in insulin resistance of obesity and type III diabetes; (5) PPARγ is highly expressed in cancer cells (e.g. liposarcoma), and upon treatment with PPARγ agonists, tumour size and tomour incidence have been shown to be significantly reduced.

All these evidence point out the pivotal role of PPARγ in regulating the adipocyte differentiation and adipogenesis, thus, lipid metabolism as a whole, which in turn, will have a marked influence in the development of pathophysiological conditions such as obesity, cardiovascular disease, atherosclerosis, non-insulin dependent diabetes and certain cancers in human. Realizing the important of PPARγ in disease development, and since the control of PPARγ gene expression is mainly occurred at the level of transcription, the aim of these works, therefore, is to :

1. determine the transcriptional start site of the human PPARγ2 gene
2. determine the interaction between the human PPARγ2 promoter region and transcription factors
3. determine the functional activity of the human PPARγ2 promoter region .

This study is important not only for advancing our understanding of the possible interactions between adipogenic/ transcription factors and the promoter region of PPARγ2 gene or the functions/activities of human PPARγ2 promoter but, in longer term, may also lead possibly to the identification of pathways for therapeutic intervention of the diseases. This will enable for the specific inhibitor(s)/drug(s) to be designed against the specific pathway(s) of a certain diseases.

Results

Identification of the transcriptional start site of the human PPARγ2 gene

RNA ligated mediated rapid amplification of cDNA ends (RLM-RACE) approach was used to map the transcriptional start site of the human PPARγ2 gene (Maruyama and Sugano, 1994; Shaefer, 1995). The method selectively allows only the 5' end of the full length mRNAto be ligated with RNA oligo following RNA treatment with Calf Intestinal Phosphatese and Tobacco Acid Phosphatase to remove phosphate group at the 5' end of truncated mRNA and 5' cap of the full length mRNA, respectively. Then, nested PCR was carried using primers designed against the RNA oligo and the 5' end of human PPARγ2 gene. A fragment with the size of 300bpwas successfully amplified, cloned and sequenced. However, data analysis revealed that the fragment was not the 5' end of the human PPARγ2 gene.

PCR optimizations with new primers were carried out several to specifically amplify the 5' end of the human PPARγ2 gene in order to map the start site of the gene. However, the specific fragment represented the 5' end of the full-length human PPARγ2 cDNA gene was not successfully amplified. It was believed that a strong secondary structure may be present at the 5' end of the gene. Similar finding was also observed when attempts were made to amplify the 5' end of PPARγ2 cDNA gene from other species (guinea pig, chicken, sheep and goat). In all cases, the sequence data demonstrated that non-specific fragments were amplified from all the species, similar to as observed in human.

Thus, an alternative method of utilizing *in silico* approach was used in order to map the transcriptional start site for human PPARγ2 cDNA gene. For this, a transcriptional start site prediction software [Neural Network Promoter Prediction software (http://www.fruitfly.org/seq _tools/promoter.html)] was used.

Analysis of the interaction between the human PPARγ2 promoter region and transcription factors

Electrophoretic mobility shift assay (EMSA) or gel retardation analysis is carried out to determine the transcription factor binding sites in the promoter region of human PPARγ2 as well as to monitor sequence-specific DNA binding activities in nuclear extracts.

Different sizes of double-stranded promoter fragments were generated by subjecting the 1kb promoter fragment to B*st*EII and E*co*RI digestion which produced fragments with the size of approximately 300bp (Fragment A), 550bp (Fragment B), 450bp (Fragment C) and 700bp (Fragment D).

Each fragment was then purified and subjected to 1.5% (w/v) agarose gel electrophoresis. Four fragments with expected sizes (300bp, 550bp, 450bp and 700bp) were produced indicating that the restriction digestion was successfully carried out. Each fragment was then radiolabelled and incubated with nuclear extracts prepared from human breast carcinoma T-47D cell line. The DNA-protein complexes were resolved on a 4% (v/v) non-denaturing polyacrylamide gel (29:1 acrylamide/bisacrylamide) containing 0.25X TBE. Following electrophoresis, the gel was dried and then exposed to Kodak X-ray film.

In all cases, at least three major DNA-protein complexes, designated C1, C2 and C3, were apparent in EMSA using nuclear extracts from human breast carcinoma T-47D cell line. None of these complexes was present when only the

radiolabelled probe was used (i.e. in the absence of nuclear extracts). However, in order to examine whether the DNA-problem complexes represented specific interactions of nuclear extract proteins with the radiolabelled probe, competition EMSA was carried out. For this, 4µg of nuclear extracts were incubated together with labeled promoter fragment and 100-fold molar excess of unlabelled promoter fragment. Only DNA-protein complexes C1 and C2 could be either completely or partially competed out whereas complex 3 was not. This competition assay strongly indicates that only C1 and C2 complexes represented the specific interaction between transcription factors present in the nuclear extracts and the respective promoter fragments. Overall, the binding activity (complexes C1 and C2) was the most prominent and highest when the nuclear extract was incubated with promoter Fragment A indicating that Fragment A contained the most binding sites for transcription factors required for constitutive expression of human PPARγ2 mRNA in human breast carcinoma T-47D cell line.

Analysis of the functional activity of the human PPARγ2 promoter region

The determination of the activity of various 5' promoter truncations was carried out by transient transfection assays. For this, a plasmid containing human PPARγ2 promoter was used as the template to amplify five different 5' promoter truncations with the size of 200bp, 360bp, 520bp, 750bp and 1kb. Each forward and reverse primer were designed to contain the digestion sites for Hind III and BgI II, respectively, to facilitate the cloning of the fragments into reporter plasmid, pGL3. Each fragment was then excised from the agarose gel, purified and size fractionated on 1% (w/v) agarose gel. The sizes of all the purified fragments were similar to that of the amplified fragments indicating that the gel extraction was successful.

The purified promoter fragments were digested with Hind III and Bgl II restriction enzymes to generate sticky ends in order to facilitate the subcloning of the promoter fragments into pGL3 reporter plasmid. The ligation reactions were then transformed into *E.coli* JM109 competent cells and plated out on LB agar plates containing ampicilin. Subsequently, colony PCR was carried out on randomly selected, well isolated white colonies in order to determine the transformed cells that contained the recombinant plasmids with the correct inserts. The colony PCR products were then analyzed on 1% (w/v) agarose gel and visualized under UV transilluminator. The sizes of the fragments generated using colony PCR, which were 200bp, 360bp, 520bp, 750bp and 1kb, matched with the size of the inserts indicating that the selected white colonies contained the recombinant plasmids with the correct inserts.

The recombinant cells were then propagated in LB medium in the presence of ampicillin and the recombinant reporter plasmids containing the inserts of various 5' promoter truncations of human PPARγ2 were then isolated. The condition for transient transfection was optimized using pGL3-control. For that, 2µg of pGL3-control was transfected into human breast carcinoma T-47D and human hepatocellular carcinoma Hep3B using Lipofectin (Invitrogen), and human histiocytic lymphoma U937 using SuperFect (Qiagen). 1µg of pRL-TK was co-transfected to serve as an internal control for the transfection efficiency. The cells were then incubated at 37°C in CO_2 incubator for 3 hours. After incubation, the medium was replaced and the cells were further incubated for another 24 hours before the cells were harvested for luciferase assays using Dual-Luciferase Reporter Assay System (Promega). Luciferase activity was then measured using a luminometer (TD-20/20 Turner Designs).

Transient transfection assays were then carried out using the optimized condition to determine the functional activity of various promoter truncations of human PPARγ2 in T-47D. For that 2µg of promoter truncations was transfected into human breast carcinoma T-47D using Lipofection (Invitrogen). 1µg of pRL-TK was co-transfected to serve as an internal control for the transfection efficiency. The cells were then incubated at 37°C in CO_2 incubator for 3 hours. After incubation, the medium was replaced and the cells were further incubated for another 24 hours before the cells were harvested for luciferase assays using Dual-Luciferase Reporter Assay System (Promega). Luciferase activity was then measured using a luminometer (TD-20/20 Turner Designs).

The relative luciferase activity of various promoter truncations of human PPARγ2 in human breast carcinoma T-47D were lower than pGL3-basic, which lacks eukaryotic promoter and enhancer sequences. Expression of luciferase activity in cells transfected with this plasmid depends on insertion and proper orientation of a functional promoter. These results show that the inserted promoter fragments may not be functional promoters in T-47D cells or may due to the low expression of PPARγ2 in human breast carcinoma T-47D.

Conclusion

Overall, the aims of these works have been achieved. The transcriptional start site of the human PPARγ2 gene has been identified i.e. nucleotide A located 176bp upstream to the translational start site. The interaction between the human PPARγ2 promoter region and transcription factors has been investigated and it showed that the 300bp fragment contained the most binding sites for transcription factors required for constitutive expression of human PPARγ2 mRNA in human breast carcinoma T-47D cell line. However, this study showed that the human PPARγ2 promoter may not be fully functional/possessed very low transcriptional activity in human breast carcinoma T-47D.

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"Chemical & Biological characterization of Indigenous Actinomycetes isolated from marine organisms of West Coast of Peninsular Malaysia"
Year 2001 MTSF Science & Technology Research Grant Recipient
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The research was undertaken to study mainly the bioactivity potential of actinomycetes derived from marine macro-organisms. Terrestrial actinomycetes have been reported to be prolific producers of antimicrobials and marine organisms were noted for their production of novel bioactive substances which may have the potential to treat a wide range of diseases. Further, the marine environment harbours a great diversity of marine macro and microorganisms, which is a good source of novel compounds; and yet marine biodiversity is one of the least studied or characterized. Thus, the diversity of actinomycetes in the marine hosts was explored via morphological, biochemical and molecular methods in an attempt to associate it with the biological activity observed.

Objectives

i) to isolate actinomycetes from selected marine organisms by selective isolation methods.
ii) to characterize the isolates obtained, by a combination of morphological, biochemical, physiological and molecular methods.
iii) to assess the antagonistic potentials of the isolates against various pathogenic microorganisms.

Methodology

Selected marine plants and invertebrates, including seaweeds, sponges, soft corals, sea cucumbers and nudibranchs were collected offshore at different depths along the beaches of Port Dickson and Pangkor Island. The specimens were firstly identified, then freeze-dried and ground to powder before plating on different selective agar media prepared using sea water collected from the same sampling sites. Out of 57 specimens processed, a total of 299 actinomycete isolates were obtained.

The isolates were further grouped and characterized by a combination of morphological (color-grouping and microscopy), biochemical (determination of diaminopimelic acid isomers), physiological (sodium chloride tolerance and sole carbon source utilization) and molecular (restriction fragment length polymorphism) methods according to standard procedures published in the literature.

Representative isolates from the grouping above were randomly selected for further assessment of their bioactivity potential. Classical antimicrobial tests (antibacterial, anti-yeast, antifungal) against various reference pathogenic cultures on agar plates were performed in two stages: primary screening via growth of the isolates and secondary screening, which involved crude extracts prepared by using organic solvents on the isolates. The crude extracts were also used in standard neutral red cytotoxicity assays against human colon and cervical cancer cell lines to assess antitumor potentials of the isolates. Promising crude extracts were further analyzed by HPLC methods to determine the chemical composition of the bioactive compound.

Results

The results showed that there is a vastly untapped sources of bioactive compounds from actinomycetes in the west coast of Peninsular Malaysia. Although characterization of the isolates showed that majority were most likely terrestrial, it was clear that actinomycete propagules, regardless of their origin, could reside and remain viable in various marine plants and invertebrates. The apparent moderate diversity observed in this study could be an underestimation due to the use of selective media and the fact that more than 99% of microorganisms in nature are not cultivated by using standard isolation techniques. Out of the 299 isolates, 76% was identified as streptomycete-like while the remaining 24% as non-streptomycetes (predominantly actinoplanetes).

Good antimicrobial activities were obtained in the primary and secondary screenings when compared to antibacterial and antifungal controls. This is in agreement with reports which state that marine-derived actinomycetes exhibit good antibiotic activities at frequencies comparable to terrestrial isolates. Selected isolates showed a high degree of antagonism against important clinical strains of *Salmonella*, *Shigella*, *Vibrio*, *E. coli* O157 and *Candida* spp.; which could be the first report of such antagonism in Malaysia. Selected isolates also showed strong bioactivity against *Ganoderma boninense*, which is the major fungal pathogen in oil palm plantations. Four crude extracts were shown to be cytotoxic against human colon cancer cell line, HT29 at effective doses, ED_{50} of less than 10 µg/ml. This could be the first report of good cytotoxic activity by marine-derived actinomycetes against HT29 cell line which indicated actinomycetes associated with marine organisms as promising lead sources of anti-cancer drugs.

HPLC analysis of 51 actinomycete crude extracts indicated that 96% was consisted of known secondary metabolites. Two extracts, AUM152 and AUM156 which showed fractions of possibly unknown compounds, also turned out to be quite similar to two known compounds, lasonolide A and piericidins, respectively when LCMS analysis was conducted. Lasonolide A was previously reported to be produced by a marine sponge and this might be a first indication of possible microbial origin of this compound.

Future Direction

RFLP analysis on selected isolates showed a high degree of polymorphism in 16S rDNA sequences and did not correlate well with findings from biochemical characterization of the isolates, nor did it provided a correlation with the observed biological activities.

Large scale re-fermentation of AUM152 and AUM156 would allow more crude extracts to be obtained for purification and verification of the unidentified compounds via structure elucidation.

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Miss Tai Yan Chin
Professor Dr. Peh Suat Cheng

Universiti Malaya

"Characterization of Genetic alteration in the Lymphoma of Mucosa-associated Lymphoid Tissue (MALT)"
Year 2001 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is the most common extranodal lymphoma, comprising of approximately 30% of the extranodal lymphoma. MALT lymphoma is postulated to have originated from accumulation of MALT at sites that are devoid of this issue in normal condition by the presence of an antigen or autoimmune antibodies. Continuous antigenic stimulation could lead to genetic aberrations that ultimately transorm the cells into neoplastic cells. Some of the genetic abberrations known to be associated with MALT lymphoma are t(11;18)(q21;21) translocation, trisomy 3, and *p53* gene mutation. The presence of t(11;18)(q211;q21) translocation has been associated with poor response *Helicobacter pylori* antibiotic therapy, but the pathogenetic importance of trisomy 3 has not been eluciated thus far. Mutation in *p53* gene might contribute to transformation of low-grade tumours to high-grade tumours.

This project investigates these genetic aberrations by fluorescence in-situ hybridization (FISH) and polymerase chain reaction-single strand conformational polymorphisms (PCR-SSCP). A total of 52 MALT lymphoma were retrieved from the archive of Department of Pathology, University of Malaya Medical Centre. The diagnosis of MALT lymphoma was confirmed by a pathologist (Peh SC) with the aid of histomorphology and immunophenotypic expressions. The tumours were graded histologically into Grade 1 (low-grade MALT lymphoma), Grade 2 (DLBCL with MALT component/transformed MALT lymphoma) and Grade 3 (DLBCL without MALT component/primary DLBCL) tumours. The LSI MALT1 probe was designed to flank the common breakpoint in MALT1 gene (18q21) that is involved in t(11;18)(q21;21) translocation, thus detecting the rearrangement of MALT1 gene.

Rearrangement occurred in 9/40 (23%) of the cases, including 7/23 (30%) Grade 1, and 2/11 (18%) Grade 3 tumours, and the remaining 12 cases failed to yield satisfactory results. Amplification of 18q21 region was detected in 10/40 (25%) cases, and one of the Grade 3 tumour carried 18q21 rearrangement as well. Trisomy 3 was detected in 9/34 (26%) cases, of which 3 tumours (2 Grade 1 and 1 Grade 3) also carried 18q21 rearrangement, and four other tumours (3 Grade 1 and 1 Grade 3) carried additional 18q21 amplification. The frequency of 18q21 rearrangement concurred with previous studies that reported a range of 29% to 60%. Pulmonary MALT lymphoma is known to be highly associated with t(11;18)(q21;q21) when compared to gastric MALT lymphoma. Therefore the lower frequency of 18q21 rearrangement in this study might be attributed to low number of pulmonary cases (1/52, 2%), and high number of gastric MALT lymphoma (42/52, 81%).

The majority of the cases with allelic imbalances (9/10, 90%) with 18q21 amplification, and 6/9 (67%) with trisomy 3 occurred in cases without 18q21 rearrangement. Therefore, this study agrees with the proposal that Grade 1 tumours

with t (11;18)(q21;q21) translocation is developed along different pathway from that of allelic imbalances. Some rare cases of Grade 1 tumours with t(11;18)(q21;q21) may transform into high-grade DLBCL with additional genetic aberration, especially trisomy 3. Amplification of 18q21 region is not likely to be associated in the transformation.

Inactivation of the tumour suppressor gene *p53* is related to the transformation of MALT-type lymphoma. It is reported that partial inactivation of *p53*, through mutation or allelic loss, is associated with the development of low-grade lymphoma. Complete inactivation contributes to high-grade transformation in MALT lymphoma. Currently, mutational analysis of *p53* gene (exon 5 to exon 8) by PCR-SSCP has been completed and data analysis is in the final stage.

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(replaced Dr. Hairul Azman @ Amir Hamzah Bin Roslan)
Dr. Mohd Hasnain Mohd Hussain

Universiti Malaysia Sarawak

"Characterization of the Starch Biosynthesis Pathway of Sago Palm"
Year 2001 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Generally, the starch biosynthesis pathway in plants involves four groups of committed enzymes: ADP-glucose pyrophosphorylase (AGP; EC 2.7.7.23), starch synthases (SSs; EC 2.4.1.21), starch-branching enzymes (SBEs; EC 2.4.1.28), and starch-debranching enzymes (DBEs; EC 2.4.2.41). AGP forms ADP-glucose from glucose 1-phosphate. SSs add ADP-glucose to the elongation end of an α(1-4)-linked glucan chain, whereas SBEs cut α(1-4)-links and rejoin them as α(1-6) branches that are subsequently trimmed by DBEs to yield short chains for further synthetic extension. However, different isoforms of SSs (soluble and granule-associated SSI, SSII, and SSIII) can participate in the production of branched glucans.

Experimental Work

a. Isolation of the gene coding for granule-bound starch synthase (GBSS)

The main work in the first year year involved attempts to isolate the granule-bound starch synthase (GBSS) gene from the tropical starch-producing sago palm, *Metroxylon sagu*. GBSS enzyme is one of the major enzymes in the biosynthesis pathway of starch molecules in plants. Determination of different isoforms of this enzyme in sago will help in characterization of the different type of starch produced in this plant. Two techniques were involved in the isolation of the GBSS gene from this plant. They were reverse transcriptase-polymerase chain reactions (RT-PCR) and cDNA library screening.

In the experiments employing the RT-PCR method, amplification of the sago total RNA produced a product of about 900 bp. Subsequent cloning and sequencing of this product showed the exact size of the amplification was 917 bp. Sequence similarities of the RT-PCR product was analysed using the BLASTX program, one of the Basic Local Alignment Search Tool (BLAST) programs provided online by the National Center for Biotechnology Information (NCBI) in United States. The results retrieved showed that the deduced amino acid sequence of the RT-PCR product shared high homologies with genes coding for granule-bound starch synthase (GBSS) from other starch producing plants. The sequence shared 72% identity with GBSS gene from rice (*Oryza Sativa*), 71% identity with GBSS gene from potato (*Solanum tuberosum*), 70% identity with GBSS gene from maize (*Zea mays*), 68% identity with GBSS gene from wheat (*Triticum aestivum*) and 68% identity with GBSS gene from cassava (*Manihot esculenta*). From the results obtained, it was concluded that the 917 bp RT-PCR was indeed part of the sago GBSS cDNA sequence. Based on this sequence, a fragment containing about 2 kb of genomic GBSS gene has also been isolated using polymerase chain reactions technique. This fragment is being used as a probe to isolate the entire locus of the sago GBSS and chromosome mapping of the gene by Southern hybridisation. This work is in progress.

The cDNA library screening experiments were conducted in an attempt to isolate the full length gene of GBSS. Screening of the library led to the isolation of one positive clone. Subsequent cloning and sequencing of this clone showed that it encodes for GBSS and was similar to the sequence obtained by the RT-PCR technique. However, comparisons of the derived polypeptide chain with GBSS from other plants indicate that the clone from the cDNA library was truncated at the upstream of the gene sequence. Based on this sequence, a fragment containing about 1.9 kb of genomic GBSS gene has also been isolated using polymerase chain reactions technique. Combining the genomic sequence obtained by this method and the sequence obtained by the RT-PCR method, a near complete genomic sequence of GBSS gene has been isolated and sequenced. Analysis of the sequences obtained using both techniques above revealed that there are 12 exons interspersed by 11 introns in the genomic sequence. The exon/intron organization of the genomic DNA segment could be determined by aligning the cDNA sequence with the genomic DNA sequence.

b. Isolation of the gene coding for starch branching enzyme (SBE)

Starch branching enzymes can be separated into two major groups based on structural and catalytic properties. One group, referred to as SBE family II or A, comprises SBEII from maize, wheat, and potato, SBE3 from rice, SBEI from pea, and SBE2 from *Arabidopsis*. The other group, SBE family I or B, comprises SBEI from maize, wheat, potato, rice , and cassava, and SBEII from pea.. In maize and Arabidopsis, it has been demonstrated that SBEII can be further divided into two types, usually classified as SBEIIa and SBEIIb, that differ slightly in catalytic properties.

In this work, isolation of total genomic DNA and total RNA from sago palm young leaves was carried out. Degenerate primers were designed from conserved regions of starch branching enzymes taken from different plant species. First strand cDNA was synthesized from the extracted total RNA and used as template for RT-PCR. One fragment with the expected size, 550bp, was amplified using the degenerate primers for starch branching enzyme. This fragment was subsequently purified from agarose gel and used for cloning purposes. Blunt-end cloning using PUC18 vector digested with *Sma*I was used in this experiment. Purified PCR product was treated with Klenow fragment to produce a blunt-ended fragment. Ligation of blunt-ended PCR fragments into the PUC18 vector was carried out using T4 ligase at 22°C overnight. The ligated product was then transformed into JM109 competent cells and plated onto LB agar supplemented with ampicilin. Xgal and IPTG were also included for blue white selection. Screening of positive clones using restriction digestion and PCR technique is still in progress. DNA sequencing will be performed in order to confirm the clones.

c. Isolation of the gene coding for ADP-glucose pyrophosphorylase (AGPase)

This research work involves an attempt to isolate the gene coding for ADP-glucose pyrophosphorylase (AGPase), the first enzyme in the starch biosynthesis pathway. Based on previously published conserved amino acid sequences of the small subunit of plant AGPase cDNA clones, a set of degenerate oligonucleotide primers were used to amplify genomic DNA coding for AGPase. Only one primer, namely forward primer A (5'-CCIGGIGCIAAYGAYTTYGG-3'), successfully produced a desired PCR product. PCR amplification using this primer gave a single band with the estimated size of 400 bp in length. The PCR product was labelled with

Digoxenin (DIG)-labelling system and used for southern hybridisation against the sago total genomic DNA digested with *Hind*III. A 6.4 kb fragment was identified and subsequently cloned into pUC19. One positive clone was identified. Plasmid DNA from this clone was isolated and analysed with several restriction enzymes in order to establish a restriction map for the 6.4 kb insert. Restriction analysis with several enzymes namely *Bam*HI, *Eco*RI, *Dra*I and *Pst*I showed that the insert carries a relatively few cut sites. Moreover, most of these cut sites are located within a 1.6 kb region of the insert.

Future Work

Future experiments including construction of a genomic library. The GBSS, SBE and AGPase cDNAs or genomic sequences that have been isolated will be used as probes to screen from the library full-length genomic loci coding for the enzymes. A full characterisation of the genomic determinants will contribute to a full understanding of their expression and regulation. Northern and southern blotting analysis will also be caried out in order to study their expression levels in different tissues of the sago palm.

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Dr. Geeta Selvarajah

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"A Genetic characterization of some *Jasminum* species found in malaysia"

Year 2001 MTSF Science & Technology Research Grant Recipient

Introduction

Malaysia imports Jasmine essential oil to be used in soaps, shampoos etc. *Jasmines* are cultivated in Malaysia, as an ornamental plant, for its flowers which are used to make garlands and in traditional medicine. Studies on *Jasminum's* medicinal properties have shown promising results. It is imperative that unimproved prospective taxa are studied for variation and variants conserved for future selection and development of suitable cultivars. Morphological and morphometric based identification alone may lead to confusion as it is dependent on plant size and environment. In this study patterns of morphological and electrophoretic data were studied to examine species delimitation and patterns of variation in some *Jasminum* species found in Malaysia.

Materials and Method

Individuals from 8 taxa consisting of 5 species and 4 varieties within species were sampled. In **meristic and morphometric** analyses, individuals were scored for: number of flowers per inflorescence, number of corolla lobes per flower, number of calyx teeth per flower, number of whorls of corolla lobes per flower, flower size, corolla tube length, calyx teeth length, pedicel length, corolla lobe length, corolla lobe width, leaf length and leaf width. In **enzyme assay**, enzyme extraction used Tris Borate EDTA, pH 8.6 (Wendel and Weeden, 1989). The electrohporetic run (in a 1 % agarose gel) was for 5 minutes at 200V and subsequently an hour at 75 V. The enzymes assayed were Peroxidase, Malate Dehydrogenase, Acid Phosphatase, Ribulose bis-posphate carboxylase, Phosphoglucomutase, Glucose Phosphate Isomerase and Esterase. Enzyme staining followed protocols of Manchenko (2003). Analysis of meristic and morphometric data was by multi-response permutation procedure (MRPP) using BLOSSOM Statistical Software. To reduce dimensions, a principle component analysis was performed using PAST Statistical Package. Ratios removed size influences of measures, while logarithmic transformation removed deviation from normality. Genetic interpretations based on electrophoretic patterns and enzyme subunit structure were analysed using POPGENE VERSION 1.31.

Results and Discussion

The present investigation shows that clear **morphological differences** exist between the eight described taxa as average within-group distance, delta equals 1.92 at a probability (Pearson Type III) of a smaller or equal to delta of 0.00. The MRPP test statistic A, a descriptor of within groups homogeneity compared to random expectation was 0.56 indicating that, while variation is not purely by chance but due to species delimitation, the grouping is not very tight perhaps due to hybridization. **Genetic variation** was high (%P = 55.5 – 77.7; Ao= 1.67 – 2.11; Ho = 0.26-0.4), possibly because variation produced by sexual reproduction or mutation was maintained by asexual propagation. This implies selection for improvement may be possible. There seems to some disagreement between clustering using electrophoretic and morphological data. Several interspecific distances were smaller than intraspecific distances, whether an artifact of sampling or a reflection of some other underlying reason is unclear. The results are a preliminary attempt to understand

the species delimitation and patterns of morphological and genetic variation in the genus *Jasminum*. Further studies involving larger sample sizes and larger numbers of markers will give a more substantial picture.

Publications

1. Geeta,S. A Genetic Characterization of *Jasminum* species found in Malaysia. 2006. Malaysian Science and Technology Congress pp 56
2. Geeta,S. Intraspecifc and interspecific variation between some *Jasminum* species found in Malaysia. 2006. Proceedings of the 8th National Symposium on Biology.

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"Distribution of Mites, Weevils and their allergen level in Rice and its raw processed products in Malaysia"
Year 2001 MTSF Science & Technology Research Grant Recipient

Introduction

Thirty percent of adult Malaysians with rhinitis had positive skin prick test to rice allergen (Gendeh *et al*., 2000). Stored rice grains may contain many allergens. The allergens may come from rice grain itself (Arai *et al*.,1998), dead bodies and / or excretion of arthropods such as mites and weevils that infested rice (Arlian *et al*., 2002) and from fungi on rice and mites (Hubert et al.,2003). Persons who consumed unprocessed or processed food infested with these arthropods are at risk and may exhibit symptoms such as asthma, rhinitis and/or allergic dermatitis which in extreme case, may lead to anaphylaxis (Olsen, 1998). Prolonged contact with infested foods may produce a mild dermatitis. The importance of mite and weevil-induced allergies caused by presence of these arthropods and their secretions in rice is still unknown in Malaysia. Since rice is the main staple food for Malaysians, there is a potential risk of allergic reactions in individuals who come into contact with stored rice. The distribution of mites and weevils in stored rice and their processed products have not been reported in Malaysia. The purpose of this study is thus to document the presence of arthropods in stored rice and their processed products from various sources in Malaysia.

Objectives

i) To determine distribution of arthropods in stored rice collected from public (non-paddy farmers), paddy farmers, aborigines and commercial outlets.

ii) To determine distribution of arthropods in processed rice products such as rice flour and rice cereals.

Materials & Methods

Samples of stored rice and their processed products were randomly collected. A sub-sample of 150 grams of each stored rice or processed products sample collected was put into a 12 cm diameter sieve that was placed approximately 7.0 cm below a lighted 60 watts frosted light bulb. A petri dish containing lactic acid was placed directly below the sieve to collect arthropods displaced from the rice. The lighted bulbs were switched on for 3 continuous days or until no more arthropods were found. Petri dishes were checked for arthropods twice a day. The whole process was repeated after 1 week to ensure complete extraction of arthropods inclusive of newly hatched larval stages. The techniques for preparation of mites for identification followed those used by Ho & Nadchatram (1984). Mites collected were suspended in 90% lactic acid and heated with constant stirring. The suspension was removed from the heat source as soon as the suspension began to bubble. The suspension was left to cool, after which a small volume at a time was transferred toa Petri dish and examined under 20x magnifications. Whole mites and mite fragments were picked up with a sharpened applicator stick. These were mounted in Hoyer's medium. Mounted slides were dried in an oven at 40oC for 10-12 days before the mites were identified. Weevils were picked up manually and identified.

Results

A. Distribution of arthropods in rice grains from public in urban areas

Samples of rice grains (150) from urban houses in central of Peninsular Malaysia were collected and screened for arthropods. Twenty-five percent (25%) of the samples were infested. Weevils were most commonly found followed by mites. Weevils and mites infested 19% and 2%, respectively. Only 4 % of the samples were infested by both arthropods. The weevils found, belonged to 2 species *Sitophilus oryzae* (82.1%) and *Sitophilus granarius* (17.9%). One medically important species of dust mites causing allergic reactions, *Dermatophagoides pteronyssinus* was identified. Adult *D. pteronyssinus* (11.1%), immature *Cheyletus* spp (33.3%) and Mesostigmatids (55.6%) were common mites found infesting rice grains. Immature stages of weevils and mites were also found.

B. Distribution of arthropods in rice grains from paddy farmers

Samples of rice grains (150) from paddy farmers in Kuala Selangor, Tanjung Karang and Sekinchan, Selangor were collected and screened for arthropods. Only 8% of the samples showed infestation. Weevils were the main arthropods in those rice grains and were represented by 2 common species, *Sitophilus oryzae* (6%) and *Sitophilus granarius* (3%). Ninety percent (90%) of the weevils were larval stages and could not be identified due to un-development of certain important structures required for identification. Mites were found in 2 samples only and were identified to be of free living group of mites.

C. Distribution of arthropods in rice grains from aborigines

Samples of rice grains (150) from aborigines in 7 settlements i.e Kemidak-Selai, Endau-Rompin in Johor; Pasoh, Jelebu in Negeri Sembilan; Kuala Lah, Sg Lah and Sg. Rual in Gua Musang, Kelantan; Pos Raya, Simpang Pulai in Kinta, Perak; and Pos Chiong, Sg Banon in Grik, Perak. Only 18% of the samples were infested with arthropods. Weevils which were most commonly found (99%) were represented by 2 common species *Sitophilus granarius* (15.2%) and *Sitophilus oryzae* (8%). Free living mites contributed only 0.2% of the infestation.

D. Distribution of arthropods in rice grains from commercial outlets

Samples of rice grains (150) from various commercial outlets (supermarket, sundry and grocery shops) in central of Peninsular Malaysia were collected and screened for arthropods. A total of 31.3% of the samples were infested. Mites were the main arthropods in rice grains from commercial sources. The mites identified were *Cheyletus malaccensis, Cheyletus fortis, Cheyletus* spp., *Suidasia pontifica, Austroglycyphagus malaysiensis* and *Grammolichus malakuensis*. Two of these mites were of medically important species.

E. Distribution of arthropods in rice flour from commercial outlets

Samples of rice flour (150) from 15 brands sold in various commercial outlets (supermarket, sundry and grocery shops) in central of Peninsular Malaysia were collected and screened for arthropods. Only 7% of the samples were infested with arthropods. Most common arthropods found were mites. The most abundant species recovered were *Tarsonemus* spp (72%) and *Suidasia pontifica* (16.7%). These 2 species were common mites known to infest storage food. Other mites found were *Suidasia oribatei, Tyrophagus putrescentiae* and Prostigmatid mites.

Distorted specimens represented 3.8% of total mites recovered. The weevils found were of 2 common species, *Sitophilus granarius* (83%) and *Sitophilus oryzae* (17%).

F. Distribution of arthropods in rice cereal-based infant food from commercial outlets

Samples of rice cereal-based infant food (150) sold in various commercial outlets (supermarket, sundry and grocery shops) in central of Peninsular Malaysia were collected and screened for arthropods. None was found infested.

An interest to investigate over due products and damaged packages / container (damp, rusty, dirty etc) was carried out from January 2002 to December 2003. A total of 42 samples from 4 brands of rice cereal-based infant food was recovered and screened. Only 1 sample was infested. Mites of the family Tydeidae were the only arthropod found.

Conclusion

There is not many related research concentrating on arthropods in rice grains and processed rice products in Malaysia. This study has shown that:

i) Samples of rice grains from paddy farmers had the least infestation of arthropods (8%), followed by samples from aborigines (18%) and public in urban areas (25%). The highest infestation of arthropods was recovered from commercial outlets (31%).

ii) Distribution of arthropods in rice grains demonstrated the presence of 3 allergenic mite species i.e *Austroglycyphagus malaysiensis, Dermatophagoides pteronyssinus* and *Suidasia pontifica*. Weevils, *Sitophilus oryzae* and *Sitophilus granarius* that are known to be allergenic were also found.

iii) Only 7% of rice flour samples collected was infested with arthropods, mostly by mites. Two common species of mites known to infest storage food, *Tarsonemus* spp and *Suidasia pontifica* were found. Other mites extracted were *Suidasia oribatei, Tyrophagus putrescentiae* and Prostigmatid mites. The weevils found were of 2 common species, *Sitophilus granarius* and *Sitophilus oryzae*.

iv) No infestation by arthropods was found in non over-due rice cereal-based infant food samples. However, an investigation of over due products and damaged packages / container showed only 1 sample (2.4%) infested. Mites of the family Tydeidae were the only arthropod found.

v) The highest infestation of arthropods from commercial outlets was from samples of rice grains (31%), followed by rice flour (7%).

vi) Weevils were found in all samples except rice cereals. Two commonly found weevils were *Sitophilus granarius* and *Sitophilus oryzae*. Both species were known to be allergenic.

vii) Dust mites occurred in rice grains from all sources except from samples collected from paddy farmers and aborigines.

Publications

1. Distribution of arthropods in stored rice grains from several sources in Malaysia.
2. The occurrence of arthropods in processed rice products in Malaysia.
3. Level of arthropod infestation of rice stored in different containers.

Dr. Ho Chai Ling
Miss Phang Siew Moi

Universiti Putra Malaysia

"Genetic Engineering of Seaweed for better properties of Alginate : Molecular cloning of mannuronan C-5-epimerase from Brown alga, *Sargassum binderi*"
Year 2001 MTSF Science & Technology Research Grant Recipient

Sargassum C. Agardh (Sargassaceae, Fucales) is a source for alginic acid, alginates, sulfated fucoidans, pigments, oils, sterols and mannitols. Despite its importance in producing various biochemicals, there is a general lack of genomic information for this genus. The objective of this study was to provide more sequence information for this genus by examining *Sargassum binderi* (Sonder) J. Agardh, a tropical brown seaweed using an expressed sequence tag (EST) approach. The primary cDNA library used in this study consisted of 9.2 X 10^5 clones with about 99% of recombinant clones as revealed by X-Gal/IPTG screening. Sequencing of 2304 cDNA clones generated 1876 readable sequences, from which 1270 tentative unique genes (TUGs) consisting of 991 singletons and 279 contigs, were obtained. The TUGs generated in this study contained a minimum of 2 ESTs to a maximum of 12 ESTs. Among the 9-12 ESTs in the contigs were peroxiredoxin, heat shock protein 70, putative type III polyketide synthase, beta actin, 14-3-3 and two elongation factors. About 40% of the TUGs have significant matches (score bits >50 and E-values < 10^{-5}) to the public databases. They were further divided into 9 subcategories: metabolism, transcription and translation, cellular processes, protein folding, sorting and degradation, signaling, membrane and transport, receptor, other functions and unknown proteins. The EST approach enabled us to identify and isolate the partial cDNAs encoding for phosphomannose mutase, GDP-D-mannose 4,6-dehydratase and fucokinase that are involved in fucose biosynthesis. These sequences are important for the understanding of fucoidan biosynthesis. There were also transcripts involved in many other pathways such as nucleoside-diphosphate-sugar epimerase, dihydrodiol dehydrogenase, monooxygenases and polyketide synthase. In conclusion, the EST approach has enabled us to isolate many transcripts from *S. binderi*, which is difficult to achieve by using single gene approach. The materials and information derived will be useful for the preparation of gene arrays for future functional genomic research.

Publications

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Universiti Malaya

"Investigation of the role of divalent cations in Dengue Virus-induced apoptotic pathway"

Year 2001 MTSF Science & Technology Research Grant Recipient

Introduction

Manifestation of dengue varies from mild dengue fever to the more fatal dengue haemorrhagic fever and dengue shock syndrome. Despite the serious implications, the pathogenesis of this disease has not been thoroughly understood. When the project was proposed, it was shown that dengue viruses induced apoptosis in vitro and also in vivo. The mechanisms, however, was not adequately explained. The apoptotic mechanisms of some viruses such as HIV, rubella virus and avian sarcoma virus revealed significant involvement of divalent cations especially in viral protein processing and host caspases enzymatic activities. Since apoptosis were shown to be directly related to specific disease states, understanding of the pathway in dengue virus-induced apoptosis was crucial. In the proposed study, different concentrations of physiologically important divalent cations such as Zn2+, Ca2+, Mg2+ and Mn2+ were added to dengue virus-infected cell cultures and the effects on the induction of apoptosis were assessed. We anticipated that results from the study could help to explain the involvement of cations in the mechanisms of dengue virus-induced apoptosis and improve our understanding of dengue virus pathogenesis. Therefore, the objective of the study was to assess the roles of divalent cations on the mechanisms of dengue virus-induced apoptosis.

Objective

To assess the roles of divalent cations on the mechanisms of dengue virus-induced apoptosis.

Methodology

C6/36 and Vero cells were cultured in Eagle's minimum essential medium supplemented with 10% fetal calf serum. Dengue virus type-2 (DENV-2) inoculum was propagated in the C6/36 cells and stocks were prepared following established protocols. Infected and mock-infected cell cultures were treated with selected concentrations (0.1, 0.5, 1.0, 2.5 and 5.0 mM) of $CaCl_2$ (Ca2+), $MgSO_4$ (Mg2+), $MnCl_2$ (Mn2+) or $ZnSO_4$ (Zn2+). Apoptosis in the cells was detected by observation of a DNA laddering pattern, and also using a commercially available terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining.

Results

DENV-2 infection of Vero cells resulted in cellular DNA fragmentation characteristic of apoptotic cells. The findings suggested that induction of apoptosis is an important cellular event in the pathogenesis of dengue. At low multiplicity of infection (MOI), which perhaps mimics the natural infection, apoptotic cells were detected in Vero cell cultures after 8 days of infection. A significant correlation between the presence of DENV-2 antigens and apoptotic cells showed that expression of DENV-2 proteins was necessary for apoptosis to occur. Zn2+-treatment resulted in acceleration of

the apoptosis which was detected as early as 24 hours post-infection. Based on these and other results, we proposed that accumulation of dengue viral proteins in the ER resulted in intracellular stress leading to apoptosis which was accelerated in the presence of Zn2+. Since the induction of apoptosis has been argued as one of the potential mechanisms whereby the host limits virus infection, the acceleration of apoptosis by Zn2+ or drugs that mimic the effects of Zn2+ could perhaps be exploited to hasten the removal of dengue virus infected cells, thus preventing further infection.

Publications

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Miss Chee Hui Yee
Professor Sazaly Abu Bakar
Miss Cheah Chen Yee

Universiti Malaya

"Molecular Investigation of Dengue Virus-Cell Interaction"
Year 2001 MTSF Science & Technology Research Grant Recipient

Dengue virus can infect a wide range of cells from mosquito, primate to human. The envelope (E) protein of dengue virus is known to act as the viral attachment protein and the binding motif on E protein is between amino acids 281 and 423 while amino acids 34 to 253 was non-binding.. Two candidate receptor molecules have been proposed, they are glycosaminoglycans and lipopolysaccharide binding CD14-associated molecule. However, other researchers have shown that those molecules are not essential for dengue virus binding or are not the only receptor. They showed that different virus strains uses different receptor for different cell types. Therefore, the receptor for dengue virus remains controversial.

The research was undertaking to map the binding site of E using the novel technique of phage display technology to determine the dengue virus precise mechanisms of protein-protein interaction.

Different regions of dengue virus 2 E binding motif were amplified from dengue virus 2 RNA using specifically designed primers. The amplified fragments were cloned into phagemid and displayed on phage.

Recombinant phages displaying different regions of E binding motif were used to determine the binding ability on various cells. Cells were incubated with recombinant phages and the unbound phages were washed off. The titer of the bound phages was determined by counting the colonies on antibiotic selective agar plate after the phages infecting *E. coli*. The recombinant phages has higher binding ability was determined.

Total solubilized protein from various cells was prepared and separated on SDS-PAGE. The proteins were transferred onto nitrocellulose membrane and probed with recombinant phages displaying different regions on E binding motif. Antibody against the phages was used to detect the binding and identify the molecular weight of the interactive protein.

The finding showed that different truncated dengue 2 virus envelope proteins have different degree of binding onto cells with recombinant phage displaying amino acids 380-423 (EB4) has the highest number of bound phages and followed by EB2, EB3 and EB5. This finding provides important information on the interacting region between dengue 2 virus envelope proteins and host cell, C3/36.

The EB4 recombinant phages however, showed non-specific binding onto immobilized mosquito C6/36 cells protein on 1D membrane and 2D membrane either using normal washing condition or more stringent washing condition with addition of 0.5 M NaCl in the washing buffer during far-western analysis. This study revealed that the recombinant phages were not suitable to be used in Far-Western analyses and alternative approaches should be considered.

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Dr. Alexander Chong Shu Chien
Dr. Tengku Sifzizul Bin Tengku Muhammad
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Universiti Sains Malaysia

"Characterization and Isolation of useful substances from Mucus secretion of a fish species demonstrating "milking" behaviour"
Year 2001 MTSF Science & Technology Research Grant Recipient

The project is undertaken to elucidate the mucus proteome of a fish species displaying external parental care.

Objectives

1. Optimization of protein separation process from fish mucus.

2. Comparison of total protein content from mucus of parental and non parental discus fish.

3. 2D electrophoresis of mucus from parental and non parental discus fish.

4. Mass spectrometry of selected proteins from parental and non parental discus fish.

Material and Methods

All parents used for breeding and larval experiment were selected from stock population maintained at Laboratory of Fish Biology, Universiti Sains Malaysia. Readily paired fishes are easily recognized from observation of territorial behavior and are separated into breeding tanks (2' x 2'x 1.5').

Fish mucus collection was done through scrapping of the dorsal-lateral part of body with clean spatula, and stored in clean glass vials on ice. Scrapping was not carried out at the ventral area to avoid possible urinal contamination. Collected mucus was then centrifuged at 12000 g for 30 minutes at 4^0C followed by storage of supernatant in -70^{o}C prior to analysis. Protein content of mucus was analysed using the Bradford Assay (reference) utilizing a commercial assay kit (BIORAD®).

2D separation of proteins was carried out using first dimension isoelectric separation followed by a 2nd separation based on molecular weight of proteins. Spots of interest were excised from preparative gels for trypsin digestion. Mass spectrometry analyses were carried out in Protein and Proteomics Center, National University of Singapore using the MALDI-TOF/TOF method.

Results

- First known separation and identification of protein mucus from fish.
- First to show that proteome in mucus may undergo changes in expression during shift from non-parental to parental stages.
- Publication in a high impact factor journal.

Unresolved problems/future direction

- A large number of unidentified proteins due to lack of proper database.
- More detailed separation of discus mucus proteins.

Publications

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Dr. Lee Ping Chin
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Universiti Malaysia Sabah

"Serine/Threonine Kinases and their Inhibitors in Mycobacterium and Streptomyces"
Year 2002 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction and Objectives

Protein phosphorylation is the principal mechanism of regulation of nearly all aspect of a cell life. This process is carried out by specific protein kinases and is often coupled to dephosphorylation, which is carried out by protein phosphatases. In prokaryotes, it involves a two-component system, consisting of histidine kinase sensors and their response regulators. In eukaryotes, serine, threonine and tyrosine protein kinases and phosphatases instead are the backbone of signal transduction pathways. Previously, these kinases and phosphatases were thought to be unique to eukaryotes. However, evidences have revealed that many prokaryotes also contain these eukaryotic phosphoester kinases and phosphatases such as in *Streptomyces* and *Mycobacterium*. These serine/threonine protein kinases coupled with its phosphatases could play the key roles in developmental process such as dormancy, determining cell number, intracellular survival, or persistency and pathogenicity in *Mycobacterium.* Understanding of the signaling mechanism will be able to facilitate the development of specific protein kinase or phosphatase inhibitors, as well as drugs that modulate the activities of these specific protein kinases and phosphatases. Therefore, this project aimed to study the basis of those kinases and elucidate their possible role in cell regulation which subsequently facilitated identification of their inhibitors.

Results

This study identified five serine/threonine kinases and one phosphatase of *Mycobacterium bovis* BCG Pasteur 1173P2 named PknI-BCG, PknJ-BCG, PknH-BCG, PknK-BCG, PknL-BCG and MPP-BCG, respectively. These kinases and phosphatase were cloned by PCR and expressed in *E. coli* for further characterization. All the kinases possess the eleven catalytic sites of Hank's Domains that conserved among serine/ threonine protein kinases. Functional analysis revealed that PknI-BCG, PknJ-BCG, PknH-BCG, PknK-BCG and PknL-BCG might be involved in bacterial metabolism regulating cell segregation and growth. In vitro phosphorylation assay using purified recombinant histidine-tagged of the kinases indicated these proteins were functional and had the ability to autophosphorylate. Phosphoamino acid analyses identified serine and threonine as the phosphorylated residue. Phosphotyrosine was not observed. This further confirmed that the cloned genes were the eukaryotic-like serine/threonine kinases of mycobacteria.

MPP-BCG was also characterized. Histidine-tagged MPP-BCG recombinant protein showed distinct phosphatase activity toward p-nitrophenyl phosphate with optimal temperature 55ºC and strictly Mn^{2+} dependent. This phosphatase contained the eleven motifs that are universally conserved and characteristic of PP2C family. MPP-BCG was not inhibited by okadaic acid or sodium orthovanadate, which are known to be specific inhibitors of eukaryotic-like phosphatases. We detected phosphatase strong activity toward the phosphoThr (pThr) and but not phosphortyrosine.

On the other hand, inhibitors for the protein kinases and phosphatases have also been screened. Sources for such screening were obtained from the crude extracts of fungi and actinomycete isolated from soil samples collected in the Sabah Rain Forests. The strategy for survival during chronic stages of infection by *Mycobacterium* entails a metabolic shift in the bacteira's carbon source to C2 substrates generated by β-oxidation of fatty acids. Under these conditions, glyoxylate shunt is up regulated to allow maintenance of the tricarboxylic acid cycle and assimilation of carbon via gluconeogensis. Isocitrate lyase is an enzyme in the glyoxylate shunt by converting isocitrate to succinate and glyoxylate. Therefore, disruption of *icl* attenuates persistence of *M. tuberculosis* in mice or inflammatory. A mutant, Δ*icl M. smegmatis* complemented with the *M. tuberculosis icl* gene was used. Both wild type and complemented bacteria were plated on minimal agar containing either glucose or acetate as the sole carbon source. Discs of filter paper soaked with crude extract from isolated *Streptomyces* were placed on the agar and inhibition zones were scored, indicating the inhibition of isocitrate lyase activity. Preliminary studies in our laboratory showed a few potential inhibitors and we have especially concentrated in extract from H7763. The crude compounds were partially extracted by solvent-solvent extraction and further fractionated using reversed phase semi-preparative HPLC column. These compounds were further purified for future studies.

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Universiti Malaya

"Haematological and Molecular characterization (Prevalent Study) of Alpha-Thalassaemia in Malaysia"
Year 2002 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Thalassaemia is a genetic blood disorder of haemoglobin synthesis and one of the most common inherited disorders in the world population. Alpha-thalassaemia is caused by deletion or mutations within the α-globin gene complex, leading to decrease or absence of α-globin chain production. Deletion of all four α-genes leads to the fatal condition Hb Bart's hydrops foetalis where the foetus dies *in utero* or very shortly after birth. In addition to foetal loss there is a significantly increased obstetric risk to the mother of an affected foetus.

The identification of the different genotypes of α-thalassaemia is crucial since prenatal diagnosis and genetic counselling should be offered to families at risk for a Hb Bart's hydrops foetalis. In Malaysia, there is however a paucity of data on the use of different haemoglobin indices and on the distinct red cell indices cut-off values between different types of microcytic anaemias and for the screening of thalassaemic carriers using molecular protocols.

This project was carried out to establish more accurate haematological tests and molecular analysis methods for thalassaemia detection and characterisation in pregnant women in Malaysia. In the process of doing so, the prevalence of the different types of α-thalassaemia in the three different ethnic groups – Malays, Chinese and Indians will also be determined.

Objectives

The objectives of the research were: (I) to establish rapid and specific molecular techniques for the confirmation of α-thalassaemia; (II) to determine the frequency and genotypes of α-thalassaemia; and (III) to determine the red cell indices predictive of α-thalassaemia in pregnant women in our population.

Methodology

DNA samples were obtained from 670 pregnant women (MCV≤89 fL and/or MCH ≤28 pg) who attended the Antenatal clinic of UMMC, Kuala Lumpur. Complete blood count results of each pregnant woman were recorded.

A duplex-PCR protocol for confirmation of the SEA deletion was optimised and developed. PCR for confirmation of the $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions, and Hb CS were modified in-house to establish more accurate, specific and cost-effective DNA amplification protocols.

All DNA samples were tested for the presence of α-thalassaemia (--^{SEA}, -α$^{3.7}$, -α$^{4.2}$ deletions and HbCS). Presence of the normal α-globin genes was also confirmed in order to differentiate the heterozygous or homozygous states of α-thalassaemia carriers. The α-thalassaemia genotypes frequency in the Malays, Chinese, Indians and other ethnic groups/populations were determined.

Comparisons of the red cell parameters between each α-thalassaemia genotype were carried out. The sensitivity and specificity of predicting and differentiating the α-thalassaemia genotypes using red cell indices were calculated using a number of cut-off values and receiver operator characteristic (ROC) curves were constructed. The effectiveness of each indices was evaluated.

Determinations of MCV and MCH cut-off values for predicting the α-thalassaemia, heterozygous α^{0}- and α^{+}-thalassaemia were carried out by selecting a properly balanced specificity and sensitivity cut-off value for each indices. Validities of the cut-off values were tested by calculating the sensitivity, specificity, correct classification, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) of each cut-off value.

Results

I. The prevalence of α-thalassaemia was 15.8% and the genotypes detected were: --^{SEA}/αα (3.7%), -α$^{3.7}$/αα (10%), -α$^{4.2}$/αα (0.6%), α^{CS}α/αα (1.3%) and -α$^{3.7}$/α^{CS}α (0.2%).
II. The --^{SEA} deletion was present in the Malays at 2.5% (10/402) and was observed to be significantly higher in the Chinese (15%, 15/100).
III. The -α$^{3.7}$ deletion was detected in the Malays (10.7%, 43/402), Chinese (10%, 10/100), Indians (7.4%, 11/148) and in 3 women from other ethnic groups/populations (one with Thai ancestry, an Arabic Malay with Yaman ancestry and a Bidayuh).
IV. The -α$^{4.2}$ deletion and Hb CS were detected at low frequencies in the Malays (1% and 0.3% respectively).
V. The results from this study indicate that the cut-off values of MCV <80 fl and MCH <26 pg are recommended for screening of α-thalassaemia in pregnant women.
VI. Pregnant women with MCV ≤74 fl and MCH ≤23.1 pg should undergo molecular tests for the determination of the double α-globin gene deletions that contribute to α^{0}-thalassaemia.
VII. Six DNA amplification protocols were established including a duplex-PCR which offers a more rapid, simple and cost-effective prenatal diagnosis technique that can be carried out in any government hospital with basic funding and technically trained staff.

Unresolved problems and future direction

A more complete study should ideally include samples from other parts of Malaysia, including the Aborigine groups. Due to unavailability of adequate blood samples, serum assays were not carried out on all the samples collected from anaemic women. The co-existence of other forms of thalassaemia and haemoglobinopathies were not available, as

haemoglobin electrophoresis was not carried out. The presence of other types of α-thalassaemia deletions (--^{FIL} and --^{THAI}) or haemoglobin variants (Hb Quong Sze or Hb Suan Dok) common in Southeast Asia could not be excluded, as DNA sequencing was not performed in this study. Presence of □-thalassaemia and other types of α-thalassaemia in the pregnant women should be carried out in future.

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"Conducting and transparent Thin Films on Plastic Substrate for Organic Light-Emitting Devices"
Year 2002 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Indium tin oxide (ITO) films have properties useful for serving as the transparent conducting layer in flat-panel display, which includes organic light-emitting devices (OLEDs). In particular, with the advent in OLEDs and photovoltaics (PV) based on organics/polymers, it draws upon the necessity of a suitable method of ITO deposition to avoid damage on the delicate organic/polymer substrates. The low-temperature environment in the pulsed laser deposition (PLD) is an option for preparing the suitable ITO coating on a plastic substrate. PLD has an advantage over other deposition techniques such as sputtering, because of its ability to produce the composition of the source material in thin film form even at room temperature and its feasibility of minimizing film contamination, because the laser beam is focused only on the source material. Most of ITO coating on glass substrate were carried out using a KrF (λ = 248nm) laser while relatively a few works have been reported for using the Nd:YAG laser at second and third harmonics (λ = 532 and 355nm, respectively). However, none reporting the use of plastic substrates using Nd:YAG laser.

The main objective of this work was to produce device-quality ITO coated samples by pulsed Nd:YAG laser for OLED application. The scopes of work were divided into deposition and characterization of ITO and OLED application. It first requires parametric studies of ITO properties with the laser wavelength, target-to-substrate distance, substrate temperature, and background gases. Secondly, fabrication of molecularly-doped single-layer OLEDs with structure ITO/(PVK+Alq_3+TPD)/Al, using the ITO films deposited by pulsed Nd:YAG laser.

ITO films were deposited on plastics (e.g. PC and PET) and glass substrates at room temperature. The shorter laser wavelength of 355nm was shown to produce samples with better electrical conductivity and optical transmittance and relatively less particulates. Due to the heating limitation ($\leq 150^{o}$C), we were not able to produce ITO-on-plastic with good enough quality for OLED application. ITO-on-glass deposited at > 150°C showed the sufficient low resistivity ($< 5\times10^{-4}\Omega$cm) and higher optical transmittance (> 90%) for OLED operation with high electroluminescent (EL) brightness. The thermally induced crystallisation with a preferred <111> directional orientation texture of the ITO film was evidenced. But the microstructure of ITO layer became much complex as it consisted of two to three sub-layers. Sodium (Na) ions were readily observed to out-diffuse from the glass and gettered by the ITO film. The ITO surface roughness varied with the deposition distance. The ITO films with resistivity < 4×10^{-4} Ωcm were obtained in argon, oxygen, nitrogen, and helium at optimum pressures. The optical transmittance of ITO was relatively very poor for the case of helium and nitrogen. The minimum resistivity for ITO thin film was inversely proportional to the molecular weight of these gases. The optimum pressure was scaled with the molecular weight of the gas, as a result of gas-phase moderation of their plume species prior to deposition on the glass substrate. While the ITO films deposited in argon

and nitrogen presented nanostructures, polycrystalline structures were obtained for the films deposited in oxygen and helium.

The ITO-on-glass sample deposited with 355-nm laser pulses was successfully tested for OLED fabrication with ITO/PVK+TPD+Alq_3/Al structure. Five different parameters affecting the ITO on OLED current-voltage (*I-V*) characteristic and EL brightness performance were tested. These parameters included the ITO resistivity, ITO surface roughness, ITO deposited in argon gas, surface etching of ITO for Na contamination, and insertion of ultra thin diamond-like carbon (DLC) layer on top of ITO.

The ITO resistivity must be $< 5\times10^{-4}$ Ωcm in order to have a good electrical contact for sufficient hole injection from ITO anode into the polymer layer for light emission. The optical transmittance of ITO must also be better that 90%. The ITO quality can be produced on the glass substrate at temperature > 150 ^{o}C in oxygen and argon gases. The OLED performance for ITO deposited in argon was slightly better in term of threshold voltage and comparable brightness with respect to the OLED fabricated on the ITO that was deposited in oxygen. The nanostructure of the ITO created in argon gas is believed to improve the hole injection and it is also believed that the argon gas avoids substrate oxidation, which may cause the oxidation of polymer layer. The insertion of ultrathin DLC layer on top of ITO improved the OLED performance as a result of more uniform distribution of injection current and reduction in hot spots. Despite a higher operating voltage and lower injection current, OLED based on DLC-on-ITO substrate was more efficient than that fabricated using the commercially available ITO. Generally, the as-deposited ITO with Nd:YAG laser was relatively inadequate for OLED application. The surface-treated ITO by dilute aquaregia solution or insertion of ultrathin DLC layer on top of ITO surface improved the OLED performance.

In the present work, nanostructures were observed for ITO that was deposited in argon and nitrogen at 250 ^{o}C and 8 cm deposition distance. A more detailed study to investigate the formation mechanism of these nanostructures, such as the study of the influence of the background gas pressure, target-to-substrate distance and substrate temperature may be performed. Furthermore, the influence of these nanostructures on the OLED can also be performed. Another interesting issue this work was the Na ions were readily been observed to out-diffuse from the glass and gettered by the ITO film. The presence of Na ions in ITO may contaminate the OLED. However, the complete issue of Na effect on OLED performance is still not fully understood because at the moment we are not able to isolate the Na and the surface-roughness effects, which can be one of the interesting issues for future study. Besides the single-layer molecularly–doped OLED, a double-layer or multilayer OLED with suitable hole transport, electron transport, and organic light emission materials can be fabricated.

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"Synthesis and characterization of Advance Nanostructure Pyroelectric Smart material"
Year 2002 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

PLZT ferroelectric ceramic is referred to as a smart material due to its capability of being both sensor and actuator. It is widely used in electronic and optoelectronic applications as a transducer and actuator. Nonetheless PLZT is also well-known for its dielectric behavior and thus it is used in a capacitor. PLZT is produced in many forms to suit different applications. PLZT in powder form is most commonly used in a capacitor. In the present age, miniaturization of electronic product is unavoidable for cost saving and to suit modern consumers' needs. In this case, the conventional PLZT powder has no longer fulfilled the present needs. Therefore, PLZT powder in nano-sized was introduced to address this issue, with the hope to stretch its limits beyond current capabilities. PLZT ceramic is typically produced by a solid-state reaction. This method, although gives a good yield, leads to compositional and structural homogeneities. In the present study, we used a novel modified-coprecipitation route which has yet to be reported, to synthesize nano-sized PLZT powder.

Objectives

1. To synthesize nanocrystalline PLZT powder using a modified coprecipitation method.
2. To characterized the physical and dielectric properties of the PLZT powder so-produced.

Methodology

PLZT precursor was synthesized using lead nitrate, lanthanum chloride, zirconium propoxide and titanium isopropoxide. Triethanol amine (TEA) was used to stabilize the mixed solution, enabling a better mixing between PL and ZT components. After filtration, the precursor powder was dried in an oven. The precursor powder was then calcined at various temperatures, determined by a TG-DTA analysis, to obtain PLZT powder in a desired phase. The samples were then examined with a number of characterization techniques, i.e. XRD, SEM, TEM, nitrogen adsorption, FTIR, for its properties in powder form. The samples of calcined powder were then pressed into pellets, and coated with Ag-Pd paste for the dielectric test before their microstructures were examined with SEM again.

Results

In this study, we have developed a modified coprecipitation route, which has never been reported in any literature. Through this method, we have successfully synthesized nano-sized PLZT powder at a lower calcination temperature to obtain a desire PLZT phase compared to other methods. Characterizations of the product by various techniques have also been carried out. XRD results showed the powder produced was in the desired phase. All XRD, TEM and

nitrogen adsorption results proved that the PLZT powder produced is in nano size. The optimized sintering temperature was obtained with a dilatometer and found to be at 1250 ^{o}C. After sintering, the PLZT pellets exhibited a relatively high dielectric constant with a rather porous structure. In the final examination of the microstructure of the sample, we discovered an interesting phenomenon where several minor phases were form at the surface of PLZT pellet. One of those phases was a temperature-stable X7R phase formed by the reaction of Ag-Pd coating and PLZT which improves the dielectric performance of the product.

Unresolved problems and future direction

Due to lack of some facilities, dielectric constant of the sample was only measured at room temperature, instead of at the Curie temperature, in which the highest dielectric constant is shown. Thus, measuring dielectric constant at the Curie temperature will be one of the main topics in the next study. The porous structure seems to give some effects on the capacitance of the materials, especially with the recent discovery of porous materials carbon aerogel which has led to the invention of ultracapacitor. These discoveries indicate the importance of the study on porous capacitor materials.

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Mr. Eugene Ong Boon Beng

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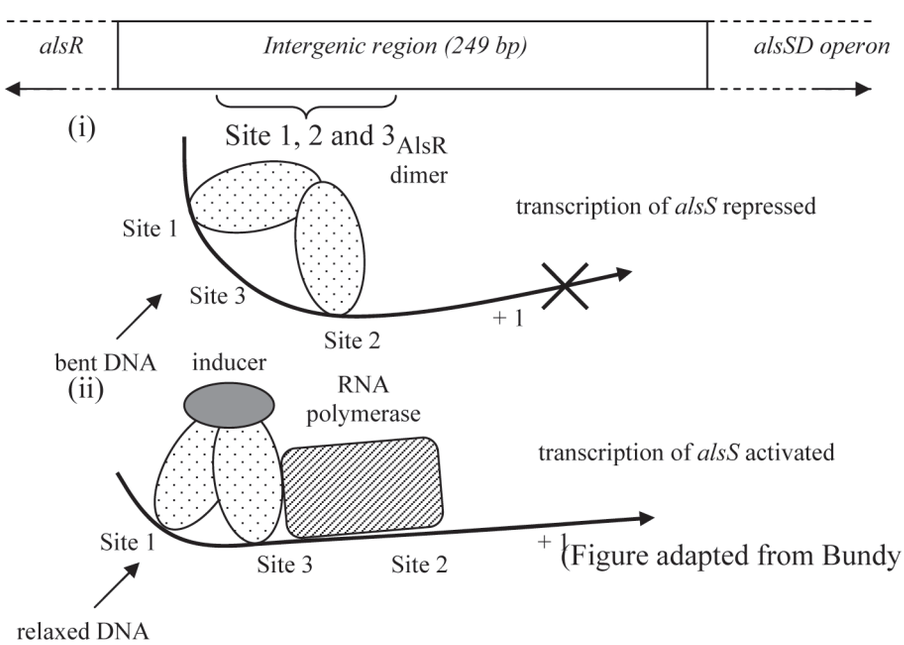
"Cloning, expression and characterization of the Bacillus subtiles alsR Regulatory Protein"

Year 2002 MTSF Science & Technology Research Grant Recipient

Objectives

To establish a recombinant overexpression system of AlsR and to localize its binding site on the *alsR* and *alsSD* intergenic region. The work culminated in an MSc thesis.

The transcriptional activator protein AlsR is similar in amino acid sequence to the LysR family of bacterial activator proteins usually sized between 300 and 350 amino acids. They are known to activate or repress the transcription of target genes or operons with diverse cellular functions such as amino acid biosynthesis, nitrogen fixation, antibiotic resistance, nodule formation of nitrogen fixing bacteria, and bacterial virulence. Here, we produced and purified recombinant AlsR by employing a plasmid-based overexpression system in *E. coli*. A crosslinking experiment with purified hexahistidine-tagged AlsR showed that it possibly acts as dimer in solution. In electrophoretic mobility shift assays, AlsR was capable of binding to the 249 bp *alsR-alsSD* intergenic region and specifically recognized its own promoter region. Binding analysis with fragments of synthesized DNA containing the divergent promoter sequence of the *alsR* and *alsSD* operon revealed that the direct binding site of AlsR was located between positions -50 and -100 relative to the putative *alsS* transcriptional start site. Computational analysis of the intergenic region revealed three significant LysR-type binding motifs (T-N11-A) about positions -15, -50, and -80 that agree to the common model of LysR-type transcriptional regulators. The occurrence of direct repeats 'TTTCCA' at the 5' end of two of the LysR binding motifs is believed to be highly significant for recognition of the binding site and encourages further investigation.



(Figure adapted from Bundy et al., 2002)

Based on the experimental results we believe that AlsR follows the *Acinetobacter sp.* BenM model of binding to DNA based on the locations of LysR binding motifs relative to the *alsS* promoter. AlsR is predicted to be able to regulate its own transcription as well as that of *alsS*. When bound to Site 1 and 2 on the intergenic region, it will repress transcription of *alsS* and also cause a bent to the DNA (i). Whereas the presence of an inducer will subject AlsR to conformational changes that also causes the shift in binding from Site 2 to Site 3 thus freeing Site 2 for binding by RNA polymerase to initiate the transcription of *alsS* (ii).

Mr. Sim Lim Chong
Dr. Azizan Aziz

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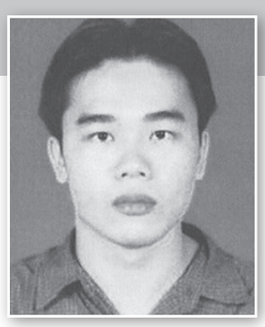

"Fabrication of new generation Thermal Interface materials, to be used in the Electronic Industries"
Year 2002 MTSF Science & Technology Research Grant Recipient

The recent advancement in packaging technology has resulted in the miniaturization of transistors, allowing more to be crammed and integrated in to a single device. This has subsequently resulted in an increase in localized heat flux, increasing the risk of devices reliability. Various methods including the use of high thermal conductive heat sinks, heat pipes coupled with thermally conductive thermal interface materials have been developed to enhance heat dissipation. However, it is still a challenge for the industries to ensure that TIM remains compliant, throughout the committed reliability requirements. Among the potential causes for the reduction in compliance, is the degradation of the materials, from the chemical and dimensional perspective.

To address the concerns highlighted above, this study has been scoped, to drive for a materials solution that is able to meet the thermal and dimensional stability required by thermal interface materials. In this study, polydimethylsiloxanes, commonly known as silicone rubber was evaluated due to its good low temperature flexibility, high thermal stability and chemical inertness. To enhance the thermal conductivity, Zinc Oxide (ZnO) and Alumina (Al_2O_3) fillers were used as reinforcements. Besides that, the ASTM D-5470 standard was developed in house to support the thermal conductivity measurements.

The sample preparation of the thermal conductive pads was carried out using the compounding methodology in accordance with the ASTM standard. First, silicone rubber was blended with various ratios of fillers, followed by the peroxide catalyst before it is compressed to individual sheets, with a mean thickness of 20um. To ensure that the samples produced meets the specifications set, a series of thermal and dimensional characterizations were carried out.

To begin with, weight loss profiles for both the thermal pads were measured with the thermal gravimetric analysis (TGA), to comprehend the influence of fillers on the thermal stability of pads. The results showed that the Al_2O_3 filled systems is less thermally stable than the ZnO systems for a dynamic ramp from 30°C to 200°C. Cross sections and failure analysis of the pads indicated that the surface area of the fillers coupled with the specific heat capacity of the fillers played a significant role in reducing the overall thermal stability of the silicone matrix, consistent with the intrinsic values of the ZnO properties. Besides weight loss measurements, the other focus area is on the thermo-mechanical response of the thermal pads under cyclic conditions. To comprehend the values and associated risk, thermal mechanical analyzer was used to measure the CTE and T_g for both the Al_2O_3 and ZnO thermal pads respectively. Results for a maximum filler loading of 40phr, indicated that the CTE values for the ZnO system is a lot lower than the Al_2O_3 based system, consistent with the rules of mixture theory. Besides that, the TMA results showed a higher cross-linking density with the ZnO systems, as observed from the higher T_g values, compared to the Al_2O_3 systems. Judging for the lower CTE and higher Tg values, its clear than the ZnO systems would impart less stress at the interface during cyclic stress test, due to the smaller magnitude of CTE mismatch with the silicon.

From the mechanics perspective, dimensional stability of the thermal pads were explored by gauging the tensile and elongation to break response for both the ZnO and Al_2O_3 thermal pads. The correlation for dimensional stability is drawn based on the assumption that a stable material would undergo more plastic deformation, hence a higher tensile and elongation at break values. From the results obtained, it was clear that the ZnO systems have a higher tensile strength and elongation at break values compared to the Al_2O_3 at a similar filler loading. The reasons are clearly correlated to our micrograph images on the fracture surface of the thermal pads, which shows a far more uniform dispersion of ZnO fillers compared to the Al_2O_3 fillers in the systems. Second level details, such as the surface energy of the ZnO fillers are the likelihood explaining why a better dispersion is achieved with the ZnO fillers as opposed to Al_2O_3 fillers for a similar compounding process conditions.

In summary, it's clear that the ZnO filled systems were more superior than the Al_2O_3 filled systems based on the discussions tabled above. Most importantly, clear correlations and visual simulations were established between the thermal-mechanical properties with the effect of increasing interfacial thermal resistance.

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"Chromosomal abnormalities in confined Placontal Mosaicism and Uniparental disomy associated with pregnancy loss"
Year 2003 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Confined placental mosaicism (CPM) is defined as the presence of chromosomal abnormalities shown in the placental tissues but is absent from the foetus. To investigate this phenomenon, previously reported methods (based on cytogenetic and endocrine research) were investigated, modified and developed to establish *in-v*itro primary placental cell culture. Eighty-eight placentas were collected and processed to disperse placental cells from villous tissue. Primary placental cell cultures were setup using either digested villous tissues or purified dispersed placental cells after Percoll density gradient centrifugation. 24 proliferating primary cultures were initiated, representing a success rate of 27.3%. Of these 24 proliferating cultures, only 14 cultures (58.3%) yielded analyzable metaphases for cytogenetic analyses. Cultures initiated from Percoll density gradient purification had strong tendency to differentiate into syncytial cells and culture proliferation stopped.

Immunocytochemistry staining against cytokerains-7 showed presence of trophoblastic cells in the culture. Cytogenetic analyses yielded female karyotypes (46,XX) with evidence of tetraploidy in the cultures initiated. Maternal cell contamination occurred in all cultures and became the dominant population in the flask. Among the cytogenetic abnormalities observed were 2 cultures with polymorphic heterochromatin at the pericentric region of chromosome 9 (9qh+), 1 mosaic culture with normal chromosomal constitution and a translocation between the long arms of chromosome 1 and 11, 46,XX[4]/46,XX,t(1;11)(q31;q22)[3], and 1 culture with chaotic mosaicism; a translocation between the long arm of chromosome 2 and chromosome 4 and a deletion on the long arm of chromosome 12, 46,XX[10]/46,XX,t(2;4)[1]/46,XX12q-[2]. The experiment suggests that maternal endometrial cells with aberrant genetic constituent may be a contributing cause in pregnancy loss.

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"Susceptibility and Molecular characterization study of the Plant Nematode Disease towards cultivated and wild Banana plants"
Year 2003 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Nematode infestation on banana plants has been identified as the key contributing factor to significant crop losses worldwide. With average annual yield losses estimated about 20% throughout the world (Sasser and Feckman, 1987), nematode infestation on bananas is inevitable, despite the establishment of numerous-combating regime. Hence, this study was carried out with its ultimate objective to formulate an alternative biological technique that could act as a replacement to the conventional nematode managements in order to minimise their infestations on banana plants. This is made possible by manipulating the naturally-developed nematode resistance/tolerance mechanisms established in plants that were reported present in *Musa* genepool (Pinochet, 1996). Conventionally, nematode parasite species infesting banana plants were identified on the basis of a combination of morphological characters by using the light microscope. However, due to its size as well as, extensive indistinguishable morphological variation established among and within species, accuracy of identification is doubted. Therefore, a molecular-based identification method is employed in order to establish a more systematic nematode characterisation process.

Thus, this study was divided into two inter-related studies i.e. banana susceptibility and molecular characterisation of plant-parasitic nematode study.

Objectives

The overall objective of this study is to develop an alternative nematode management method by manipulating the naturally established nematode resistance/ tolerance mechanisms in *Musa* genepool.

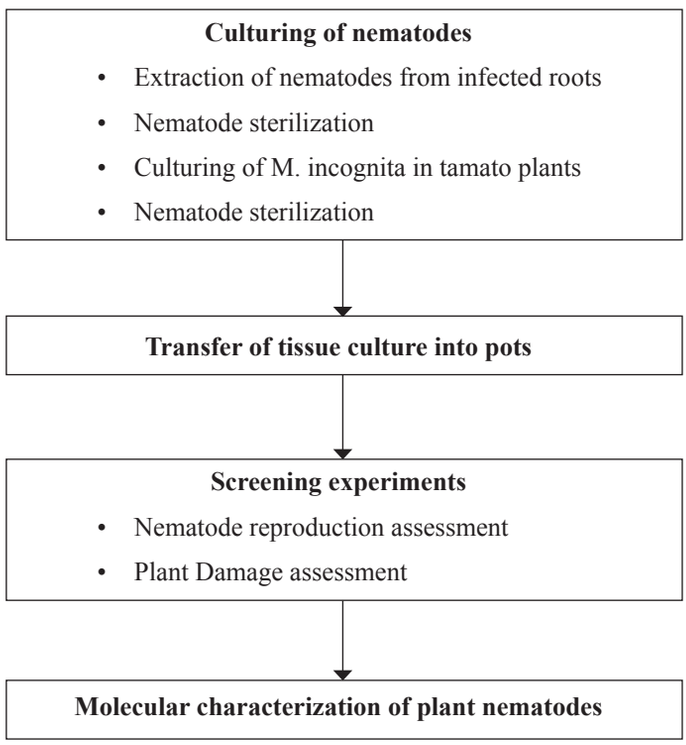
Specific objectives;

1) To expand the current resistance/susceptibility study with the inclusion of other banana varieties by using the same treatment in order to obtain a conclusive inference of the role of *M .incognita* and the host status of A genome banana plants.

2) To investigate and acquire in depth understanding of the host-pathogen interaction during nematode infestation.

3) To develop species-specific DNA-based identification system, which is crucial for plant-parasitic nematode identification assay

Methodology



Results

Susceptibility/Resistance Screening Study

Meloidogyne incognita was chosen as the treatment factor for the screening experiment conducted as it was found to be predominant in the roots and soil of banana plants in Peninsular Malaysia. These root-knot nematodes were inoculated into the soil of three banana varieties namely Pisang Mas, Malaccansis and Jari Buaya. The minimum of four and the maximum of 16 replicates of each variety were subjected to the treatment. After 60 days of inoculation, host plants were harvested and the Gall Index (GI) together with the degree of host suitability (R) was determined by following the protocol described by Sasser *et al.,* 1984. From the experiment conducted, it was observed that all three cultivars inoculated with *M. incognita* demonstrated susceptibility towards the challenge with all host plants showing the average GI >2 and R >1.

Despite the proven analysis of the previous studies on the host status of banana nematodes, our findings revealed that the interaction between *Meloidogyne incognita* and three banana varieties namely Mas, Malaccansis and Jari Buaya to be contradictory to previous studies. It was previously perceived that Pisang Jari Buaya was resistant towards nematode infestation while Mas should be susceptible when being challenged with any nematode species. However, results showed that out of twelve replicates of Mas that were subjected to *M.incognita* inoculation; three of them demonstrated resistance towards the infestation. This presumably results from somaclonal variation developed amongst the plantlets that were originated from tissue cultured products. Jari Buaya on the other hand was found to be susceptible towards nematode infestation while the hypersusceptibility demonstrated by Malaccansis is a novel finding.

Molecular Characterisation of Plant-parasitic Nematodes

The ITS (Internal Transcribed Spacer) regions of ribosomal DNA serve as versatile genetic markers and display a concerted evolution pattern whereby copies of these genes from a single individual tend to be similar to one another. As oppose to that, copies of this spacer regions were found to be distinct amongst different nematode species studied. Therefore, universal amplification coupled with the ability to amplify ITS regions from individual nematodes suggests that any species population or ecological community of nematodes can be analysed using a molecular approach based on the rDNA ITS region.

In this study, the fragment of rDNA comprising two ITS regions and the 5.8S gene were amplified by using two pairs of universal primers as described by Fallas *et al.* (1996) and Kaplan *et al.* (2000) to assess the diversity established between nematode isolates that range from different genera. For this purpose, 12 nematode species were studied namely *Helicotylenchus spp, Hoplolaimus spp, Macrophostonia spp, Meloidogyne incognita, Paratylenchus spp, Pratylenchus coffeae, Radopholus similis, Rotylenchulus reniformis, Tylenchida spp., Tylenchus spp.* and *Tylenchorynchus spp.*. These nematode individuals were subjected to molecular manipulations which include Polymerase Chain Reaction (PCR), cloning and sequencing.

Amplification of ITS1 and ITS 2 regions from the DNA of plant-parasitic nematodes yielded expected PCR product sizes. Initial results suggested that the size of the amplified products can determine the nematode species from which the DNA was obtained. It is observed that ITS regions of plant-parasitic nematodes are generally distinct from one genus/species to another. However, intra-specific heterogeneity and intra-individual microheterogeneity found in both ITS 1 and ITS 2 sequences merit further investigations

Conclusion

- All three cultivars inoculated with *M. incognita* demonstrated susceptibility towards the challenge with all host plants showing the average GI of >2 and R >1 with the growth and physiology of the plants seemed to remain in healthy condition for the period of 60 days post-inoculation.
- The size of the amplified ITS1-5.8S-ITS2 PCR products can determine the nematode species from which the DNA was obtained.
- ITS region of plant-parasitic nematodes are generally distinct from one genus/species to another.

Unresolved problems and Future directions

This study has highlighted banana cultivars that are resistant and susceptible. However, it is now pertinent to further investigate what are the factors that contribute towards the make-up that makes a banana variety susceptible and resistant. Future directions will be to look into the biochemical exudes from resistant plant and genetic markers for future application in banana crop improvement.

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Universiti Sains Malaysia

"Investigation of Chitosan as an Immobilization Maatrix for Enzyme"
Year 2003 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

This research is carried out to determine the possibility of chitosan as a matrix for glucose oxidase immobilization.

The materials used were high and low molecular weight chitosan, glucose oxidase, glutaraldehyde, glacial acetic acid, lactic acid, maleic acid, mutarotated glucose, hydrogen peroxide and 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS).

Phase one of the experimentation included the physical and mechanical characterization of chitosan membranes prepared using high and low molecular weight chitosan with different organic acids. The second phase was consisted of immobilization of the enzyme onto chitosan membranes performed under the optimum condition from phase one. The selected membranes were immobilized with glucose oxidase (GOD) using absorption and crosslink methods. The retention activity of both immobilization techniques was compared using ABTS-spectrophotometric method. The immobilized membranes were subjected to FTIR and SEM evaluations. In constructing GOD-chitosan electrode, the GOD-chitosan membrane was fastened onto surface of a platinum electrode with an O-ring. Amperometric detection of glucose was performed using a potentiostat connected to an integrator-plotter and a digital multimeter. The conventional three electrodes were comprised of a silver/silver chloride (reference electrode), a platinum wire (counter electrode) and a platinum working electrode with the GOD-chitosan layer. The parameters carried out to investigate the optimum conditions for the test electrodes were applied potential, membrane thickness, glutaraldehyde concentration, enzyme concentration, temperature, pH and buffer concentration. The test electrodes were characterized for their response time, Michaelis-Menten constant, repeatability, reproducibility, and the effect of possible interferences from electroactive substances. Finally, the accuracy and recovery of the test electrodes were determined using rat serum sample and ABTS method was used as a reference method.

Both high and low molecular weight chitosan membranes prepared from acetic acid exhibited significantly higher mechanical strength and elongation at break as compared to those prepared in lactic acid and maleic acid. FTIR spectra and SEM micrographs showed the existence of intermolecular interactions between chitosan and GOD. Higher catalytic activities were observed on GOD-FCHIT (enzyme immobilized in high molecular weight chitosan membrane) than GOD-SCHIT (enzyme immobilized in low molecular weight chitosan membrane) and for those crosslinked with glutaraldehyde as compared to the adsorption technique. Enzyme loading higher than 0.6 mg could decrease its activity. The highest response for glucose was observed at 0.21 ml/cm^2 membrane thickness for GOD-FCHIT/PT biosensor and 0.35 ml/cm^2 membrane thickness for GOD-SCHIT/PT biosensor. The optimum experimental conditions for analyzing glucose at pH 6.0 using the biosensors were found to be at 35 °C with an applied potential of

0.6 V. Under such conditions, response times of 85 s and 65 s were observed for GOD-FCHIT/PT and GOD-SCHIT/PT respectively. The apparent Michaelis-Menten constant (K_M^{app}) was found to be 12.7370 mM for GOD-FCHIT/PT and 17.6920 mM for GOD-SCHIT/PT. This indicated that the GOD-FCHIT/PT had greater affinity for the enzyme. Moreover, GOD-FCHIT/PT showed higher sensitivity (52.3666 nA/mM glucose) when compared with GOD-SCHIT/PT (9.8579 nA/mM glucose) at S/N>3. A better repeatability and reproducibility were achieved by GOD-FCHIT/PT than GOD-SCHIT/PT in the glucose measurement. GOD-FCHIT/PT was found to give the highest enzymatic activity among the electrodes under investigation. The performance of the biosensors in the determination of glucose in rat serum was evaluated. Comparatively better accuracy and recovery results were obtained for GOD-FCHIT/PT. Hence, GOD-FCHIT/PT showed a better performance when compared with GOD-SCHIT/PT. In conclusion, chitosan membrane has the potential to be a suitable matrix in the development of glucose biosensor.

Due to time and financial constraints, *in vivo* test using human volunteers to evaluate the biosensor performance was not carried out. It is hoped that this project can be further developed to finally produce biosensor strips with commercial value which is affordable and easy to use.

Miss Ida Shazrina Ismail
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"Molecular characterization of Bovine Peroxisome Proliferator activated Receptor-Gamma2 (PPARγ2) Promoter"
Year 2003 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Peroxisome proliferator activated receptor gamma 2 (PPARγ2) is a ligand-activated transcription factor which belongs to the PPAR subfamily of nuclear receptor superfamily. It regulates transcription by forming heterodimer complex with retinoic X receptor (RXR) and binding to peroxisome proliferator response element (PPRE) in the regulatory domain of target gene. PPARγ2 has been extensively studied for over a decade and has been implicated in adipocyte and mammary gland development, and various pathological disorders including obesity, insulin resistance, inflammation and atherosclerosis. The control of PPARγ2 gene expression mainly occurs at the transcriptional level, hence, it is vital to characterise the promoter region of bovine PPARγ2 gene because of its economical importance. In this study, bovine PPARγ2 promoter with the size of approximately 1.1 kb was successfully cloned. Two transcriptional start sites located at 173 bp and at 143 bp upstream the start codon of the gene were also identified. Sequence analysis revealed potential binding sites for transcription factors Sp1, C/EBP, Smad4, Oct-1, GATA, CREB, IRF and STAT. In addition, three putative TATA boxes were found distally located from the transcriptional start site of the gene and the nearest TATA box relative to the transcriptional start site, i.e. at position -363, was shown to be functional by using electrophoretic mobility shift assay (EMSA). Transient transfection analysis demonstrated the presence of transcriptional activities in various promoter fragments, proving that the cloned promoter is a functional promoter.

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"Production and characterization of novel Polyhydroxyalkanoate (PHAs) from locally isolated micro-organisms"
Year 2003 MTSF Science & Technology Research Grant Recipient

Malaysia being the leading producer of palm oil is also the major processor and exporter of palm oil products in recent years. The oil palm processing industries in Malaysia offer regular palm oil processed at different points, and some of the by-products are also eliminated from different stages of processing. These products and by-products are being used for other purposes in order to create zero waste products.

One of the creative projects for zero emission from palm oil industry is converting the palm oil products and by-products into polyhydroxyalkanoate (PHA), a biodegradable and biocompatible thermoplastic. PHA is synthesized by microorganisms during nutrient limited growth conditions. For example, when nitrogen source is limiting and in the presence of excess carbon source. In order for PHA to be commercially competitive, factors such as low cost of production must be fulfilled. Hence, our ultimate aim is to achieve high production of PHA using palm oil products that are less costly and readily available in Malaysia.

In our work to isolate a PHA-producing bacterium, a novel method of bacterial isolation was developed based on mixed microbial culture approach. A consortium of microorganisms from oil polluted wastewater samples was directly cultivated to promote the PHA production prior to isolating the microorganisms. The isolation of a potential bacterial strain was performed by subjecting the cultivated mixed microbial culture to sucrose density gradient ultracentrifugation. This resulted in the fractionation of the bacterial cells according to the type of PHA that they contained. By using this new approach, an isolate identified as *Burkholderia* sp. USM JCM 15050 was obtained. This bacterium was capable of converting palm oil products and glycerol into poly(3-hydroxybutyrate), P(3HB) efficiently. Up to 70 wt% and 68 wt% of P(3HB) could be obtained when 0.5 % (v/v) crude palm kernel oil (CPKO) or palm kernel oil (PKO) was fed, respectively.

In order to further understand the role of lipase-hydrolyzed compounds in either promoting or inhibiting both cell growth and PHA biosynthesis, the main composition of fatty acids and glycerol in palm oil products were tested separately as the sole carbon source. Feeding of different types of fatty acids ranging from caproic acid ($C_{6:0}$) to oleic acid ($C_{18:1}$) that contained even-numbered carbon chain revealed that lauric acid ($C_{12:0}$) and myristic acid ($C_{14:0}$) encouraged both cell growth and P(3HB) production, with the P(3HB) content amounting to 69 wt% and 38 wt% of the dry cell weight, respectively. The ability of this bacterium to utilize glycerol was also determined by feeding different purities of glycerol derivatives respectively as the sole carbon source. Results showed that the highest P(3HB) content achieved was 60 wt% with approximately 2.5 g/L of the dry cell weight.

In future, more efforts should be expended to transform heterologous PHA synthase gene into the *Burkholderia* sp. in order to produce a wide variety of PHA homopolymers and copolymers. The introduction of heterologous PHA synthase gene will also help to further elucidate the metabolic pathways involved in the production of PHA from palm oil products. Further studies are also required to increase the PHA productivity of the isolate in order to make palm oil derivatives economically viable for the production of biobased and biodegradable PHA.

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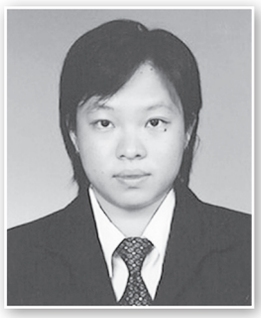

"Molecular characterization of Polyhydroxyalkanoate Biosynthesis Genes of Cyanobacteria : Towards Photosynthesis Production of Bioplastics"
Year 2004 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Polyhdroxyalkanoate (PHA) is a bio-based thermoplastic synthesized by various microorganisms including some of the blue green algae (cyanobacteria) in the presence of excess carbon source. PHA can be decomposed and assimilated by many microbial species (biogegradable) and do not cause toxic effects in the host (biocompatible).

Spirulina platensis used in this study is capable of synthesizing polyhydroxyalkanoate (PHA) under nitrogen-starved condition with the maximum accumulation of up to 10 wt.% of the cell dry weight (CDW) under mixotrophic culture condition. However, ongoing study found the nitrogen-starved cells accumulated large amounts of PHA granules under fluorescent and phase contrast microscopic analysis. This result was in contrast with the gas chromatography determination. It is proposed that the *S. platensis* accumulated starch-like granule under PHA favourable condition. In order to investigate the starch-like granules in *S. platensis*, we performed Nile blue A staining on the various starch granular and surprisingly it gave red fluorescing. These results prompted us to identify and characterize polyglucose biosynthetic genes of *S. platensis* which is reported for the first time.

The recombinant *Escherichia coli* clones harboring partial digested genomic DNA of *S. platensis* exhibited the presence of a novel type of insoluble, polyglucose-like granule. Whilst in GC-MS analysis, freeze-dried cells of recombinant *E. coli* gave positive results with the identified glucose compound which was not observed in the sample from wild-type *E. coli*. The freeze-dried cells of Nile blue A stained recombinant *E. coli* was red fluorescing under fluorescent miroscopic analysis. The fluorescing intensity of the recombinant *E. coli* was closely similar with starch granular. In molecular genetic level, the partial digested genomic DNA of *S. platensis* was subcloned and gycosyl transferases group 1 was identified. These findings suggest that the polyglucose metabolism is available and functionally active in heterologus *E. coli* host. Of particular importance is that the identification and characterization of the polyglucose biosynthetic genes will make it possible to genetically engineer metabolic pathways to improve polyhydroxyalkanoate accumulation in *S. platensis* in future.

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Dr. Sim Yoke Leng
Dr. Azhar Ariffin

Universiti Malaya

"Kinetics and Mechanism of Cleavage of N-Substituted Phthalimide and Phthalamic acid in mixed Aqueous-Organic Solvent"
Year 2004 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Objectives

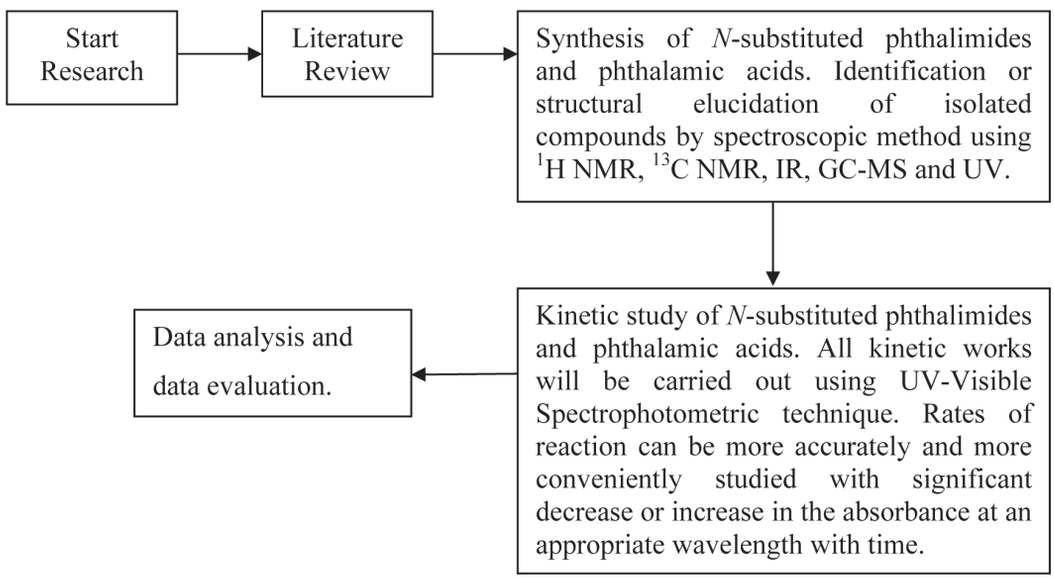
a) To understand the importance of inter- and intramolecular general acid-base (GA-GB) catalysis in aqueous and mixed aqueous-organic solvent which is similar to many biological systems.

b) To study the cleavages of *N*- substituted phthalamic acids, which are partial model to many interesting and mechanistically well-defined class of enzyme-catalyzed reactions.

Introduction

Inter- and intramolecular catalysis occurs in many enzyme-catalyzed reactions[1-3] as well as the hydrolysis of phosphate esters and bases in RNA / DNA cleavage.[4-7] Because of the characteristic importance of intramolecular catalysis, the mechanistic aspects of these catalyses in organic reactions have been studied extensively.[8-10] Recently, there has been considerable interest in the study of systems involving the intramolecular participation of carboxyl groups, in ester and amide hydrolysis, as models for hydrolytic enzymes.[11-13] On the other side, the understanding of the mechanism of enzyme-mediated reactions becomes important in drug design synthesis and in some biological reactions related to biotechnology.[14-16]

Methodology Flow Chart

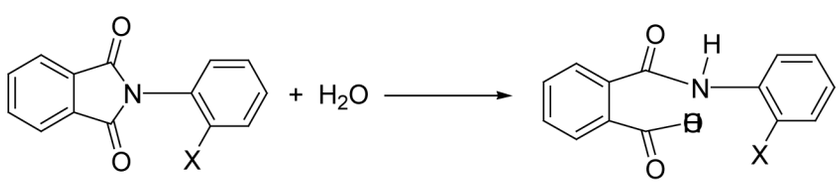


Results

N-(2-Hydroxyphenyl)phthalimide (**N-2OHPhPT**), *N*-(2-methoxyphenyl)phthalimide (**N-2MPhPT**) with their corresponding phthalamic acids and *N,N'*-disubstituted phthalamides were synthesized through standard and simplified procedures, respectively.

Pseudo-first-order rate constants (k_{obs}) for the conversion of **N-2OHPhPT** to **N-2OHPhPTH** decrease nonlinearly from 114 × 10^{-3} s^{-1} to 62.3 × 10^{-3} s^{-1} with the increase in CH_3CN content from 2 – 90 % v/v CH_3CN at 1.0 × 10^{-3} M NaOH. On the other hand, k_{obs} for alkaline hydrolysis of **N-2MPhPT** was observed to decrease from 64.7 × 10^{-3} s^{-1} to 4.72 × 10^{-3} s^{-1} with the increase in CH_3CN content from 2 – 80 % v/v CH_3CN at 2.0 × 10^{-3} M NaOH. Such significant differences in the rate of alkaline hydrolysis of *N*-substituted phthalimides may be attributed to the occurrence of different rate law in these reactions, i.e. rate = k_w [**1**$^-$][H_2O] and rate = k_{OH} [**2**][HO^-] in the hydrolysis of **1** and **2**, where **1** and **2** represent **N-2OHPhPT** and **N-2MPhPT**, respectively.

2-OH and 2-OMe substituents show different behavior in the aqueous cleavage of **N-2OHPhPT** and **N-2MPhPT** in alkaline medium. The hydrolysis of **N-2OHPhPT** involves intramolecular general base (IGB) assistance where *o*-O^- group of ionized **N-2OHPhPT** acts as IGB and H_2O as the reactant which gives a rate enhancement of > 8 × 10^4 – fold compared to that of **N-2MPhPT**. Second-order rate constant (k_{OH}) for hydroxide ion – assisted hydrolysis of ionized **N-2OHPhPT** and **N-2MPhPT** are 3.0 and 29.2 M^{-1} s^{-1}, respectively. The solvent deuterium kinetic isotope effect (dkie) on the rate of alkaline hydrolysis of **N-2OHPhPT** and **N-2MPhPT** reveals that the respective values of k_{OH} / k_{OD} are 0.84 and 0.78 for alkaline hydrolysis of these imides. The values of $k_w^{H_2O} / k_d^{D_2O}$ is 2.04 for the reactions of ionized **N-2OHPhPT** with H_2O and D_2O, respectively.



X = OH (**1**)
X = OMe (**2**)

X = OH (**3**)
X = OMe (**4**)

IGB – assisted rate enhancement due to *o* – O^- in **1** is > 8 × 10^4 – fold

The effects of concentrations of Me_4NCl, Pr_4NCl, Me_4NBr, Pr_4NBr and Bu_4NBr on the rate of alkaline hydrolysis of **N-2MPhPT** reveal the formation of ion-pair complexes between solvent-separated loose ion-pair forms ($M^{n+} \cdots X^{k-}$) and **N-2MPhPT**. The bulky hydrophobic tails of tetraalkylammonium salts and their halide anions inhibit the reaction by blocking the OH^- nucleophilic attack on the electrophilic site of **N-2MPhPT**. Me_4NCl showed slight inhibition because of the small shielding effect created by the methyl group. However, Bu_4NBr presents the greatest inhibition on hydrolysis rate because of its strong binding affinity with **N-2MPhPT**.

For the acidic cleavage of *N*-substituted phthalamic acids, the values of k_{obs} for the cleavage of **N-2OHPhPTH**, obtained at 4.9×10^{-2} M HCl, 35°C and within CH_3CN content range 2 – 80 % v/v in mixed aqueous solvent are smaller by nearly 1.5 – to 2 – fold than k_{obs} for the cleavage of **N-2MPhPTH**, obtained under almost similar experimental conditions. These observations show the absence of expected intramolecular general acid catalysis due to 2-OH group in **N-2OHPhPTH**. The k_{obs} values for the cleavage of **N-2OHPhPTH** and **N-2MPhPTH** decrease by more than 20 – fold with the increase in the CH_3CN content from 2 to 80 – 82 % v/v in mixed aqueous – CH_3CN solvent.

Finally, we have studied a series of amines (acetate, phosphate, *N*-methyl morpholine, Tris-(hydroxymethyl)-aminomethane (TRIS), morpholine, 1,4-diazabicyclo[2.2.2]octane (DABCO), 2-methoxyethylamine, ethanolamine, piperazine, 3-amino-1-propanol, methylamine, dimethylamine, piperidine) with their pK_a values ranging from 4.53 to 11.05 on the cleavage of **N-2OHPhPT** and **N-2MPhPT**. Aminolysis is another interesting part of research and the way to work out a mechanism will be a great challenge to us. Different primary, secondary and tertiary amines are needed to conduct a Bronsted plot throughout the end of our research. This particular study entails a lot of hard work which we wish to communicate in the near future to prove the occurrence of inter- or intramolecular catalysis in our studies.

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Miss Chew Guat Siew

Associate Professor Tengku Sifzizul Tengku Muhammad

Universiti Sains Malaysia

"Differential regulation of PPARs Family in Murine J774.2 Macrophage Cell Line by Cytokines : Molecular mechanisms of Atherosclerosis"

Year 2004 MTSF Science & Technology Research Grant Recipient

(Funded by Toray Science Foundation, Japan)

Introduction

The research was undertaken to study how PPAR family which consists of PPARα, γ, and β/δ as potent negative regulators in the progression of atherosclerosis are able to coordinate with each other in the development of drugs for coronary heart disease. Because cytokines are known to modulate the progression of atherosclerosis by regulating the function of the various cells present in lesions, their action on macrophage PPAR isoforms are important in the pathogenesis of atherosclerosis research. However, to date, no systematic studies have been carried out to functionally investigate the effects of cytokines on PPAR gene expression. It is, thus, absolutely essential to understand comprehensively the molecular mechanisms responsible for the effects of cytokines in the modulation of PPAR gene transcription.

Objectives

The primary objective of this research was to advance our understanding on the mechanisms involved in the regulation of expression of this important trancription factor by determining the regulation of PPAR gene expression and protein contents as well as the PPAR-DNA binding activities which may lead to the identification of novel pathway(s) for therapeutic intervention.

Methodology

(i) Materials

The murine J774.2 macrophage cell line was purchased from the European Collection of Cell Cultures (ECACC). The recombinant murine TNF-α, IFN-γ, IL-1β and IL-1α were purchased from National Institute for Biological Standards and Control, UK. All the cell culture reagents were purchased from GIBCO/BRL. Antibodies against different PPAR isoforms were from Santa Cruz Biotechnology, Inc.

(ii) Methods:

(a) *Cell culture and isolation of total cellular RNA*

J774.2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (HI-FBS), 100U/ml penicillin and 100μg/ml streptomycin, and incubated at 37˚C in a humidified atmosphere containing 5% (v/v) CO_2 in air. The cells were washed twice with PBS and incubated for 4h in medium containing reduced 0.5%(v/v) HI-FBS prior to incubation with various concentrations (0-1000 U/ml) of individual cytokines for 24h. Total cellular RNA was prepared from the cells using Tri-Reagent LS (Molecular Research Center) according to the manufacturer's instructions.

(b) *Semi-quantitative RT-PCR*

The mRNA expression levels of widely established PPAR isoforms were carried out using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) as described (Tengku Muhammad et al., 2000; M.L. Tan et al., 2005)*.

*Tengku-Muhammad, T-S., Hughes, T.R., Ranki, H., Cryer, A., Ramji, D.P. (2000). Differential regulation of macrophages CCAAT- enhancer binding protein isoforms by lipopolysaccharide and cytokines. *Cytokine.* **12**, 1430-1436.

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(c) *SDS-PAGE and Western blot analysis*

Total cellular proteins from the stimulated and unstimulated cells were extracted and determined its concentration. The method to be adopted to examine the regulation pattern of PPARs protein levels is called Western Blot. Equal ammount of protein from each sample will be run on SDS-PAGE, transferred onto PDVF membrane, blotted with antibody against PPARα, γ, and β/δ, and finally developed using ECL method.

(d) *Identification of the PPAR family members involved in DNA-protein interactions.*

To determine PPAR isoforms activities, we used a BD TransFactor Colorimetric kit (BD Biosciences Clontech, Tokyo, Japan). Using an ELISA (enzyme-linked immunosorbent assay) -based format, the TransFactor kit detected DNA binding by specific PPARαβγ family members of transcription factors coated in a 96-well plate. The absorbance of the plate was measured at 655 nm with a microtiter plate reader (Anthos ht II, type. 12500, Anthos Labtec Instruments).

Results

The regulation of PPAR family in macrophage by cytokines plays a critical role in the pathogenesis of atherosclerosis. The action of four cytokines TNF-α, IFN-γ, IL-1β and IL-1α on the expression of PPAR mRNA, protein and functional DNA binding activity in the murine J774.2 cell line was therefore studied. Exposure of cells to TNF-α and IFN-γ produced a reduction of PPARα and PPARγ mRNA levels and a corresponding increase in the expression of PPARβ. By contrast, IL-1β induced the PPARα and PPARγ mRNA but showed a corresponding decrease in the expression of PPARβ. IL-1α, however, had no significant effect on all PPAR isoforms. Electrophoretic mobility shift assay (EMSA) showed a close correlation between the PPAR mRNA and protein expressions in the PPAR-DNA binding activity of the cytokine-treated macrophages. Additionally, incubation of nuclear extracts in 96 wells coated with PPRE consensus sequence from the Colorimetric TransFactor ELISA successfully detected a similar PPAR-DNA binding activity pattern as EMSA. Since the changes observed in PPAR protein content and DNA binding activity in cytokine-treated macrophages followed closely the corresponding changes in PPAR mRNA expression, the results strongly suggest that the PPAR expression and binding activity were mainly regulated at the levels of mRNA metabolism. These studies,

therefore indicate that TNF-α, IFN-γ, IL-1β and IL-1α are important regulators of macrophage PPAR mRNA and protein expression which affect PPAR-DNA binding activities. Thus, this study provides novel insights into the potential mechanisms that may be responsible for the molecular regulation of macrophage PPAR gene expression by cytokines in murine J774.2 cell line, and suggests a potential target for therapeutic intervention against arteriosclerosis.

Unresolved problems and future direction

This study has successfully demonstrated that macrophage PPAR was differentially regulated by cytokines. The changes observed at PPAR mRNA levels were paralleled with PPAR protein expression and subsequently at the levels of DNA binding activity clearly indicate that mRNA metabolism was primarily responsible for the observed changes. Therefore, a possible cell/tissue-specific mechanisms and pathways may exist from this study on modulation of PPAR gene expression by cytokines.

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Miss Mok Pooi Ling
Professor Dr. Cheong Soon Keng

Hospital Universiti Kebangsaan Malaysia

"In vitro expression of Erythropoietin by Human Mesenchymal Stem Cells"
Year 2004 MTSF Science & Technology Research Grant Recipient

The objective of the proposed research is to study the capability of human Mesenchymal Stem Cells (MSC) as a vehicle for gene delivery. We choose to experiment the erythropoietin (Epo) gene because it is small. The size of the functional encoding gene is approximately 590 bp.

Mesenchymal stromal cells (MSC) are pluripotent progenitor cells that can be found in human bone marrow. MSC are suitable for gene delivery because (i) they are easily isolated and expanded in culture, (ii) they are able to maintain in undifferentiated state unless exposed to certain differentiation stimulators, and thus can be kept in large volumes for a long period. (iii) genetically altered MSC could also be easily recovered after installation *in vivo*, and (iv) the ability of newly introduced genes within MSC and their progeny to be expressed in a less restrictive fashion than other cells, thereby expanding the potential application in treating medical disease. Previous researches have also shown that they have low immunogenicity and even suppress allogeneic T cell responses. This is an important feature in therapeutic gene delivery because we would not want the transfected cells that efficiently express therapeutic genes to be eliminated by the immune systems in an allogeneic patient, and thus, brings down the success of *in vivo* gene therapy. If the human MSC could maintain and successfully express therapeutic genes, MSC can then be expanded *in vitro* and thus be supplied (implanted) to individuals who have gene abnormality or protein deficiencies (e.g. adenosine deaminase deficiency) urgently without having to perform MHC matchings.

This research also aims to study the transfection efficiencies of Minimally, Immunogenically Defined Gene Expression (MIDGE). MIDGE is a new vector, linear, double-stranded DNA molecules consisting solely of the expression cassette, capped with hairpin structures at the end. The application of MIDGE in gene delivery has not been widely studied, and thus we would like to explore the potential of MIDGE in gene delivery, replacing viral or plasmid cloning and expression systems. The MIDGE vector offers the following advantages over retroviral or plasmid vector gene transfer:

1) MIDGE contains only the necessary elements (expression cassette) required to express the desired gene, with no resistance markers (antibiotic encoded gene) or other unwanted genes.
2) Immunostimulatory sequences, such as CpG can be minimized. CpG exists in plasmid and they stimulate the release of cytokines typical for the response of organisms to bacterial infection. This represses transcription from transferred genes and activates the innate immunation system.
3) MIDGE vector has better chance of delivery into the nucleus of expressing cells because it is smaller in size.
4) Risk of oncogenes activation is avoided.

In the current study, mesenchymal stromal cells were tested for their capability to carry and deliver therapeutic gene such as erythropoietin gene *in vitro*. MSC were isolated from the bone marrow of patients with non-malignant blood disorder. The isolated MSC were characterized based on their morphology, cytochemistry, immunochemical properties by flow cytometry, and differentiation into adipocytes, chondrocytes and osteoblasts. The expanded MSC was then transfected with erythropoietin (EPO)-encoded plasmid pMCV1.2 and EPO-encoded MIDGE (Minimalistic Immunologically Defined Gene Expression) vector by electroporation. Following transfection, pMCV1.2-transfected MSC expressed up to 4779.4 mU/mL EPO per 1.0 µg of vector per 1 x 10^5 cells on Day 1 and the yield dropped sharply to 28.53 mU/mL EPO per 1.0 µg of vector per 1 x 10^5 cells on Day 22. However the cells still maintained the low expression for 3 months in culture. Meanwhile the MIDGE vector-transfected MSC expressed highest amount of EPO on Day 6, up to 2683.76 mU/mL EPO per 1.0 µg of vector per 1 x 10^5 cells, and the yield dropped to 641.56 mU/mL EPO per 1.0 µg of vector per 1 x 10^5 cells after 3 months post-transfection. The results showed that MIDGE vector is more effective and stable than the plasmid (pMCV1.2) in delivering erythropoietin gene into MSC. Further studies showed that the supernatants containing EPO obtained from the transfected cell culture were able to induce the differentiation of hematopoietic stem cells into erythroid colonies. In conclusion, MSC hold promise to be a cell factory for the production of biological molecules and MIDGE vector is superior to plasmid in transfection experiments involving erythropoietin gene *in-vitro.*

Miss Christabel Loni Jiram
Professor Dr. Vikineswary Sabaratnam
Professor Dr. Thong Kwai Lin

Universiti Malaya

"Biological and Chemical Diversity of Actinomycetes from Coral Reefs Marine Organisms of the East Coast of Peninsular Malaysia"
Year 2004 MTSF Science & Technology Research Grant Recipient

Actinomycetes were isolated from marine sponges of Tioman Island, Malaysia, taxonomically characterized (culturally, morphologically, physiologically, chemotaxonomically) and identified up to species level using 16s rDNA sequencing. A total of 407 actinomycetes isolates were recovered from 39 marine sponges collected from nine sites in Tioman Island using a selective isolation protocol developed in this study. Isolates were assigned into 15 color groups. 106 culturally different representatives were then selected under the binocular light microscope for further characterization. Isolates were further dereplicated to 64 physiologically different isolates. 21 (32.8%) isolates with L,L- diaminopimelic acid (DAP) and no diagnostic sugars pattern in the whole-cell wall hydrolysate were tentatively streptomycetes (chemotype I), while 43 (67.2%) isolates with meso-DAP with observed three different sugars pattern were tentatively non-streptomycetes (chemotype II/D, IV/A and III/B). Scanning Electron Microscopy revealed 44 morphologically different isolates (1 streptomycetes and 43 non-streptomycetes). According to BLAST analysis on partial 16s rDNA sequences, 37 of the 44 representative isolates had different sequences. Slight modifications to the standard protocol led to the first discovery of four *Salinispora* strains from the Malaysian marine sponges along with other rare actinomycetes such as the *Saccharomonospora, Pseudonocardia*, *Microbispora*, *Mycobacterium*, *Blastococcus* and an uncultured bacterium clone. Out of 38 crude extracts tested against 13 bacterial strains and 3 fungal strains, 34 (64.2%) exhibited antibacterial and antifungal activities. Eight (18.9%) of the isolates from the *Streptomyces, Salinospora, Saccharomonospora*, *Micromonospora* and *Pseudonocardia* groups possessed broad spectrum bioactivity. These discoveries proved indeed that the Malaysian waters do harbor a gene pool of novel actinomycetes with its bioactive compounds yet to be explored.

Dr. Latifah Binti Saiful Yazan
Associate Professor Raha Binti Abdul Rahim

Universiti Putra Malaysia

"The mechanisms of Damnacanthal-induced Apoptotic cell death in the T-Lymphoblastic Leukaemia Cells (CEM-SS)
Year 2004 MTSF Science & Technology Research Grant Recipient

Introduction and problem justification

Damnacanthal, an anthraquinone has been reported to possess inhibitory effects on the cancer promoting *ras* gene (Hiramatsu *et al.,* 1993), *p*561 gene (Faltynek and Schroeder, 1995) and other anticancer properties (Toledo *et al.,* 1984; Aoki *et al.,* 1999). Recently, it was found to be cytotoxic to the T-lymphoblastic leukemia cells (CEM-SS) (unpublished). It induced apoptosis in the cells. Nevertheless, the mechanisms underlying the process remain unclear. The understanding on the mechanisms on how an agent induces apoptosis in cancer cells provide new avenues for cancer diagnostics, prognostic and treatment.

Objective

The objective of this study was to determine the mechanisms of damnacanthal-induced apoptotic cell death in the acute T-lymphoblastic leukaemia cells.

Methodology

Compound

Damnacanthal was kindly supplied by Professor Dr. Nordin Md Lajis from the Institute of Bioscience, Universiti Putra Malaysia. The compound was dissolved in dimethylsulfoxide (DMSO) to give a stock concentration of 10 mg/mL.

Cells

The T-lymphoblastic leukemia cells (CEM-SS) were obtained from the National Cancer Institute (NCI), USA.

Determination of cytotoxicity

Cytotoxicity of damnacanthal was determined according to Shier (1991).

Determination of mode of cell death

DNA from the untreated and damnacanthal-treated CEM-SS cells (30, 10, 3 and 1 μg/mL) was isolated using the Apoptotic DNA Ladder Detection Kit (Chemicon, USA). Agarose gel electrophoresis was carried out for the analysis of fragmentation of DNA.

Determination of cell cycle arrest

Determination of cell cycle arrest was performed according to Klucar and al-Rubeai (1997). The cell cycle kinetics was analyzed using flow cytometry machine (FACSCalibur flow cytometer, Becton Dickinson) with CellQuest software.

Determination of involvement of caspase 2, 3, 6, 8 and 9

Assay was carried out using the Apo*Target*™ Caspase Colorimetric Protease Assay from Invitrogen™.

Effects of damnacanthal on the expression of p53

Effects of damnacanthal on the expression of p53 was determined by using the Human p53 ELISA Bender Medsystem (Austria).

Effects of damnacanthal on the expression of Bcl-2

Effects of damnacanthal on the expression of Bcl-2 was determined by using SDS-PAGE followed by Western Blotting.

Results

Damnacanthal from *Morinda elliptica* was cytotoxic to the T-lymphoblastic leukemia cells (CEM-SS) as being detected by using the trypan blue dye exclusion technique. The compound induced apoptosis in the cells, forming the distinctive ladder-like DNA pattern on agarose gel. By flow cytometric analysis, damnacanthal arrested the cells at the G2/M phase. Caspase 2, 3, 8, 6 and 8 were involved in the process. Nevertheless p53 did not play a role. Surprisingly, the expression of Bcl-2 was not affected, suggesting that Bax might be involved in the programmed cell death.

Unresolved problems and future direction

Even though damnacanthal was confirmed to be cytotoxic and induced apoptosis in the acute T-lymphoblastic leukemia cells (CEM-SS) by a few mechanisms such as activation of caspases, regulation of p53 and expression of Bcl-2, there are many more molecules and pathways that are needed to be studied to increase the understanding underlying the process. Future research should be carried out *in vivo* (using animals) before a clinical trial being performed.

Publication

Latifah, S.Y., Hisham, A.H., Raha, A.R. and Nordin, M.L. The mechanisms of damnacanthal-induced apoptotic cell death in the T-lymphoblastic leukaemia cells (CEM-SS) (manuscript in preparation).

Miss Goh Fen Ning
Professor Ngeow Yun Fong
Dr. Stacey Yong Foong Yee

Universiti Malaya

"Development of a rapid detection and quantification assay for *Legionella pneumophila*"
Year 2004 MTSF Science & Technology Research Grant Recipient

Legionella spp. are a common cause of community-acquired respiratory tract infection and occasional cause of nosocomial pneumonia. Surveillance of *Legionella* in water system is essential to reduce the risks of an outbreak. Direct plating, heat treatment, and acid buffer treatment are the 3 methods commonly used to isolate legionellae from water. This study showed that direct plating method had the lowest recovery rate of legionallae. Heat treatment and acid buffer treatment both have 100% and 80% recovery rate respectively. However, acid buffer treatment produced a more consistent and reproducible result, as acid treated samples are free of interference from background bacteria. Fifty-five legionellae from 5 cooling towers around Malaysia were isolated and *L. pneumophila* is the commonest species (80%) isolated followed by *L. gresilensis* (7.3%), *L. anisa* (5.5%), *L. gormanii* (1.8%) and *L. busanensis* (1.8%).

Speciation of legionellae by latex agglutination, 16S rRNA and partial *rpoB* gene sequencing were performed. Latex agglutination was found to have a high level of false negative (17%) and it is not suitable to be used for the routine detection of *Legionella*. 16S rRNA is suitable to be used for differentiating *Legionella* into species level, but it lacks discriminative power to further assign them into the respective serogroup. Partial *RpoB* gene is more discriminatory than 16S rRNA but it is not conserved enough to be shared by some of the more distant *Legionella* species. Thus, it is important to compare the sequences of more than 1 gene in the speciation of *Legionella* as the different genotyping systems may serve to complement each other.

IcmX gene, a well-studied virulence factor *L. pneumophila* was detected in 4 species of *Legionella*, which have not been previously reported to harbour this gene. The species are *L.busanensis*, *L. anisa, L. gormanii* and *L. gresilensis*. The sequences of the *icmX* genes in this species shared 98 – 99% homology to the gene in *L. pneumophila* SG1. The findings from this study also suggested that *icmX* gene may be acquired through horizontal transfer. Further studies will be needed to investigate the differences in the ability to cause infection between species that lack *icmX* gene and the ones that habour it.

Miss Maria Lourdes T. Lardizabal
Professor Dr. Mohd. Sofian Azirun

Universiti Malaya

"Toxicology studies of Plant extractives from Azadirachta Excelsa (Jack.) Jacobs against House Flies (Musca domestica L.)"
Year 2005 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

The common housefly is well known for being a significant worldwide public health pest which act as mechanical vector of human and animal pathogens. Due to continuous exposure of insecticides, houseflies develop resistance against nearly all common insecticides. Thus, the application of botanical insecticide is an alternative solution that could solve this problem besides the use of synthetic IGR's. Extracts from *A.excelsa* was chosen in this study as earlier report by Schmutterer and Ermel (1995) indicated the discovery of a new active compound, namely marrangin (azadirachtin L), along with azadirachtin. These compounds are believed to have IGR effect, as well as cause anti-feeding and anti-oviposition on insects. The general objective of this study was to investigate the insecticidal properties of *A.excelsa* as a new source of botanical insecticide. The specific objectives were:

i) To identify active compounds in *A.excelsa* extractives.
ii) To study the bioefficacy of *A.excelsa* extractives.
iii) To investigate the mode of action of crude *A.excelsa* extractives against growth of *M.domestica*.
iv) To study the histopathology effect of sentang extractives against house fly larvae.

The study was conducted by obtaining crude extracts from the seed kernels and leaves of *A.excelsa*, using polar and non-polar solvents. GC-MS analysis was performed to identify compounds present in the extract. Bioassays were conducted to test the toxicity effect and mode of action of sentang extract on the adult and larvae of the houseflies. Histology studies was conducted at the end of the study to investigate the effect of sentang extract towards the internal structure of the treated housefly larvae. Results obtained showed that sentang extracts contain fatty acids group, mainly hexadecanoic acid. Azadirachtin and marrangin was not detected. Topical application of extracts caused decreasing mortality percentage as the concentration level descends. Mortality in adult male houseflies was higher compared to females. Mode of action showed that sentang extract acts as an insect growth regulator (IGR) which produced malformed pupa and emerging adults. Histology results demonstrated that the extracts affected some damages on the cuticle formation and tissues of the larvae treated. In conclusion, results obtained from this study demonstrate that sentang extract is a potential source of IGR insecticide which is an alternative control for houseflies. The irregular fruiting season of sentang trees and the sensitivity of active compounds present in the extract towards environmental factors are constraints in using sentang extract as potential source of insecticide. This requires further studies to investigate the degree of sensitivity of the active compounds towards environmental factors and to produce synthetic forms of the active compounds.

Three journal publications and one seminar will be produced from findings of this study. Currently, all findings of this study will produce a PhD thesis entitled the Toxicology effect of Sentang extracts on Houseflies, which is expected to be completed and submitted by November 2009.

Miss Nazura Binti Zainuddin
Associate Professor Siti Aisah Alias
Dr. Lee Choon Weng

Universiti Malaya

"Isolation and characterization of biologically active metabolites from Marine Fungi"
Year 2005 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

In recent years a number of studies dealing with activity of marine fungi have been reported with diverse and unique compounds with activities such as antimicrobial, anticancer, anti-inflammatory activity and antiviral agents. Marine fungi are expected to produce a high number of active secondary metabolites with unique structures. This is the first research conducted in Malaysia, therefore, it was undertaken in order to reveal the potentially active metabolites from Malaysian marine fungi. The first step in conducting the research by isolating and culturing of identified and unknown marine fungi from a wider habitats and substrates in marine environment and then to screen and investigate marine fungi isolates for their ability to produce antimicrobial and cytotoxicity activity. Finally, antimicrobial metabolites isolated from the fungus *Fasciatispora nypae* by using bioactivity-guided fractionation were investigated.

Isolated fungal cultures were evaluated for their antimicrobial activity using two methods: i) plug assay, ii) disc diffusion assay. The plug assay was used as a preliminary screening method for antimicrobial activity and revealed 82 strains out of 152 marine fungi possessed antimicrobial activity against yeasts and bacteria. From the number of active marine fungi, 7 species showed a broad spectrum antimicrobial activity against yeast and bacteria. Most of the strains of marine fungi exhibited antibacterial activity against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*. In this study antibacterial activity was greater than antifungal activity.

In the disc diffusion assay, crude ethyl acetate extracts of five marine fungi namely, *Caryosporella rhizophorae, Fasciatispora nypae, Melaspilea mangrovei, Leptosphaeria* sp. and Ascomycete sp.19 (NF) were selected for antimicrobial testing after the activity was confirm from the previous preliminary screening. Incubation time and culture conditions were considered for this part. Results showed that incubation time and culture conditions affected the production of secondary metabolites of marine fungi and varied between species. *Fasciatispora nypa* produced good activity after 35 days from static culture against *Candida albicans and Saccharomyces cerevisiae,* whereas *C. rhizophorae* produced active metabolites from shake culture with good activity achieved after 35 days incubation period against *Escherichia coli* and *B. subtilis*. *Leptosphaeria* sp. and *M. mangrovei* produced good active metabolites after 15 days cultivation against *B. subtilis, S. aureus* and 30 days against *B. subtilis*, respectively from static culture. Ascomycete sp.19 (NF) produced active metabolite at stationary phase with high activity achieved at 15 days incubation period against *B. subtilis*. The culture conditions such as the cultivation of the fungi in the shaken or stationary phase may not be enough to optimise their production of active secondary metabolites in this study. It was demonstrated that increasing the optimization of physical factors such as temperature, salinity, pH value and light and chemical factors such as employing different media components, precursors and inhibitors can be useful in yielding high bioactive

compounds (Miao *et al*., 2006). Therefore, in order to obtain high antimicrobial activity, these physical and chemical factors should be carried out in future. A few previous studies show that bioactive metabolites can be extracted from the mycelia and culture filtrate. However, only the culture filtrate was extracted with ethyl acetate in this study. Therefore, the extraction of marine fungi should be carried out both from the mycelia and the culture filtrate.

Cytotoxic activity was screened by an *in vitro* assay system of growth inhibition against breast cancer cell line (MCF-7) and study showed that only two marine fungi, *F. nypae* and *C. rhizophorae* exhibited cytotoxic effect against MCF-7 tested at 100 µg ml^{-1}, where the ED_{50} value of *C. rhizophorae* was 14.5 µg ml^{-1}, and, ED_{50} value of *F. nypae* was 24 µg ml^{-1}. In the present study, only one breast cancer cell line (MCF-7) was used to study the cytotoxic activity of marine fungi. Although only *Caryosporella rhizophorae* showed active cytotoxic activity against this cancer cell line, but this does not determine that the rest of the marine fungi are not toxic. Therefore, several cancer cell lines should be carried out for cytotoxic activities in future.

Bioassay-guided fractionation was performed on the crude extract of *Fasciatispora nypae* in column chromatography and yielded three active fractions; fraction 2, fraction 5 and fraction 6. Chemical analysis for structures elucidation of the extract from *F. nypae* using one and two dimensional nuclear magnetic resonance (NMR) techniques and Gas Chromatography/Mass Spectrometry (GC/MS) resulted in the isolation and structure elucidation of a new benzopyran derivative which displayed close structural similarity to the known acremine C. This pure compound was successfully isolated and determined as 2,2,7-trimethyl-2H-1-benzopyran-5-ol. Interestingly, although this compound has been reported previously in the literature (Choi and Yoon, (2005) but this is the first time that this compound reported was isolated from a natural product and reported to display antimicrobial activity against yeasts and bacteria, with weak activity against *S. aureus, C. albicans* and *S. cerevisiae* and moderately active against *B. subtilis* and against and active against *Schizosaccharomyces pombe* in disc diffusion assay. However, the MIC value of this compound showed that it exhibited weak activity against all bacteria and yeasts with MIC value of >12.5 mg ml^{-1} against *S. aureus, C. albicans, S. cerevisiae and B. subtilis* and 10.4 mg ml^{-1} against *S. pombe.* Previous studies reported that compound with a chromene ring have various activities and this was proven in this study. Bioassay-guided fractionantion of *F. nypae* has led to isolation of one active compound. However, there are another two fractions; fraction 5 and fraction 6 that also exhibited antimicrobial activity. Fraction 5 exhibited good activity against test microorganisms compared to fraction 6. Due to small amount of fraction 5, the fraction 5 could not be furthered purify. Therefore, future study will undertake to isolate active secondary metabolite from fraction 5, which have many minor compounds.

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Professor Dr. Mary Anne Tan Jin Ai
(replaced Miss Rita Ling Hui Lian)
Associate Professor Umah Rani Kuppusamy

Universiti Malaya

"Oxidative stress and antioxidant status in β-Thalassaemia major patients at the University of Malaya Medical Centre"
Year 2005 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

In Malaysia, β-thalassaemia is a public health problem and it is common in the Chinese and Malays with a carrier rate of about 4.5%. Beta-thalassaemia results in a reduced (β^+-thalassaemia) or absence (β^0-thalassaemia) in the synthesis of β-globin chains and thus, causes ineffective erythropoiesis and premature haemolysis of red blood cells. There are three clinical phenotypes produced - β-thalassaemia trait, β-thalassaemia intermedia and β-thalassaemia major. Beta-thalassaemia major patients develop severe haemolytic anaemia and require life-long blood transfusions. The resulting iron overload due to repeated transfusions requires intensive chelating therapies using desferrioxamine or deferiprone to remove the excess iron. In the absence of chelation therapy, the accumulating iron results in free radical formation that will induce progressive damage to organs. The excess iron catalyzes reactive oxygen species formation that attacks lipids, proteins and DNA that will interfere with normal cell function. Living organisms counteract reactive oxygen species by having complex antioxidant systems.

Limited studies have been carried out on the antioxidant status and degree of molecular peroxide damage in β-thalassaemia major patients in the UMMC. Results from this study will benefit the patients as the levels of their oxidative stress and antioxidant status will be known.

Objectives

1. To determine the oxidative stress levels in β-thalassaemia major patients who are:
    - on subcutaneous chelation therapy and those who are
    - not on any iron chelation therapy.

    The extent of protein oxidation, DNA strand breaks and lipid peroxidation will be measured to estimate oxidative damage.

2. To confirm the β-globin gene mutations responsible for β-thalassaemia major in the patients studied.

Methodology

Subjects

Ethics approval for this study was obtained from the Medical Ethics Committee of the University of Malaya Medical Centre (UMMC) in accordance with the Declaration of Helsinki. A total of 65 patients diagnosed with β-thalassaemia major based on clinical presentation with regular blood transfusions were recruited. A patient information sheet was

prepared and given to each patient/parents when the study was explained to them. Written consent was obtained from each patient in the presence of his/her parents if the child was below 18 years. The study comprised of 38 patients under regular iron-chelation therapy with desferrioxamine and 27 patients who were not on any chelation therapy. Blood from 24 healthy individuals were used for age-matched controls.

Biochemical studies

Lipid hydroperoxide concentrations were measured using ferrous ion oxidation xylenol orange (FOX-1) assay in conjunction with triphenylphosphine (TPP) and butylated hydroxytoluene (BHT). Oxidation of plasma proteins was quantified by determination of AOPP, which were measured by spectrophotometry on a microplate reader, and calibrated with chloramine T solutions. The concentration of AOPP was expressed in chloramines units (μmol/l). The ferric reducing antioxidant potential (FRAP) assay, which measures non-enzymic antioxidant, was performed on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to the ferrous form (Fe^{2+}-TPTZ). These assays were determined in plasma.

The enzymic assays to determine catalase, glutathione peroxidase and xanthine oxidase activity were estimated spectrophotometrically according to known established methods. Glutathione peroxidase activity was determined by coupled enzyme reaction in which glutathione (reduced form) reacts with hydrogen peroxide to produce oxidized glutathione. Catalase activity was estimated based on the decomposition of hydrogen peroxide at 240 nm. Enzymic antioxidants activity measured in red blood cell (RBC) and peripheral blood mononuclear cell (PBMC) lysates respectively, were expressed as μmol/ g Hb and μmol/ g protein. Xanthine oxidase activity, determined in PBMCs, were based on the oxidation of the chromogen ABTS [2,2′-azino-di (3-ethylbenzthiazoline-6-sulphonate)] (absorbs at 410 nm) through coupled reactions catalysed by uricase and peroxidase.

Molecular study

DNA extraction and purification

DNA from blood samples were extracted in Tris-EDTA (pH 8) using sodium dodecyl sulphate and proteinase K, and digested overnight at 37°C. DNA was purified using phenol-chloroform-isoamyl-alcohol and precipitated in 4 M sodium acetate and ethanol. Extracted DNA samples were stored at -70°C for analysis using molecular techniques.

DNA analysis using the Amplification Refractory Mutation System (ARMS)

The mutations responsible for β-thalassaemia major in patient DNA will be characterised using the Amplification Refractory Mutation System (ARMS) and gap-PCR.

Results

Oxidative-antioxidant indices among the Chinese β-thalassaemia major patients attending the University Malaya Medical Centre, Kuala Lumpur who were either on desferrioxamine-chelation or without chelation therapy, were examined. Oxidative stress in these patients was evident as advanced oxidized protein product and lipid hydroperoxides were elevated whereas glutathione peroxidase activity and FRAP were reduced. The catalase activity in the PBMCs in these patients was elevated, possibly as a compensatory mechanism for the reduced glutathione peroxidase activity

in both RBCs and PBMCs as well as the reduced catalase activity in the RBC. The superoxide producing xanthine oxidase activity measured in the PBMC of thalassaemic patients was not significantly different from the controls. Only the lower FRAP level and a higher AOPP level in the non-chelated patients compared with the chelated patients were indicative of a lower oxidative stress level in the chelated patients. The ferritin level in the chelated patients were as high (comparable to the non-chelated patients) and the liver enzymes were not reduced or normalized.

It is evident from this study that desferrioxamine chelation therapy in the β-thalassaemia major patients was only partially effective in reducing oxidative stress. The iron over-load in these patients which is the result of regular blood transfusions requires a better management regime.

Future direction

An alternative or adjuvant therapeutic agent to minimize iron overload may be developed/identified.

Molecular characterization of the β-globin gene mutations responsible for β-thalassaemia major in the patients is currently been carried out.

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Miss Nor Hayati Binti Abdullah
Dr. Ling Sui Kiong
Miss Mazura Md Pisar

Forest Research Institute Malaysia

"Chemical analysis of Prismatomeris Malayana and its anti-inflammatory activity"
Year 2005 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

The preliminary bioactivity screening studies carried out in FRIM have shown that the crude extracts of *Prismatomeris malayana* exhibited strong anti-inflammatory properties (not reported). From the study, it was found that the leaf extract inhibited 12-o-tetradecanoylphorbol-13-acetate (TPA) induced ear oedema and platelet activating factor (PAF) receptor binding inhibitory activity at 96.03 % and 76.9%, respectively. From the previous study reported on this species, not much was on the anti-inflammatory activity and the compound involved. Therefore, the aim of this study is to further investigate the chemical constituents in relation to the biological properties of *Prismatomeris malayana*, which will provide more comprehensive an updated information essential for developing and upgrading the plant into a potential herbal product in the local healthcare market or it may act as a primary study in the aid of related drug development.

Objectives

1. To evaluate the biological activities of *Prismatomeris malayana* related to anti-inflammation.
2. To identify anti-inflammatory constituents of *Prismatomeris malayana*.
3. To prepare HPLC fingerprint of *Prismatomeris malayana* based on the selective bioactive marker(s).

Methodology

1) Plant sample preparation

- Plant samples (leaves) will be collected from identified sites and identified by FRIM botanist and deposited at the FRIM Herbarium.
- Plant samples will be dried in oven at 40^{o}C and ground.
- Plant samples will be extracted in methanol and recovered under vacuum.
- Plant extract will be subjected to solvent-solvent portioning (hexane, chloroform, ethyl acetate and butanol).

2) Fractionation and purification

- Combination method and technique of column chromatography employing MCI gel CHP 20P, Chromatorex ODS, Sephadex LH-20, silica gel, etc. or HPLC will be used to fractionate and purify the compounds of interest.

3) Bioactivity assessment

The extracts, fractions, or pure compounds will be evaluated for the anti-inflammatory activities using the assay systems described:

TPA induced moused ear oedema assay

12-O-tetradecanoylphorbol-13-acetate, TPA (1 µg) dissolved in acetone (20 µl) was applied to the ear of male ICR albino mice (25-30g) by means of a micropipette. The plant extracts were applied topically to the inner surface of the right ear at 2 mg/ear about 30 min before each TPA treatment (Fig. 2.0). The other ear which acted as a control was applied with sample vehicle. The resulting oedema was measured 8 hours after TPA treatment (fig.3.0). The results were expressed as percentage inhibition (IE%). IE% of test sample was calculated as the ratio of the weight increase of the ear sections. Each value used was the mean of individual determinations from 7 mice. Indomethacin was used as a positive control for this study.

Hyaluronidase inhibitory assay

The assay was performed according to the Sigma protocol with slight modifications (7). The assay medium consisting of 1.00-1.67 U hyaluronidase in 100 µl 20mM sodium phosphate buffer was preincubated with 5 µl of the test compound (in DMSO) for 10 min at 37^{o}C. Then the assay was commenced by adding 100 µl hyaluronic acid and incubated for a further 45 min at 37^{o}C. The undigested hyaluronic acid was precipitated with 1 ml acid albumin solution. After standing at room temperature for 10min, the absorbance of the reaction mixture was measured at 600nm. The absorbance in the absence of enzyme was used as the reference value for maximum inhibition. The inhibitory activity of test sample was calculated as the percentage ratio of the absorbance in the presence of test compound vs. absorbance in the absence of enzyme. The performance of the assay was verified using apigenin as a reference under exactly the same experimental conditions.

4) Structural elucidation

- The structure of pure compounds will be elucidated based on spectroscopic analysis using ultraviolet (UV), infrared (IR), mass spectroscopy (MS), 1D and 2D nuclear magnetic resonance (NMR) spectroscopy, chemical reactions and by comparison of their data with literature values or standard samples.

5) HPLC method for qualitative and quantitative analysis of marker compound(s) and standardization of crude extracts.

- Optimisation of extraction processes will be carried out to get the highest concentration of the active constituent(s).

Results

1. Isolation of ursolic acid from this species with anti-inflammatory activity. Further study can be carried out on this compound in order to develop it into new anti-inflammatory drug.
2. From the evaluation on this plant species, it can be concluded that it has a potential to be developed into herbal care product with anti-inflammatory property.
3. The optimized extraction method can be used as a tool in order to produced standardized extract rich in triterpenoid saponins content.

Unresolved problems and future direction

1. Development of chemical profile by using HPLC method. It can be used in the quality control of the extract or product by monitoring the quantity or concentration of ursolic acid.
2. Development of this species into herbal product with high and active anti-inflammatory properties.

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Dr. Hairul Azhar Abdul Rashid
(replaced Dr. Sulaiman Wadi Harun)
Dr. Mohd Ridzuan Mokhtar

Multimedia University
"Thulium-doped Fiber Amplifier : Fundamental Studies"
Year 2005 MTSF Science & Technology Research Grant Recipient

Introduction

The telecommunication industry has witnessed tremendous growth in the demand for capacity over the last few years. The demand for increased transmission capacity can be met by dense wavelength division multiplexed (DWDM) lightwave system that makes use of the entire low-loss window in silica fiber by increasing the total optical bandwidth. Expansion into S-band (1480 – 1520 nm) on the shorter wavelength side of the C-band is considered the most likely option. Optical fiber amplifiers play an important role in telecommunication networks because they compensate for the insertion loss of the optical fiber used for transmission lines and for the loss due to optical components used at optical nodes. Their amplification band determines the operating wavelength region of the network. The S-band amplification can be achieved by Thulium-doped fiber amplifier (TDFA).

Thulium-doped fiber amplifiers, which is the focus of this research project can provide high gain and low noise characteristics. They are usually fluoride-doped fiber having 2000 ppm Tm+ concentration and can reach small signal gain beyond 20 dB and noise figure < 7 dB.

Objectives

1) To design a Thulium Doped Fiber Amplifier (TDFA) that can give high gain and low noise figure in S-band region.
2) To characterize and analyze the TDFA using a numerical model

Methodology

1) The design of the new TDFA focuses on using the dual pumping schemes. Based on current literature review, the common pump wavelengths for TDFA are 800nm, 1050nm and 1400nm. In this work, a secondary pump at 1560nm is combined with the main pump at 1050 nm to increase the efficiency of the TDFA.
2) A numerical model for the dual pump TDFA is constructed using the Runge-Kutta and Relaxation method.
3) Characterization of TDFA – The performance parameters such as gain, noise figure and efficiency is characterized against design parameter such as input signal power, input signal wavelength, pump power and TDF length.
4) Analysis of result – The numerical modeling results is analyzed, focusing on the design and performance parameters' relationship. An optimized design of the TDFA is achieved for the dual-pump TDFA.

Results and Discussion

We demonstrate a numerical model of TDFA based on dual pumping scheme using 1050 nm and 1560 nm pumps in steady state conditions. Both pumps work in forward direction, co-propagating with the input signal. We compare and discuss our results with published experimental results.

As reported previously, 1050 nm pumping alone can be used to provide amplification but it needs high pump power because of low ground absorption. The value of 1560 nm pump power is fixed at 40 mW and the 1050 nm pump is changed from 50 mW to 200 mW. It is clear from Figure 1 that 40 mW is enough for 1560 nm pump power to increase the population inversion of 3H4, then small amount of 1050 nm pump power can up-convert to provide ASE level up to -40 dBm.

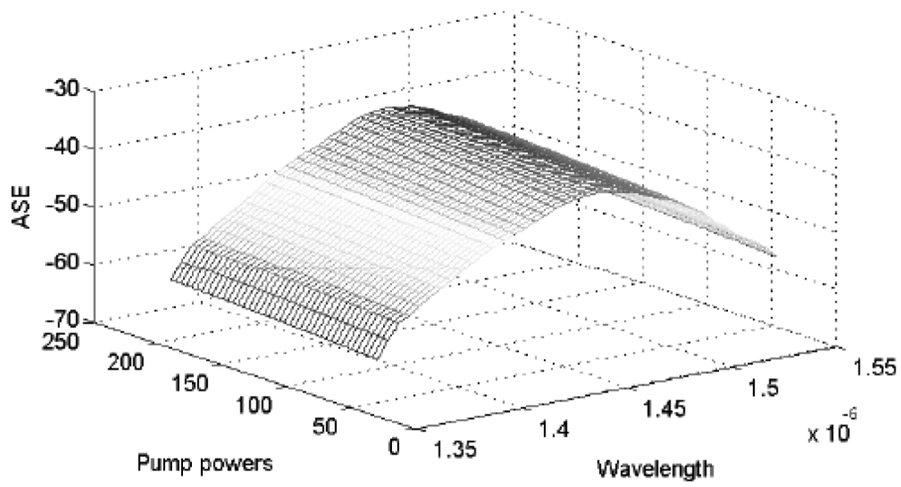

Figure 1: ASE spectra with 1560 nm pump fixed at 40mw and 1050 nm pump changing from 50 mW to 200 mW

In Figures 2 and 3, the gain and noise figure of small signal (-30 dBm) at S-band wavelengths in the above pump power condition. The noise figure increases as the gain decreases across the S-band wavelengths. We obtain a gain around 1460 nm input signal of about 30 dB and 5dB noise figure. The gain and noise figure profile agrees closely to experimental results from earlier literature.

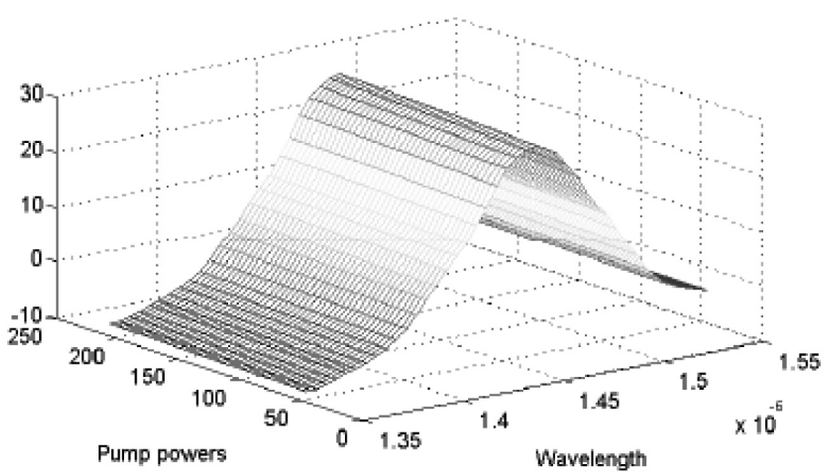

Figure 2: TDFA gain with 1560 nm pump fixed at 40mw and 1050 nm pump changing from 50mw to 200mw. Input signal power is -30dBm

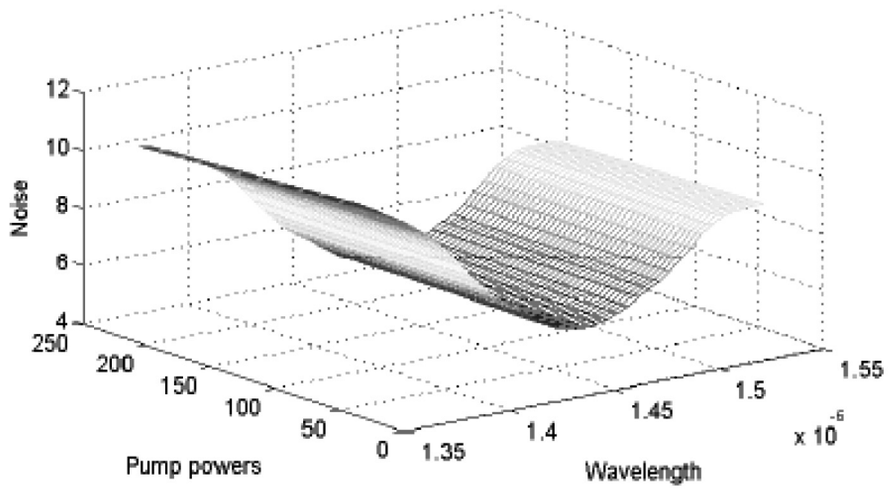

Figure 3: TDFA noise figure with 1560 nm pump fixed at 40mw and 1050 nm pump changing from 50mw to 200mw. Input signal power is -30dBm

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1. S. D. Emami, S. W. Harun, F. Abd-Rahman, H. A. Abdul-Rashid , S. A. Daud and H. Ahmad, "Effects of an auxiliary pump on the performance of TDFA", accepted for publication in Laser Physics (2008).
2. S. D. Emami, S. A. Daud, F. A. Rahman, H. A. Abdul-Rashid, S. W. Harun, "Numerical Modeling of Dual-Wavelength (1050 nm + 1560 nm) pumped Thulium Doped Fiber Amplifier", Proceedings of Multimedia University Symposium on Information and Communications 2007 (M2USIC 2007), Kuala Lumpur, Malaysia, 2007.

Dr. Teh Geok Bee
Dr. Saravanan Nagalingam

Universiti Tunku Abdul Rahman

"Investigation of Silicon Nanocrystals : Synthesis, Structural and Properties characterizations "
Year 2005 MTSF Science & Technology Research Grant Recipient

Silicon nanoparticles have attracted much attention due to their potential application in the optoelectronic devices. Since the discovery of room-temperature luminescence from anodized porous silicon, this phenomenon from nanosized silicon is now widely investigated. Various techniques can be employed in producing nanosized silicon crystals such as colloidal systems using organic liquids, gas evaporation techniques, pulsed laser ablation, self-assembly techniques, silane plasma formation techniques, plasma-enhanced chemical vapour deposition and photoelectrochemical techniques.

Colloidal systems using organic liquids to synthesise the nanosized silicon crystals is desired as a simple dilution will eliminate the interparticle interaction of the nanocrystals. Thus, ensure the narrow distribution of particle sizes. Colloidal systems also eliminate the possibilities of unintended oxidation by capping the nanocrystals with a layer of solvent molecules. Selection of inactive solvents such as hexane, acetone and alcohols is found to be able to control the interparticle interaction of active metals such as calcium, zinc and magnesium.

Luminescence of infra-red or red region was due to quantum size effect, while a surface effect was presented for blue or green luminescence as a possible mechanism. If the photoluminescence (PL) originates from quantum confinement, then the PL will vary according to the change in the average size of the nanoparticles. As the nanoparticles aggregate, the size increases thus shifting the PL to the lower energy region. It is favourable to use colloid for surface modification of Si nanoparticles. We envisage that the emission peak position and its intensity will be affected by the selection of capping solvent molecules.

Objectives

- To investigate the feasibility of using micro-emulsion techniques in preparing unique silicon nanocrystals in room temperature conditions
- To elucidate the factors influencing the growth of nanocrystals via micro-emulsion techniques
- To investigate the photoluminescence properties of silicon nanocrystals in relation to their particle sizes
- To elucidate the structural properties of silicon nanocrystals via X-ray diffractometry
- To investigate, if any, structural defects or interfaces of silicon nanocrystals using high resolution transmission electron microscopy (HRTEM)

Results

Photoluminescent silicon nanocrystals have been produced via the microemulsion systems. CTAB and TOAB are useful to create stable inverse micelles to control the particle sizes of the silicon crystals. The use of $LiAlH_4$ is found to be less favourable as compared to $NaBH_4$ due to the Al contamination.

Publications

1. G. B. Teh, N. Saravanan, B. Y. Foo, R. F. Louh, C. J. Chang & W. L. Yuan, *"Investigation of Photoluminescent Properties of Silicon Nanocrystals Produced Via Microemulsion Techniques",* Proceedings of the 41st IUPAC World Chemical Congress, Lingotto Conference Centre, Turin, Italy, 5th – 11th August, 2007.
2. B. Y. Foo, G. B. Teh, *"Investigation of Silicon Nanoparticles: The Effect of Capping Molecules on Silicon Nanopaticles"*, 2nd International Conference for Young Chemists, Universiti Sains Malaysia, Penang, Malaysia.18th -20th June, 2008.
3. G. B. Teh, B. Y. Foo, N. Saravanan, "*"Investigation of Silicon Nanocrystals : Synthesis, Structural and Properties Characterisations",* Proceedings of Malaysia Science and Technolgoy Congress (MSTC), Kuala Lumpur, Malaysia, 4th – 6th September, 2007.

Manuscripts under preparation/review:

1. G. B. Teh, B. Y. Foo, N. Saravanan, R. F. Louh, C. J. Chang, W. L. Yuan & R. D. Tilley, *"Efficacy of utilizing mixed CTAB/TOAB cationic surfactants to produce photoluminescent silicon nanocrystals",* Journal of Cluster Science, 2008, submitted and under review.
2. B. Y. Foo, G. B. Teh & R. D. Tilley, "Colloidal Synthesis of Silicon Nanocrystals Demonstrating Quantum Confinement Phenomenon", 2008, pending re-submission.

Dr. Sreeramanan Subramaniam
Professor Dr. Helen Nair

AIMST University

"Genetic Engineering of Phalaenopsis Orchid for Fungal Disease Resistance"
Year 2005 MTSF Science & Technology Research Grant Recipient

We are focusing on *Phalaenopsis violacea* species, native orchid plant in Sarawak, Malaysia. In Malaysia, *Phalaenopsis* plants and flowers still not as popular as other types of orchid due the fungal infections caused by humid climate and high night temperature. In our current research project, we examined the fundamental concept of producing transgenic *Phalaenopsis violacea* resistance to fungal diseases by using protocorm like-bodies (PLBs). Biochemical analyses were carried out first on the leaves and flower samples of *Phalaenopsis violacea* orchid plants. Anthocyanins and anthocyanidins, chlorophylls, phenolics, proteins and sugar contents were used to analyse and provide the general idea regarding selection of superior qualities for the tissue culture purposes. The UV-spectrophotometric technique was used for anthocyanins and anthocyanidins pigments quantification.

Through the biochemical and statistical analyses, certain selected *Phalaenopsis violacea* orchid that found to be the most superior plant will be continue for the next stage. In order to speed up the whole process of propagation, the leaves of adventitious shoot induced to form PLBs using different types and concentrations of cytokinin and auxin in the solid medium and manipulating the effect of light, and presence of organic extracts as to reduce amount of phenolic compounds in culture medium. The establishment of target tissues (PLBs) make possible to the studies for antibiotic and herbicide sensitivity as well as genetic transformation of PLBs by *Agrobacterium*-mediated transformation. It has been suggested that of all the four selective agents tested, hygromycin and basta are potential selection agents for *Phalaenopsis violacea* PLBs. Both showed an earliest sign of toxicity in fast inhibition response at low concentration level. Under natural conditions, chemotaxis of *Agrobacterium* to wounded plant cells is the first event required for bacterial infection and disease development, a process of primarily importance for an opportunistic pathogen. Therefore, the absence of directed movement of bacteria in the presence of wounded *Phalaenopsis violacea* explants could at least partially explain why this plant species not forms tumors in nature.

It was observed that *Agrobacterium* was attracted to wound exudates from different types of explants. Once *Agrobacterium* reaches the neighbourhood of wounded tissues, the next step required for the development of plant tumors is its attachment to plant cells. *Agrobacterium* was able to bind to wounded as well as to unwounded plant cell surfaces, questioning the long debated requirement of plant cell damage for transformation, at least during the initial phase of bacterial colonization. In addition, the binding process was quantified, which provided a further evidence for the specific ability of virulent *Agrobacterium* to colonize tissues from PLBs of *Phalaenopsis violacea.* At this stage, since *Agrobacterium* was found to migrate towards wounded *Phalaenopsis violacea* explants and to specifically bind to exposed cell surfaces, there was no evident reason why *Agrobacterium*-mediated gene transfer leading to transgene expression in *Phalaenopsis violacea.* The subsequent step of T-DNA transfer leading to transgene expression in *Phalaenopsis violacea* cells was studied by means of transient expression of reporter genes. Reporter

genes, *gusA* and *gfp* provide a clear indication of the expression, transient or stable of transferred genes in transgenic cells. Optimization of several parameters affecting transient GUS and GFP expressions were evaluated to determine the efficiency of *Agrobacterium*-mediated transformation system in *Phalaenopsis violacea.* Optimization of several factors affecting transient *gusA* and *gfp* genes expression such as duration of preculture period, co–cultivation period, different temperatures for gene transfer, L-cysteine concentrations, acetosyringone concentrations, density of bacterial cultures (OD_{600nm}), calcium concentration during co-cultivation period, types of wounding, dark and light conditions during co-cultivation, influence of PLBs sizes, post cultivation period, pH during co-cultivation period and silver nitrate concentrations for regeneration were evaluated to determine the efficiency of *Agrobacterium*-mediated transfer during the early stages of transformation in this orchid species.

Publications

1. Loke Foke Ling and Sreeramanan Subramaniam (2007). Biochemical Analyses of *Phalenopsis violacea* Orchids. *Asian Journal of Biochemistry*. 2(4): 237-246.
2. Sreeramanan, S., Vinod, B., Sashi, S., and Xavier, R. (2008). Optimization of the transient *gusA* gene transfer of *Phalaenopsis violacea* orchid via *Agrobacterium tumefaciens*: An assessment of factors influencing the efficiency of gene transfer mechanisms. *Advances in Natural and Applied Sciences*. 22(2): 77-88.
3. Sreeramanan, S, Vinod, B., Ranjeetha, P., and Xavier, R**.** (2009)**.** Chemotaxis movement and attachment of *Agrobacterium tumefaciens* to *Phalaenopsis violacea* orchid tissues: An assessment of early factors influencing the efficiency of gene transfer mechanism. *Journal of Biosciences.* 20 (1): 43-54.

Miss Sew Yun Shin
Dr. Tan Chon Seng
Dr. Vilasini Pillai

Malaysian Agricultural Research and Development Institute

"Molecular farming of Transgenic Papaya for Production of recombinant Hepatitis B Virus Surface Antigen (HBsAg)"
Year 2005 MTSF Science & Technology Research Grant Recipient

Introduction

Hepatitis B is the most common serious liver infection in the world. It is caused by infection with the hepatitis B virus (HBV). It is estimated that there are 350 million chronic carriers of HBV worldwide with 2.4 million in Malaysia and 20 million new infections occur annually. No current medical treatment is totally effective in the treatment of chronic HBV infection. The most effective means of controlling the spread of HBV is prophylactic or post-exposure immunization with hepatitis B vaccines.

The earliest vaccines which were derived from the plasma of chronic HBV had raised various difficulties. Genetic engineering and molecular biology tools have made possible to engineer and express recombinant hepatitis B surface antigen (rHBsAg) in bacteria, yeast and mammalian cells for vaccine production. However, those technologies have several drawbacks. Bacteria lack the ability for extensive post-translational modifications that are required by most eukaryotic proteins for complete antigenicity. While mammalian cells expression system has a certain amount of risk associated with contamination such as live, pathogenic animal viruses.

With the advancement of transgenic plant technology, gene encoding immunogenic protein can be transferred into the nuclear genome of a plant system such that the plant is capable of producing the desired immunogenic protein. Transgenic plants have been used to produce recombinant protein such as cytokines, hormones, monoclonal antibodies, industrial and therapeutic enzymes, and vaccines. Transgenic plants have the potential to express recombinant proteins in large amount, which are inexpensive and safe for disease diagnosis and therapy. Plants on the other hand do not serve as direct hosts for human or animal pathogens and thus much safer to handle.

Objectives

1. To isolate Hepatitis B surface antigen (HBsAg) gene fragment
2. To construct a HBsAg gene cassette for papaya plant transformation
3. To transform and produce transgenic papaya containing recombinant HBsAg for molecular farming

Methodology

1. Isolation of HBsAg gene fragment

A pair of specific PCR primers namely 5' HpBSF and 3'HpBSR with overhang restriction enzyme (RE) sites NcoI and SalI respectively were designed based on the sequence of 685 bp Hepatitis B virus surface antigen (HBsAg) gene that was available in the public domain database. The DNA fragment containing HBsAg gene was PCR amplified using the specific primers designed from a former protein expression construct donated by Dr. Tan Wen Siang from UPM.

2. Construction of plant expression vector

The PCR amplified DNA fragment containing HBsAg gene and RE overhangs was cloned into an intermediate TOPO cloning vector. The plasmid of TOPO-HBsAg clone was subjected to DNA sequencing and the sequence the HBsAg gene fragment was analyzed and verified. Subsequently, plasmid TOPO-HBsAg was cut by RE NcoI and SalI to release the HBsAg gene fragment before subcloned into a modified intermediate vector namely CMVCP.PJK. The DNA fragment containing HBsAg together with 35S promoter and NOS terminator was released by cutting the plasmid with KpnI and cloned into plant transformation vector pCAMBIA 2300.

3. Plant transformation and regeneration

The embryogenic calli were derived from immature zygotic embryos which were collected from Carica papaya Eksotika 1 fruits. The constructed transformation plasmid namely pCAM2300-HBsAg was introduced into *Agrobacterium tumefaciens* LBA4404 and transformed into embryogenic callus according to the standard papaya agrobacterium transformation method in Biotechnology Research Centre, MARDI. After transformation, the transformed and non-transformed embryogenic calli were transferred to plasmolysis medium for 1 day and co-cultivation medium for 3 days. Later the embryogenic calli were transferred onto embryo induction medium containing carbenicillin, cefotaxime and timentin for 14 days. After two weeks, the transformed embryogenic calli were transferred onto selection medium containing kanamycin, cefotaxime and carbenicillin for 4 months. Subculturing of the transformed embryogenic calli onto fresh selection media was carried out every 3 weeks. Non-transformed (served as negative control) papaya embryogenic calli were grown onto selection as well as onto control media. After 4 months, the transformed and non-transformed calli were transferred onto regeneration medium containing carbenicilin for plant regeneration.

Results

The Hepatitis B virus surface antigen (HBsAg) gene fragment with approximately 700bp had been isolated by PCR with specific primers namely 5' HpBSF and 3'HpBSR from a former construct containing HBsAg gene fragment (donated by Dr. Tan Wen Siang from UPM) (Figure 1). The gene fragment with overhang NcoI and SalI had been cloned into TOPO TA cloning vector and sent for DNA sequencing. The identity of HBsAg DNA fragment was verified by sequencing results.

Subsequently, plasmid TOPO-HBsAg was digested by RE NcoI and SalI to release the HBsAg gene fragment and subcloned into a modified intermediate vector namely CMVCP.PJK. In order to release the DNA fragment containing 35S promoter, HBsAg and NOS terminator, the plasmid was digested with KpnI before cloning into plant transformation vector pCAMBIA 2300. The gene cassette namely pCAM2300-HBsAg has been successfully constructed and the map of the gene cassette is shown in Figure 2. The plasmid of the construct pCAM2300-HBsAg was digested with few restriction enzymes such as NcoI, SalI, EcoRI, KpnI and PstI to validate the correct cloning sites of the gene cassette. Approximately 15 *Carica papaya* variety Eksotika 1 immature green fruits were collected from a papaya commercial field at Nilai. A total of 300 embryogenic calli were obtained aseptically from seeds of the papaya fruits collected. These immature zygotic embryos were cultured onto callus induction media namely 1.2a10C for about 4 weeks in order to derive papaya somatic embryogenic cultures.

The gene cassette constructed namely pCAM2300-HBsAg was introduced into *Agrobacterium tumefaciens* LBA4404 and transformed into approximately 250 papaya embryogenic calli according to the standard papaya agrobacterium transformation method. Around 50 embryogenic calli served as negative control without infection of agrobacterium. After transformation, we noticed that the growth of non-transformed embryogenic calli was slowly retarded on the selection media containing kanamycin and died after several rounds of media subculturing. For the transformed embryogenic calli, we noticed that around 80% of the transformed calli were able to grow on the selection media. However, after few rounds of subculturing on the selection media, it was noticed that the growth of transformed calli slowed down as compared to the non-transformed calli on control media.

After 4 months, all the transformed and non-transformed calli were transferred onto the regeneration medium containing NAA, BAP and carbenicillin. The non-transformed calli were regenerated into papaya plantlets. However the growth of the transformed embryos seemed to be retarded and failed to regenerate (Figure 3). Hence, our first attempt of transformation was not successful. This could be due to two reasons in which the Agrobacterium LBA 4404 strain used was no longer virulent and optimization on the current papaya Agrobacterium transformation protocol is needed for this particular gene construct. The co-researcher of the project, Dr. Vilasini suggested using particle bombardment transformation method instead of the Agrobacterium mediated transformation method.

Unresolved Problems

The first attempt of papaya transformation was unable to produce transgenic papaya with pCAM2300-HBsAg construct made. Hence we would like to go for second attempt with some optimization of the previous papaya transformation protocol. However, there were few limiting factors that had hindered us to proceed with the second attempt as bellows:

1. Insufficient balance of funding from TORAY awards to sustain the subsequent project activities since papaya agrobacterium-mediated transformation method is costly especially with the consumption of few expensive antibiotics such as cefotaxime, timentin, carbenicillin and kanamycin,.
2. Inadequate materials for transformation which is immature green papaya fruits var. Eksotika 1 sample for zygotic embryos collection due to outbreak of papaya dieback disease at most of the papaya plantations since 2006.

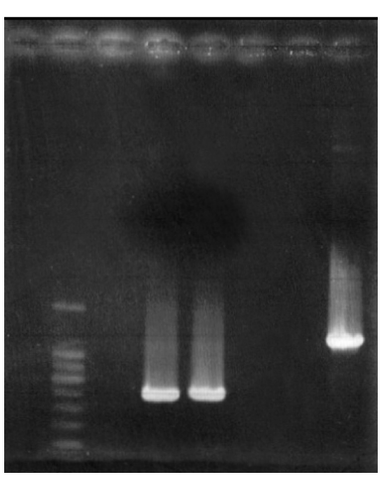


Figure 1. Lane M: Promega 100bp DNA ladder. Lane 1: PCR amplified HBsAg DNA fragment with approximately 700bp using specific primers namely 5' HpBSF and 3'HpBSR from a former construct donated by Dr. Tan Wen Siang.

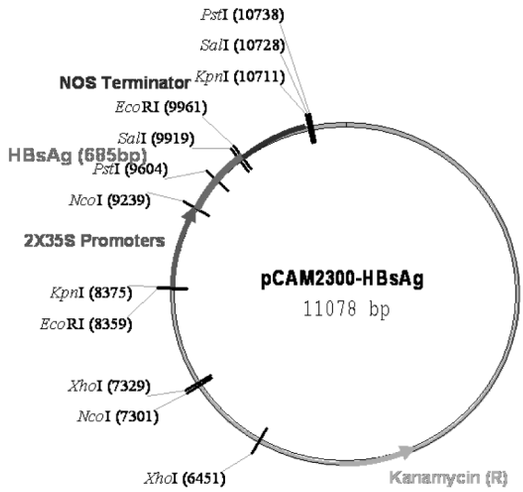
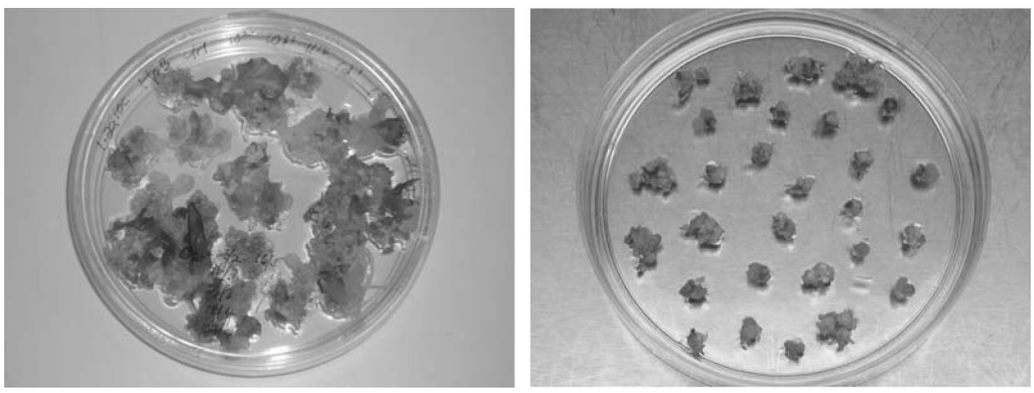


Figure 2: The map of the gene cassette pCAM2300-HBsAg that has been successfully constructed.



Non-transformed Transformed

Figure 3. The non-transformed versus transformed papaya embryogenic calli with gene cassette of pCAM2300-HBsAg on the papaya regeneration media.

Significant Results

1. Gene cassette namely TOPO-HBsAg whereby gene fragment of Hepatitis B virus surface antigen (HBsAg) cloned in TOPO intermediate vector.
2. Gene cassette namely pCAM2300-HBsAg whereby gene fragment HBsAg in cloned in pCAMBIA 2300 plant transformation vector.

Dr. Chow Wen Shyang

Universiti Sains Malaysia

"High Performance Recyclable Poly (Butylenes Terephthalate)/ Organo-Montmorillonite Nanocomposites"

Year 2005 MTSF Science & Technology Research Grant Recipient

Introduction

In recent years, polymer nanocomposites have attracted great interest. Nanocomposites offer new technological and economical benefits. The incorporation of nanometer scale reinforcement (e.g. layered silicates of clay, nanofibre, nanotubes, metal nanoparticles in polymeric materials) may dramatically improve selected properties of the related polymer. These nanocomposites exhibit superior properties such as enhanced mechanical properties, reduced permeability, and improved flame retardancy.

This research were focused on the study of thermoplastics nanocomposites based on poly(butylene terephthalate) **(**PBT). PBT is a thermal engineering plastic. PBT nanocomposites were produced via polymer melt intercalation method (extrusion and injection molding) by incorporation of organophilic modified montmorillonite (organoclay, OMMT). Naturally occurring montmorillonite is the most abundant member of the smectite family of clays. Ion exchange is widely practiced to modify the montmorillonite's surface to increase its compatibility with mostly hydrophobic polymer. Direct polymer melt intercalation is the most attractive because of its low cost, high productivity and compatibility with current processing techniques (e.g. extrusion and injection molding). It is believed that the mechanical properties of a nanocomposites is governed by several factors, these may include (i) interfacial interaction between polymer and clay, (ii) dispersion and distribution of clay, (iii) degree of exfoliation/intercalation of the clay, and (iv) anisotropicity of the clay. The exfoliation of the clay in polymer matrix could be induced by shearing (via extrusion and injection molding processes).

From the literature review, most of the nanocomposite produced containing a mixture of exfoliated/intercalated/ agglomerated structure. This can be evidenced by Transmission Electron Microcopy (TEM), X-Ray Diffraction (XRD), and Scanning Electron Microscopy (SEM). This is may be attributed to the shearing-induced exfoliation is limited by one-step extrusion and injection molding. Thus, in this research work, attempts were made to study the shearing-induced exfoliation on the PBT/OMMT nanocomposites by repeated extrusion processes. It is hypothesizing that higher degree of exfoliation could be achieved by repeated shearing process (e.g. analog to recycling). Hence, it is expected that the retention-ability of mechanical properties for recycle-PBT/OMMT nanocomposites could be enhanced by intercalation and exfoliation phenomenon.

Objectives

1. To improve the mechanical and thermal properties of poly (butylene terephthalate) by organo-montmorillonite.
2. To develop a high performance recyclable PBT/OMMT nanocomposites by direct melt intercalation method.
3. To study the mechanical retention-ability of PBT/OMMT nanocomposites upon subjected to repeated extrusion and injection molding processes.

Methodology

Generally, PBT/OMMT nanocomposites were prepared through extrusion (melt compounding) followed by injection molding. Several techniques and characterization methods were carried out to determine the mechanical, morphological and thermal properties of PBT nanocomposites, these including tensile tests, flexural tests, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Dynamic Mechanical Thermal Analysis (DMTA), and Differential Scanning Calorimetry (DSC). Attempt was made to study the recycle-ability of PBT nanocomposites.

Results

Tensile modulus and strength of PBT/OMMT was slightly improved attributed to the confinement of polymer chains and increment of crystallinity for PBT. Intercalated PBT/OMMT nanocomposites were successfully formed. Two times twin-screw extrusion followed by injection molding did not influence the thermal properties of PBT/OMMT nanocomposites significantly. The percentage retention in tensile properties of PBT/OMMT(E2) intercalated nanocomposites is more than 96%. Thus, repeated extrusion and injection molding processes could enhance the shear-induced melt intercalation of OMMT silicate layers in poly(butylene terephthalate). This approach could be analog to recycling process, where the PBT materials parts can be re-extruded and re-injection molded. The retention-ability of the PBT/OMMT materials is comparable excellent. A high performance intercalated PBT/OMMT nanocomposites with high recycle-ability was produced by direct melt intercalation method.

Future Direction

In the future, the research and development of recyclable PBT/OMMT nanocomposites will be carried out by using different approaches, e.g.

(i) the using of suitable compatibilizer,
(ii) blending with other engineering plastics and
(iii) the using of different types of inorganic nanofillers.

Publications

1. W.S. Chow (2008). Cyclic Extrusion of Poly(butylene terephthalate)/Organo-Montmorillonite Nanocomposites: Thermal and Mechanical Retention Properties. Journal of Applied Polymer Science, 110(3): 1642-1648.
2. W.S. Chow (2008). Tensile and Thermal Properties of Poly(butylene terephthalate)/Organo-montmorillonite Nanocomposites. Malaysian Polymer Journal 3(1): 1-13.

Dr. Hii Yii Siang
Professor Law Ah Theem

Universiti Malaysia Terengganu

"The study of a Symbiotic Bacterial interaction in Biodegradation of Tapis A Crude Oil"
Year 2005 MTSF Science & Technology Research Grant Recipient

Introduction

Oil pollution is a continuous threat to marine and coastal ecosystems. Oil toxicity is harmful to wildlife, health, tourism, fishing and the livelihoods of those in the vicinity of the spill. Since 1970's oil and grease, which are predominantly hydrocarbons, has been staying high in the list of prioritized pollutants in Malaysian marine environment. There is an increasing trend of hydrocarbons contamination reported in our environment. Most oil that is spilled at sea is degraded by microorganism. Success of a bioremediation is very much depends on the bioremediation agent; in this case - the oil degrading bacteria. Under batch culture condition, it is normal that the oil degrading bacterium undergo four different phases: lag phase, log phase, stationary phase and death phase. The retardation phases (stationary phase and death phase) are believed to be due to the nutrient deficiency, unfavorable conditions and production of self inhibiting metabolites (Madigan et al., 2000). Biodegradation is a series of complex biochemical reactions. The efficiency of the oil-biodegradation is believed to be able to improve when there are several different microorganisms working together as a consortium. However, there is not well understanding and study on how HCBs interact with other microorganisms in crude oil degradation. Hence, this study aims to reveal the interaction of the symbiotic bacterial interaction.

Objectives

1. To study the kinetics of *Tapis* Blended crude oil degradation by using local isolates of oil-degrading bacteria.
2. To study the effect of symbiotic bacteria on *Tapis* blended crude oil degradation.
3. To determine factors that influent the activity of the oil degrading bacteria in the environment.

Methodology

Active oil degrading bacteria and the associated heterotrophic bacteria was isolated from Malaysia marine environment. Biodegradation of crude oil by oil bacteria was conducted in a 4L bioreactor (Sartorious). Sterilize synthetic medium containing Tapis blended crude oil was use to grow the bacteria. The experiment was conducted at 28°C, pH 8.1, salinity 30ppt. Bacteria density, total organic carbons and the remaining crude oil were determined every 48 hours. Specific growth rate of the bacteria will be determined using the Michaelis-Menton equation. The concentration of the total organic carbon (TOC) was determined by using Total Organic Analyzer (Shidmazu, TOC-5000). The petroleum hydrocarbon was determined based on UNEP (1992) technique by using GC-MSD (Shimadzu, QP5000). Statistical analysis were performed to compare biodegradation rate of consortium bacteria and single inoculation.

Results

Active oil degrading bacterium, AR3 was selected from a total of 29 active strains of hydrocarbonclastic bacteria isolated from Malaysian marine environment as reported in the previous progress report. This oil-degrading bacterium, AR3 is found symbiotically interacted with two other non-oil-degrading but heterotrophic bacteria; OG1 and OG2. OG1

and OG2 are oil-resistant and active marine heterotrophic bacteria, however they are not able to utilize hydrocarbons as their carbon source. Identification based on the 16r DNA sequence shows that AR3 is 99.9% similar to Pseudomonas psedoalcaligene; while OG1 and OG2 are Erythrobacter citreus but different strains. It is interesting to find that, the symbiotic interaction between the bacteria is able to enhance the biodegradation rate of AR3 on *Tapis* blended crude oil. The AR3-OGs bacterial consortium is able to degrade 68.6% of total *Tapis* blended crude oil within 6 days as compared to 59.9% of total *Tapis* blended crude oil by AR3 alone. The remaining fractions are believed to be asphaltenes and resin which are highly resistant to microbial degradation. Maximum specific growth rate (μmax) of AR3 was triggered by the symbiotic interaction of the bacterial consortium; it increased from 0.068±0.02 h^{-1} to 0.091±0.007 h^{-1}. Other than that, the symbiotic interaction among the heterotrophic bacteria also promoted degradation of carcinogenic polycyclic aromatic hydrocarbons (PAHs). Results reveal that, the bacterial consortium is able to remove 9.260 mg L-1 (67.7%) of the total 16 priorities PAHs listed by USEPA while the oil-degrading bacteria alone is only able to degrade up to 7.001 mg L-1 (51.2%) of the USEPA priorities PAHs. Biodegradation of crude oil in the marine environment could be retarded by the self-inhibiting metabolites produced by the oil degrading bacteria while degrading crude oil. The self-inhibiting mechanism is probably one of the limiting factors that slow down oil degradation in the marine environment. Introduction of the symbiotic bacteria is able to remove the inhibiting metabolites and thus overcome the hold back mechanism. Removal of the self inhibiting metabolites further improved the oil-biodegradation. The symbiotic bacterial consortium formulated in this study is having superior performance on oil-degradation. It can be used to develop a new oil-combating approach for oil spills in the marine environment. Due to the finding of this study, the bacterial consortium was employed for developing immobilized cells to combat oil pollution in the country.

Unresolved problems and future direction

This study revealed the hold back mechanisms in the oil-degrading bacteria. Application of a non-oil degrading bacteria could enhance biodegradation by removing the self-inhibiting compounds. However, the inhibiting compounds are remained unknown. Further research could be focused on isolation and identification of the inhibition compounds. Knowing the inhibitory compounds could lead to better understanding of the self-inhibiting mechanisms in biodegradation.

Publications

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"Synthesis and characterization of Inorganic-organic Nanocomposite comprising metal chalcogenicles (Cus & Cds) and mesogenic diols liquid crystals as well as their Polymers for photonic applications"
Year 2005 MTSF Science & Technology Research Grant Recipient

Introduction

The research was undertaken to study effect of CdS nanoparticles in the thermotropic liquid crystal monomers as nanocomposites for photonic applications.

Objectives

The purpose of this research project is to synthesize and characterize a series of metal chalcogenides such as cadmium sulphide (CdS) as well its interaction with mesogenic diols (diol-vanilin & diol-*para*-hydroxybenzaldehyde (PHB)) for the purpose of photonics applications. Therefore, in order to fulfill the purpose, the research commences with the synthesis, characterization and evaluation of the metal chacalogenides and azomethines (bisphenol-vanilin & bisphenol-PHB).Subsequently, the mesogenic diols (monomers) which exhibit liquid crystal (LC) properties are also beeen synthesized and characterized accordingly. With the above products, the novelty of this research comes when the inorganic-organic nanocomposites are synthesized and characterized accordingly. These new nanocomposites will then be studied based on their photonic characterizations.

Methodology

(i) **Materials:** Unless otherwise stated, all chemicals are commercially obtained and are used without further purification or distillation. The starting materials for the synthesis of CdS are $CdCl_2.2.5H_2O$ and thiourea. *P*-hydroxybenzaldehyde, 1-chloro-6-hexanol, sodium carbonate, dimethylformamide, 1-butanol and diethyl ether are purchased for the synthesis of the organic materials. Doubly distilled water is used throughout the experiment.

(ii) **Methods**:

(a) Preparation of metal chalcogenides

CdS synthesized using the chemical precipitation method. The obtained samples are characterized with XRD, SEM and TEM.

(b) Synthesis of bisphenol-vanilin and bisphenol–*p*-hydroxybenzaldehyde (PHB)

The synthesis of two type of bisphenol namely bisphenol-vanilin and bisphenol-PHB are synthesized according to Mohammed (2001)*.

* Mohammed, I.A. (2001). In *Synthesis and characterization of novel high thermal stability poly(azomethine urethane)s*. Ph.D Thesis. Penang: Universiti Sains Malaysdiol vanilin.

(c) Synthesis of mesogenic diols (monomers)

Two mesogenic diols can be synthesized from the products in (b) by reacting them with 6-chloro-1-hexanol.

Note: The characterizations of products (b)-(c) are analyzed as follow: The spectral analysis is determined using FTIR spectrometer and FT-NMR spectrometer. Differential Scanning Calorimetry (DSC) is used to carry out thermal characteristic and finally, the mesophase textures of these compounds are studied using hot stage polarizing optical microscope (POM).

(d) Synthesis of inorganic-organic nanocomposites

The main approach is adopted to prepare nanocomposites which CdS nanoparticles are generated *in situ* in the presence of thermotropic liquid crystal monomers(c) using simple reflux system. The obtained nanocomposites' photonic characterization is then studied using photoluminescence spectroscopy.

Results

The preparation CdS: diol vanilin or diol PHB nanocomposites are successfully synthesized. The size distribution for nanocomposites is also narrower compared with that of as-obtained CdS nanoparticles. The analyses from POM and DSC reveal that mass composition from 0.1: 1.0 up to 0.5:1.0 of CdS: diol vanilin nanocomposites show their enantiotropic nematic phase. Otherwise, when the mass composition of CdS in diol vanilin at 0.6: 1.0 and greater, the liquid crystal property is affected. POM result reveals that the liquid crystal property of diol PHB vanishes completely when the mass composition is at 0.5:1.0 (CdS: diol PHB) (w/w). PL's emissions for CdS: diol vanilin or diol PHB nanocomposites are found to be in the 700-800 nm, suggesting that deep trap defects occur in both cases. However, the PL analyses show unique findings in both cases. PL for CdS: diol vanilin nanocomposite in irrespective mass composition of the nanoparticles has resulted in suppression of PL. On contrarily, addition of CdS in diol PHB nanocomposite enhances the PL till 0.3:1.0 but the PL emission drops when the mass composition is 0.3:1.0 onwards. XPS confirms the stoichiometry for Cd/S is almost 1 which agrees well with the formulation of CdS.

Unresolved problems and future direction

Aggregation and agglomeration of the nanoparticles in the matrix remains a challenge in the chemical synthesis process. Materials such surfactant, polymer and so forth are employed to cap the nanoparticles have been reported in many publications to circumvent this problem. Besides that, researchers are working towards more environmental friendly techniques to produce their new materials. Therefore, we foresee green methods would become more popular in the future to fabricate such materials. All in all, incorporating CdS in thermotropic liquic crystal monomer in the current project has showed a great potential in the photonic applications.

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"Comparison of the relationship between the expression of IL-12 Beta 2 Receptor (IL-12β2R) and CD30 on surface of T-Lymphocytes and disease manifestation in systemic Lupus Erythemathosus (SLE) and Rheumatoid Arthritis (RA) patients from Hospital Seremban – A Pilot Study"
Year 2005 MTSF Science & Technology Research Grant Recipient

The aim of this project is to study the relationship between the IL-12β2R and CD30 gene expression and disease manifestation of rheumatoid arthritis (RA) patients. Rheumatoid arthritis is an autoimmune disease in which autoantibodies are produced against the various self-antigens. Recent evidence suggests that a large number of autoimmune diseases are mediated by T-helper cells type 1 (T_H1) cells either through the cell-mediated immune mechanisms or through the T_H2 cell-dependent autoantibodies pathway. There are some debates on the clonal nature of the autoreactive T_H cells and whether these cells belong to the T_H1 or T_H2 subsets. Previously, there was no easy way to discriminate between T_H1 and T_H2 populations. Recent studies suggest that T_H1 cells selectively express the IL-12 beta-2 receptor (IL-12-β2R) while T_H2 and T_H0 cells predominantly express a cell surface antigen known as CD30. These unique expression patterns permit us to discriminate T_H1 and T_H2 immune responses in RA patients. In this study, we aimed to investigate the expression of IL-12-β2R or CD30 in RA patients and healthy subjects and to determine the correlation between RA activity and the T_H1/T_H2 immune responses in the RA patients.

Forty-three (43) RA patients and 46 sex- and age-matched healthy volunteers were recruited for this study. Total RNA was extracted from the blood of all subjects and the yield and quality of the RNA was analyzed spectrophotometrically. The IL-12β2R and CD30 gene expression were assayed by real-time RT-PCR technique. β-actin was used as housekeeping gene and negative control consisting no RNA was included in every run. Relative gene expression for IL-12Rβ2 ad CD30 was normalized against β-actin gene. Expression data was analyzed using computer programme provided by manufacturer (Bio-Rad) and statistical analyses were performed using SPSS v11.5. Tests of normality i.e. Kolmogorov-Smirnov and Shapiro-Wilk test showed that the expression data was not normally distributed. Therefore, non-parametric test i.e. Mann-Whitney test was used to evaluate the differences between gene expression and p-value was set at 0.05 for statistical significance.

The gene expression of IL-12β2R in patients and controls did not differ significantly whereas CD30 gene expression was significantly higher in control cohort compared to the patients ($p<0.001$). Gene expression of IL-12β2R and CD30 in patients with different disease status was also compared. IL-12β2R gene expression was significantly lower ($p=0.001$) in patients with active RA ($n=21$) than patients with RA under control ($n=22$). However, we did not detect a significant different in CD30 gene expression between patients with active and under control.

The state-of-art of real-time PCR approach permits us to determine the expression of multiple genes in a same reaction. This approach eliminates the incongruity between reactions hence gives us a more sensitive detection in gene expression. The sensitivity of real-time PCR did, in fact, confer us a great challenge to optimize the experiment. However, we managed to overcome it with the help of bioinformatics' tools in designing primers wit higher specificity and sensitively. As for the future direction, more samples can be included in each comparison group; this will allow more stringent statistical analyses and will provide us more information about the differences.

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"Structure and Electron-Phonon interaction studies in CuSe and SnSe based semi-conductor compounds"
Year 2005 MTSF Science & Technology Research Grant Recipient

Introduction

It is evident that for the future well-being of nations, a supply of energy based on a renewable source which is economically and environmentally acceptable has to be developed. Successful production of an efficient metal chalcogenide solar cell and modules requires the coupling of fabrication techniques with a basic understanding of the devices. There is a need to develop a greater fundamental sciences and engineering basis for the selenium based semiconductor material devices and processing requirement. In this work, we have fill the information gap on literature about the fundamental study of the structural and electron-phonon interaction studies in polycrystalline CuSe and SnSe material in order to find out the fabrication and design parameters, which are imposed by current technology, material specifications and irradiation conditions to maximize the solar cell efficiency.

Objectives

1. To synthesize the polycrystalline CuSe and SnSe semiconductor compounds.
2. To characterize the electrical and thermal properties values of the CuSe and SnSe using parallel plate and photoflash technique.
3. To investigate the temperature dependence of the structural, electrical and thermal properties of the CuSe and SnSe at low and high temperature.
4. To evaluate the phase transition of CuSe and SnSe and investigate the electron-phonon interaction and the possible existence of thermal percolation.

Methodology

CuSe and SnSe powders were synthesized by chemical precipitation method. Manipulations and reactions were carried out in air. The obtained products were then characterized by XRD (Philips X'pert PRO PW3040) using Cu K_{α} (λ = 1.5418 Å) radiation. The diffraction patterns of these samples were obtained by scanning the samples with an interval of scanning angle (2θ) from 20^{o} to 60^{o} with a scanning speed of 0.005^{o}/s. The synthesized CuSe and SnSe powders were pressed into pellet shape using a hydraulic press (SPECAC USA, model 15011) of 3 ton pressure. The electrical conductivity and thermal diffusivity of the pellet sample under study were measured by a standard two probe and photoflash technique respectively at temperature range of 100 – 523 K.

Results

Copper (II) Selenide and Tin (II) Selenide metal chalcogenide semiconductor compound have been successfully synthesized via low cost chemical precipitation method. The XRD patterns revealed that the CuSe and SnSe compounds have been obtained. All the peaks obtained from the synthesized CuSe were well matched with the JCPDS data (File No. 34-0171) as Klockmannite which belongs to the hexagonal system. SnSe compound showed the orthorhombic system as all the peaks obtained from the XRD were well matched with JCPDS data (File No. 32-1382). The synthesized CuSe and SnSe exhibited a stable structure at temperature range of 100 - 398 K and 100 – 473 K respectively where no selenium peaks, unassigned or additional peaks were observed. CuSe compound has shown some changes in its structural as an additional peak is observed at temperature above 423 K.

To establish the electron-phonon interaction studies in the CuSe and SnSe semiconductor compounds, the thermal diffusivity and electrical conductivity measurement have been carry out from 100 K to 523 K. The dc electrical conductivity values of CuSe and SnSe were found to be in the range of 3.03×10^{-2} - 4.78×10^{-2} S/cm and 3.83×10^{-7} - 6.79×10^{-3} S/cm respectively as the temperature increased from 100 to 523 K. The variation of the conductivity with temperature for CuSe and SnSe compounds reveals that the samples follow an extrinsic semiconductor pattern. CuSe and SnSe compounds show a similar behavior where the thermal diffusivity value tends to decrease as the temperature increase from 100 to 523 K. The thermal diffusivity values of CuSe were determined to be in the range from 5.98×10^{-3} to 1.20×10^{-2} cm^2/s while the thermal diffusivity for SnSe were determined to be in the range from 3.80×10^{-3} to 1.37×10^{-3} cm2/s.

The electron-phonon interaction study plays an important role in formulating fundamental understanding of the electronic and thermal properties at the material interface. It is well known that the electron-phonon interactions and scattering play an important role for various properties of the semiconductor compound as temperature increases. At the low temperature range, the thermal and electrical properties of the CuSe and SnSe compound become heavily influenced by the scattering of phonons, while at the high temperature range, the carriers are accelerated. This acceleration caused the carriers to gain energy and free to scatter with other electrons, phonon, impurity atom, etc. The understanding of the electron-phonon interactions in our compounds has not only an important theoretical meaning, but also practical significance for device applications. Thus, our theory and results will be valuable for further understanding the thermal and electronic properties of the CuSe and SnSe semiconductor compound

Unresolved problems and future direction

Based on the fundamental results we have obtained, there is still need some time to fabricate CuSe and SnSe thin film and obtain a suitable deposition condition which could produce good quality films for solar cell purposes. Our future directions are as follows:

1. Synthesis ternary compound (Cu_2SnSe_3) using the combination of CuSe and SnSe binary metal chalcogenide compound.
2. Study in depth the layer by layer, thickness, annealing and substrate effect which could optimized the quality of the films deposited.

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"A Study on the Sensing Mechanisms of Solid State Chemical Gas Sensors for High Temperature Operations"
Year 2006 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Objective

A study of the interfacial properties of MOSiC devices in order to understand the sensing mechanism of silicon carbide based gas sensors.

Methodology

The metal-oxide-silicon carbide (MOSiC) device structure is used to in the study. The substrate is a highly doped (~ 0.02 ohm-cm) *n*-type 4H-SiC. The epilayer is 10 µm thick and has a doping concentration of around 3×10^{15} cm^{-3}, deduced from Schottky barrier diodes. The oxide layer is grown by thermal oxidation, and two different structures, with oxides targeted at 50 nm and 20 nm thicknesses are produced.

Electrical characterization of the MOSiC devices is carried out by capacitance-voltage (*C-V*) and conductance-voltage (*G-V*) measurements at a high frequency of 1 MHz and a small signal *a.c.* voltage of 300 mV. The sweep rate is 10 mV/sec. The LCR meter is Agilent E4980A. The electrical signal is probed by two micromanipulators in a MMR probe station. The probe station has a stage heater and a temperature controller which enables precise control of temperature from room temperature to 700K. The hydrogen sensing test is carried out by feeding a 2% hydrogen into the probe chamber. The concentration is chosen to enable saturation of the sensing response but it is below its lower explosive limit (LEL) which is 4% in air.

Results

The interface state densities deduced are in the order of 10^{11} $cm^{-2}eV^{-1}$ which is within the acceptable range for sensor response.

The sensor response results show a considerable voltage shift of the C-V curve under the influence of hydrogen, compared to measurements in air without hydrogen. It is found that sample with a thinner oxide has a higher sensitivity, as evident from the higher voltage shift compared to that of sample A.

The detection principle of field effect sensors with catalytic metal gates is based on the change in the electric field in the structure introduced by the gas. A familiar explanation of the response of the Pd-SiO_2-SiC sensor to hydrogen which appears in many published work is the formation of a polarized layer at the metal/SiO2 interface. Since the polarized charge build-up at the metal-SiO_2 interface decreases the potential of the metal gate, this would lead to a parallel shift of the C-V curve towards lower gate bias but would not change the shape of the curve.

However the shape of the C-V curves also change, where the slope become steeper, which indicate a change in the interface trap density. It was first investigated by Tobias, *et. al*. It is proposed that the hydrogen can passivate neutral defects at the interface and prevents them from charging/discharging.

Other than the two effects, we propose there may be a third effect. The response can be attributed to the possible existence of negatively charged states below the Fermi level. As the protons (H^+) reaches the SiO_2/SiC interface, these defect trap levels give up their electrons to the protons. The argument can be supported by examining the CV results at different temperature and oxide thickness. Coupled with the passivation effect, the interface state's influence on the C-V is reduced which explains the steeper slope of the CV response curves.

Conclusion

We have investigated the effect of interface trap levels on hydrogen sensing mechanisms of MOSiC. The voltage shift and the changing of the shape of the response curves due to hydrogen can be due to three mechanisms. When molecular hydrogen dissociates at the metal gate, a polarized layer is created at the metal/SiO2 layer. The next mechanism is the possible passivation of the interface states by hydrogen. We have shown there may be a third possible mechanism, which is the existence of negatively-charged interface traps at the SiO2/SiC interface which may change its occupancy in response to the protons (H^+) reaching the interface. Both the passivation of uncharged and neutralization of the charged interface trap levels contribute to the steeper slope of the hydrogen response curves.

Future work and publication

Currently the sensor does not have a heater. A built-in heater and temperature sensor will be built monolithically on the SiC wafer to enable easy hydrogen sensing measurements at elevated temperatures.

The manuscript is currently prepared for submission to an international journal.

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"Development of Silicon Carbide (SiC) Nanowire for Nano-Electronic Applications"
Year 2006 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Silicon carbides (SiC) nanowires have been successfully synthesized using a modified chemical vapour growth (CVG) approach, which conducted in a vacuum. In the process of synthesizing SiC nanowire, activated carbon powder and Si wafer were used as a source materials and it was heated in low pressure atmosphere and at high temperature. By controlling the physical parameter namely growth temperature (1200-1350^{o}C) and ambient (with and without Ar flow), deposition time (1-4 h), and crucible height (9, 13, and 16 cm), the morphology and chemical composition were evaluated by using field-emission scanning-electron microscopy with energy dispersive X-ray spectroscopy, X-ray diffractometer spectroscopy, transmission electron microscopy, and Raman spectroscopy. The nanowires with diameter and length in the range of 10-30 nm and lengths up to several ten of micrometers were produced and the phases of the nanowires can be engineered by changing these parameters.

A possible model of SiC nanowire growth mechanism had been proposed. According to this model, the nanowires were grown according to the following proposed mechanisms:

(1) diffusion of C/CO into Si substrate,
(2) weakening of Si bond and atomic kick-out,
(3) formation of Si-C in vapour phase,
(4) formation of saturated SiC layer,
(5) formation of pyramid-like SiC nanostructure and (6) formation of SiC nanowires.

Publications

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Miss Chew Guat Siew

Professor Tengku Sifzizul Tengku Muhammad

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"In search of signal transduction pathways that mediate the cytokine inhibitory effect on PPARα gene expression in Liver : Towards the elucidation of molecular mechanisms of the development of atherosclerosis"

Year 2006 MTSF Science & Technology Research Grant Recipient

(Funded by Toray Science Foundation, Japan)

Introduction

This proposed project was undertaken to enhance the knowledge of the signal transduction pathways that mediate IL-6 inhibitory effects on human liver PPARα in relation to the development of atherosclerosis.

Objectives

i) The objectives of research are:

ii) to determine the effects of IL-6 on PPARα gene expression in human liver cells,

iii) to elucidate the signal transduction pathways mediating the regulatory effects of IL-6 on PPARα gene expression,

iv) to verify the specificity of the identified components of the signal transduction pathways mediating the regulatory effects of IL-6 on PPARα gene expression,

v) to identify cis-acting elements of PPARα promoter that mediate the regulatory effects of IL-6-activated components of signal transduction pathways on PPARα gene expression,

vi) to delineate the trans-acting factors activated by the identified signal transduction pathways that mediate IL-6-inhibitory effects on hepatic PPARα.

Methodology:

a) Determination of the effects of IL-6 on PPARα gene expression

Human hepatocarcinoma HepG2 cell line was utilized in this study since PPARα was constitutively expressed in this cell line. The total cellular RNA samples were extracted using Tri-Reagent LS and the PPARα mRNA expression was determined using iCycler iQ® Real-Time PCR Detection System (BioRad).

b) Identification of signal transduction pathways mediating IL-6 inhibitory effects on human liver PPARα gene expression

A panel of inhibitors with specific action on components of known 'upstream' signal transduction pathway(s) were used to identify intracellular signaling routes which may potentially be involved in the suppression of PPARα gene expression by IL-6. HepG2 cell line were pretreated with the individual inhibitor in the presence of IL-6 for a further 24h before total RNA were extracted to determine the level of PPARα gene expression using Real-Time PCR.

c) Identification of the phosphorylated/activated components of 'downstream' signal transduction pathways

The 'downstream' signal transduction pathways were determined by phosphorylation of the kinases of 'upstream' signal transduction pathways. Thus, Western Blot analysis were carried out using anti-phospho antibody of the members of 'downstream' signal transduction pathways followed by anti-rabbit antibody-HRP conjugate. To further verify the involvement of the components in mediating the pathway, the action of IL-6 were blocked with the inhibitors identified previously.

d) Characterisation of transcription factors which interact with the identified cytokine responsive elements (CREs)

The final step of the phosphorylation of the components of 'downstream' signal transduction pathways is to activate the transcription factors that specifically bind to the promoter region of PPARα gene. Electromobility shift assay (EMSA) was employed to characterise the sequence-specific DNA binding proteins from liver cells, HepG2 that interacted with the identified Cytokine Response Elements (CREs).

e) Identification of cis-acting elements of PPARα promoter that mediate the regulatory effects of IL-6-activated components of signal transduction pathways on PPARα gene expression

In order to determine the cis-acting elements presence on the promoters that were responsible in mediating the action of the IL-6-activated components of signal transduction pathways in reducing the PPARα mRNA expression, transient transfection analysis was employed.

Results

The regulation of PPARα by IL-6 is of potential crucial importance in the development of PPAR. However, the precise nature of the signal transduction pathways triggered by IL-6 to suppress PPARα expression remains currently unclear. Real-Time PCR clearly demonstrated that IL-6 significantly suppressed PPARα mRNA expression in a dose dependent manner with the highest suppresion of 40% at 1000U/ml as compared to the untreated cells. The specific inhibitors, such as AG490 (JAK-STAT pathway), Worthmanin, LY294002, Akt IV Inhibitor and Rapamycin (PI3K pathway) and the MAP Kinase inhibitors; SB203580, U0126 and SP600125 (p38, p44/42 ERK and p46/54 JNK pathways) were shown to attenuate the inhibitory effect of IL-6, thus, suggesting that these inhibitors blocked the inhibitory action of IL-6 on PPARα expression. In addition, through the Western Blot analysis, we also proved that IL-6 stimulated phosphorylation via these pathways and lastly, activating STAT1 and STAT3 at the downstream level.

The specificity of IL-6 induced phosphorylation was further confirmed when the above mentioned inhibitors were used to inhibit these IL-6-induced signalling pathways. In addition, transient transfection analysis revealed that the promoter region B2 (-765/+34) contained the IL-6 responsive elements. By carrying out site-directed mutagenesis experiments of the STAT site within the region was observed to play a crucial role in the IL-6-mediated suppresion of promoter B2 activity. Interestingly, co-transfection experiments not only showed that STAT act as a corepressor to PPARα constitutive expression, but also function to enhance the IL-6-inhibitory action on PPARα. Electrophoretic mobility shift assays (EMSA) further revealed that exposure of HepG2 cells to IL-6 dramatically induced the binding of proteins to regions in the STAT site.

Addition to that, major reduction of binding activity was seen when inhibitors was treated to the HepG2 cells. In conclusion, the use of specific inhibitors against JAK-STAT, PI3K and MAP Kinase pathways demonstrated that IL-6 inhibited the mRNA levels of PPARα via these pathways, which in turn, activated and induced the binding of STATs in the PPARα promoter. Thus far, this study has provide novel insights into the potential pathways that may be responsible for the molecular regulation of macrophage PPARα gene expression by IL-6 in HepG2 cell line, and suggests a potential target for therapeutic intervention against atherosclerosis.

Unresolved problems and future direction

This study has successfully formed the basis and provided the vital fundamental information regarding the development of novel signal transduction pathways for therapeutic intervention against the molecular mechanisms of atherosclerosis. Therefore, we anticipated that the present study will provide a lead in the future direction especially in elucidating the potential role of the inhibitors as therapeutic agents in the intervention of atherosclerosis.

Publications

1. Chew, G. S., and Tengku Muhammad, T.S., (2007) Molecular mechanisms of acute phase response: Regulation of human peroxisome proliferator activated receptor alpha expression by interleukin-6 via the JAK/STAT pathway in HepG2 cells. Proceedings of the 9th Symposium of Malaysian Aplied Biology. 30-31 May 2007, Penang, Malaysia.
2. Chew, G. S., Tengku Muhammad, and Alexander Chong, S.C., (2008) Molecular mechanisms of Acute Phase Response: Identification of signal transduction pathways mediating the cytokine inhibitory effect on human peroxisome proliferator activated receptor alpha (PPARα). Proceedings of the 2nd USM Penang International Postgraduate Convention 2008. 18-20 June 2008. Penang, Malaysia.
3. Chew C. H., Chew, G. S., Nazalan Najimudin and Tengku Muhammad, T. S., (2007) Interleukin-6 inhibits human peroxisome proliferator activated receptor alpha gene expression via CCAAT/enhancer-binding proteins in hepatocytes. The International Journal of Biochemistry & Cell Biology 39 (2007) 1975-1986.

Miss Cindy Teh Shuan Ju
Professor Dr. Thong Kwai Lin

Universiti Malaya

"Development of DNA and Protein based Assays for
Salmonella Enterica Serovar Paratyphi A"
Year 2006 MTSF Science & Technology Research Grant Recipient

Salmonella Paratyphi A (*S.* Paratyphi A), a causative agent of paratyphoid fever is endemic in Malaysia and recent reports from Asian countries indicate the importance of this pathogen. In this study, we aimed to develop a more rapid and easy detection method of *S.* Paratyphi A, then to evaluate the specificity and sensitivity of the assay, and lastly to clone and express a specific fragment of *S.* Paratyphi A. Therefore, 4 pairs of primers which specifically detect *S.* paratyphi A had been designed and optimized in a multiplex PCR. This multiplex PCR demonstrates a 100% specific detection of *S.* Paratyphi A, detection limit of 3.95 X 10^4 CFU/ml for bacterial culture, 1.1 X 10^5 CFU/ml for spiked blood without enrichment, 1 X 10^4 CFU/ml for spiked blood with 5 hours enrichment, 1.9 X 10^5 CFU/ml for spiked stool without enrichment and 1.9 X 10^3 CFU/ml for spiked stool with 5 hours enrichment. Amongst the four primer sets, SPA3 was the most specific primer for *S.* Paratyphi A detection as it showed no amplification for non-Paratyphi A strains. This specific fragment amplified with primer SPA3 was further cloned into pBAD vector and expressed in an *E. coli* system. A 24 kDa protein was successfully expressed in soluble form and the antigen was able to detect polyclonal antibodies from sera of paratyphoid fever patients by Western-blotting analysis. Overall, the multiplex PCR developed in this study is a promising new technique for rapid, specific and sensitive detection of *S.* Paratyphi A. Further experiments are essential to have a better understanding of the expressed protein. Functional and structural study of the protein will be carried out in future.

Publications

1. Hong-Yu Ou, Cindy Teh Shuan Ju, Kwai-Lin Thong, Norazah Ahmad, Zixin Deng, Michael R. Barer and Kumar Rajakumar. (2007). Translational Genomics to Develop a *Salmonella enterica* Serovar Paratyphi A Multiplex Polymerase Chain Reaction Assay. *J Mol Diagn.* 9(5). 624-630.
2. Cindy Shuan Ju Teh, Kek Heng Chua, Savithri Devi Puthucheary, Kwai Lin Thong. (2008). Further Evaluation of a Multiplex PCR for differentiation of *Salmonella* Paratyphi A from other *Salmonellae*. Jpn J Infect Dis. 61(4). 313-314.

Dr. Phebe Ding
Dr. Hamid Sulaiman

Universiti Putra Malaysia

"Betacyanin Pigments and Colour Expression in red-flesh Pitaya *(Hylocereus polyrhizus)*"
Year 2006 MTSF Science & Technology Research Grant Recipient

The red colour of red-fleshed pitaya (*Hylocereus polyrhizus*) is due to a red colour pigment known as betacyanin. The betacyanins of pitaya have potential as food colourants and as natural antioxidant in food, cosmetics and pharmaceutical industries. Betacyanins of pitaya are highly appealing as compared to those from red beet and amaranth. Thus, the peel and flesh of pitaya fruit could be utilized for pigment extraction and this gives the fruit an additional value. To our knowledge, no work has been carried out to screen and profile the pigments pattern of this fruit. Therefore, the objectives of this study were to determine i) peel and flesh colour of pitaya fruit ii) profiles and total contents of betacyanins in the peel and flesh of pitaya fruit iii) relationships between colour measurements and betacyanins concentration of red-fleshed pitaya at each stage of fruit development and iv) to examine the usefulness of tristimulus colour measurements as predictors of pigment composition so that these measurements can be used instead of tedious determinations of pigment composition in red-fleshed pitaya fruits. Red-fleshed pitaya fruits were harvested for analysis at 5 days interval beginning from 25 to 35 days after flower anthesis (DAA). Peel and flesh of pitaya fruit at each DAA were extracted and concentrated prior to analysis. Then, fruits were analyzed for peel and flesh colour, total betacyanins content, protein content and pigment were separated by using HPLC method. The experimental design was a complete randomized design with three replications. Data obtained were analyzed by using analysis of variances. Differences within each factor were determined by least significant difference. Linear and quadratic regressions were used to analyze the relationships between DAA and each variable, whereas correlation was used to analyze the relationship between each variable. As DAA progressed from 25 to 35 DAA, the peel colour of pitaya fruit turned from green to red, while the flesh turned from creamy white mixture with red to full purplish red. There were significant relationships between DAA and colour (L*, C* and h°), betacyanins and protein content of peel and flesh of red-fleshed pitaya fruit. The DAA and peel L*, C* and h° related significantly with $R^2 = 0.56$, 0.78 and 0.99, respectively. There were also significant relationships between DAA and flesh L*, C* and h° with $R^2 = 0.97$, 0.69 and 0.81, respectively. Significant relationships were also obtained between DAA and betacyanins content of both peel and flesh with $R^2 = 0.99$, respectively. Significant relationships also occurred between DAA and protein content of both peel and flesh with $R^2 = 0.75$ and 0.89, respectively. A total of three types of betacyanins were separated for peel and flesh of pitaya fruit at 30 and 35 DAA as epxressed by three peaks while for 25 DAA, only one type of betacyanins was separated as epxressed by a single peak. The three major peaks (peak 1, 2 and 3) obtained for 30 and 35 DAA of red-fleshed pitaya eluted at about 5.7, 9.6 and 13.2 min, respectively, whereas for 25 DAA, the single peak observed at 9.6 min. Peak 3 was identified as isobetanin while peak 2 was identified as betanin. Peak 1 cannot be identified due to standard limitation. There were significance differences in total betanin concentration of peel and flesh as DAA progressed where fruit peel and flesh of 35 DAA possessed a highest content of betanin with 8.72 and 11.70 mg/ml, respectively. This result suggested that peel and flesh of pitaya fruit at 35 DAA contain more pigments with fully developed colour and possesses highest content of betacyanins as compared to those at 25 and 30 DAA, respectively.

The difference in pigment concentration between DAA might explain the differences in chromaticity and red hues of the peel and flesh colour of red-fleshed pitaya fruit. There were significant correlations between peel L*, C*, h°, flesh L*, C* and h°. There were also significant correlations between betacyanins content and peel C*, h° and flesh L*, C*, h°, respectively. Betacyanins content was positively correlated with protein and betanin content of both peel and flesh. The colour measurements (L*, C* and h°) were all significantly correlated with each other and were also significantly correlated with betacyanins content except L* values of peel colour. Therefore, C* and h° values could be used as predictor of betacyanins content for peel and flesh of red-fleshed pitaya fruit. This result suggested that tristimulus colour measurement can be adequately used to estimate the betacyanins content of red-fleshed pitaya fruit quickly, easily and nondestructively. It may also be sufficiently accurate for screening populations and could be used in the field as well.

Dr. Chan Kok Gan

Universiti Malaya

"Molecular Studies on Quorum Quenching System in Soil Bacteria"
Year 2007 MTSF Science & Technology Research Grant Recipient
(Funded by Toray Science Foundation, Japan)

Introduction

Bacterial cell-to-cell communication, famously known as quorum sensing is one of the most defining phenomena in molecular microbiology in the last two decades. Quorum sensing regulates myriad virulence factors production, making it an interesting target as novel anti-bacterial targets. Quorum quenching refers to the disruption of quorum sensing, and aims at attenuating quorum sensing-dependent virulence determinants without the use of antibiotic, hence reducing the emergence of multi-drugs resistant pathogens. My research was undertaken to isolate novel quorum quenching bacteria from various environmental habitats. These isolates can serve as novel anti-quorum sensing agents. I have started working on quorum sensing since my PhD work in Nottingham University under the supervision of Prof Paul Williams, the pioneer in this field, with the financial support from the prestigious Commonwealth Scholarship.

Objectives

1. To design an enrichment medium for isolation of *N*-acylhomoserine lactone (AHL)-degrading bacteria.
2. To characterize novel quorum quenching bacteria from environmental samples.
3. To determine AHL-degradation mechanism by these novel quorum quenching bacteria.

Methodology

- Design of selection medium for the isolation of quorum quenching bacteria.
- Design of high throughput quorum quenching assay to screen for quorum quenching activity
- Molecular characterisation of quorum quenching bacteria via 16S rDNA gene sequencing and phylogenetic analysis
- Application of bioinformatics tools in the study of bacterial identification

Results

In this work supported by MTSF, I have designed and published a quorum quenching bacteria selection medium. This discovery has significant impact on the work on quorum quenching as my work enables diverse quorum quenching bacteria readily isolated from Malaysian environmental habitats. My work has made possible the rapid isolation of quorum quenching bacteria beyond natural habitats extending to human body including oral cavity. My finding will spearhead the study of quorum quenching and will lead the path to the discovery of more novel quorum quenching bacteria and mechanisms that can interfere quorum sensing. Currently, we are actively characterizing the various isolates selected and determining their quorum quenching mechanisms as well as exploring the isolates as biocontrol.

The rapid quorum quenching essay reported in this work will also contribute major breakthrough in screening of quorum quenching bacteria activities as it is a high throughput and simple assay to perform. This will contribute

to shorten the time to study quorum quenching bacterial activities, thereby reducing the amount of manpower and materials used.

Unresolved problems and future direction

I have successfully designed KG medium to select and enrich quorum quenching bacteria from environmental samples. Numerous quorum quenching bacteria have been isolated by me which are yet to be characterized. The future work will direct to the determination of quorum quenching mechanisms, to explore these bacteria as biocontrol and to study their application in attenuating quorum sensing-dependent virulence determinants.

Publications

1. Kok-Gan Chan, Wai-Fong Yin, Choon-Kook Sam, and Chong-Lek Koh (2009). "A novel medium for the isolation of *N*-acylhomoserine lactone-degrading bacteria." *J Ind Microbiol Biotechnol* 36: 247-251.
2. Cheng-Siang Wong and Kok-Gan Chan (2008). Degradation of *N*-Acyl-Homoserine Lactone Quorum-Sensing Molecules by Yeast. The 30th Symposium of Malaysian Society for Microbiology 2008 – Microbes: Biotechnology Engine for Health and Wealth Creation. 16th-19th August 2008, Hyatt Regency Resort, Kuantan, Pahang, Malaysia.
3. Kok-Gan Chan (2009). Isolation of novel quorum quenching bacteria from various habitats in Malaysia. "UK -Malaysia Symposium on Current Strategies in Antimicrobial Therapies". 18-19th March 2009. University of Malaya, KL.
4. Cheng Siang Wong and Kok Gan Chan (2009). Enzymatic degradation of quorum sensing signal molecules by *Bacillus* sp. enriched from Malaysian rainforest soil using novel selection KG medium. Second International Conference and Workshops on Basic and Applied Sciences Regional Annual Fundamental Science Seminar 2009. 2-4 June 2009, Johor Bahru, Malaysia.

Miss Lim King Ting
Professor Dr. Thong Kwai Lin
Dr. Rohani Md Yasin

Universiti Malaya

"Determination of the Beta-Lactam Resistance Mechanisms in *E.Coli* and *Klebsiella* sp."
Year 2007 MTSF Science & Technology Research Grant Recipient

The emergence of multidrug resistant (MDR) *Escherichia coli* and *Klebsiella pneumoniae* poses a serious antibiotic management problem as ESBL genes are easily transferred from one organism to another via plasmids.

The objectives of this study are to determine the antimicrobial resistance profiles of *E. coli* and *K. pneumoniae* strains isolated from various hospitals in Malaysia and to study their genetic diversity using PCR-based fingerprinting techniques and PFGE. The presence of several resistance genes and integrons was also determined by PCR and their transmissibility was examined by conjugation and transformation experiments.

A total of 47 *E. coli* and 51 *K. pneumoniae* strains obtained from clinical specimens from various public hospitals in Malaysia were analyzed. Resistance profiles were obtained by disk diffusion tests. Detection of ESBLs genes and integron-encoded integrases was performed by PCR. Transformation test was performed by electroporation. The strains were subtyped by PFGE and PCR fingerprinting using RAPD, REP and ERIC.

Multidrug resistant strains (that are resistant to 2 or more classes of antibiotics) accounted for majority of the isolated strains (i.e., 36 *E. coli* and 31 *K. pneumoniae*). A large proportion of the *E. coli* (40 of 47) strains and all *K. pneumoniae* strains were producers of extended-spectrum □-lactamases (ESBLs). However, all the *E. coli* strains were sensitive to imipenem as were 98% of the *K. pneumoniae* strains. This indicates that imipenem appeared to be an effective agent against multidrug resistant *E. coli* and *K. pneumoniae,* in in-vitro.

PCR detection using gene-specific primers on the ESBL-producing strains showed that *E. coli* mainly harboured bla_{TEM} (35 of 40 strains) while bla_{SHV} was dominant in *K. pneumoniae* (46 of 51 strains). Other ESBL-related genes detected were bla_{OXA-1} (2 *E. coli* and 5 *K. pneumoniae* strains) and bla_{CTX-M} (19 *K. pneumoniae* and 8 *E. coli* strains).

Conjugation experiment carried out for selected *E. coli* and *K. pneumoniae* strains indicated that the ESBL phenotype and some of the antibiotic resistance genes were transferable and were likely plasmid-encoded. Results from the DNA fingerprintings showed that the strains obtained from sporadic cases of infections were very diverse and heterogeneous. REP-PCR and PFGE were found to be more discriminatory than RAPD and ERIC-PCR in subtyping *E. coli* and *K. pneumoniae.*

Publications

1. Lim KT, Yeo CC, Yasin RM, Balan G, Thong KL. 2009. Molecular typing of β-lactamase CTX-M-producing *Klebsiella pneumoniae* and *Escherichia coli* strains isolated in Malaysia. Book of Abstract 8th International Symposium of Antimicrobial Agents and Resistance, Bangkok, 18-20 March 2009, pp 209.
2. Lim KT, Yasin RM, Yeo CC, Thong KL. 2009. Genotypic Characterization of Multidrug-resistant *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* Book of Abstract UM expo penyelidikan 2009. Dewan Tuanku Canselor, University of Malaya. 13-15 January 2009. (Poster Presentation). Bronze Medal Awarded.
3. Lim KT, Yasin RM, Yeo CC, Thong KL. 2008. Genetic Characterization of pathogenic *Escherichia coli* strains isolated in Malaysia. Book of Abstract 13th Biological Science Graduate Congress 2008, National University Singapore, Singapore, 15-17 December 2008 (Poster Presentation).
4. Lim KT. Yasin RM, Yeo CC, Thong KL. 2008. Phenotypic and Genotypic Characterization of Extended-spectrum beta-lactamases producing bacteria. Book of Abstract 13th International Congress on Infectious Diseases Malaysia, 20-22 June 2008 (Poster Presentation).
5. Lim KT, Yasin RM, Yeo CC, Puthucheary SD, Thong KL. 2007. Determination and Characterization of ESBL producing *Klebsiella* strains isolated in Malaysia. Book of Abstract 12th Biological Science Graduate Congress, Malaysia, 17-19 December 2007 (Poster Presentation).

MTSF

PROSPECTUS

YEARLY SCHEDULE

ACTIVITIES

SUPPORTERS

WEBSITES

APPLICATION FORMS

Malaysia Toray Science Foundation (MTSF) is a registered and privately funded charitable organization devoted to supporting research initiatives and education that promote the advancement of science and technology in the country. Since its establishment in 1993 through a RM4 million endowment from Toray Industries, Inc., Japan, every year MTSF expends approximately RM600,000 derived from its operating income and contributions from the Toray Malaysia Group of Companies to fund its programmes.

Our Vision

To make a positive contribution to the betterment of Malaysian society and economic growth through national scientific and technological advancement and development of human capital.

Our Mission

The advancement of knowledge in the field of science and technology through the following strategic goals *:*

- To reward Malaysian scientists whose outstanding achievements have contributed to scientific and technological progress.
- To stimulate and encourage basic research in science and technology in Malaysia.
- To foster educational initiatives for the development of creative and effective Science education.

Our Annual Programmes

- ***Science and Technology Award***

  Awards of RM30,000 – RM60,000 each to one or two selected Malaysian scientists in recognition of their outstanding achievements in Science and Technology.

- ***Science and Technology Research Grants***

  Five to ten grants of up to RM60,000 each for Malaysian researchers below 40 years of age pursuing basic research in science and technology, limited to the fields of natural sciences, including the environment, but not including clinical medicine and mathematics.

- ***Science Education Award***

  Prizes ranging from RM2,000 up to RM10,000 each for Malaysian Science educators in secondary schools and pre-university colleges.

SCHEDULE OF MTSF YEARLY ACTIVITIES

JANUARY	-	**ANNOUNCEMENT OF NEW PROGRAMS**
	-	**DESPATCH OF APPLICATION FORMS**
MAR/APR	-	**PROMOTIONS / ROADSHOWS**
	-	**FINAL ANNOUNCEMENT OF PROGRAMS**
MAY 31	-	**CLOSING DATE FOR APPLICATION**
JULY	-	**SHORTLISTING OF APPLICANTS**
SEPTEMBER	-	**INTERVIEW & SELECTION OF WINNERS**
NOV/DEC	-	**PRESENTATION CEREMONY**
	-	**GRANT RESEARCH REPORT SESSION / SYMPOSIUM**

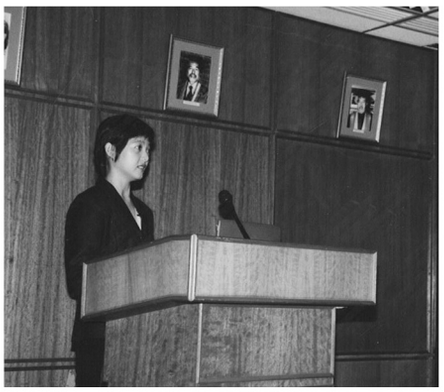
ANNOUNCEMENT OF NEW PROGRAMS VIA POSTERS

MTSF PRIZE PRESENTATION CEREMONIES


MTSF

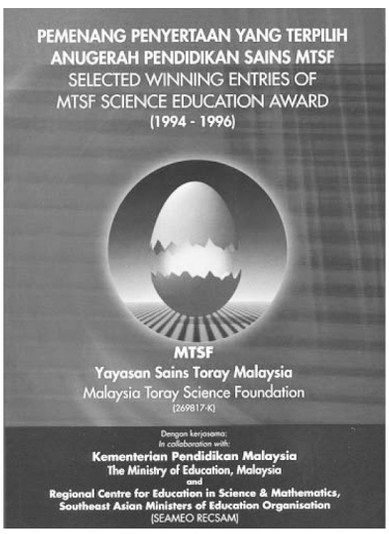

Why is allergy a problem?


MTSF
Malaysia Toray Science Foundation
www.mtsf.org




MTSF

SCIENCE EDUCATION AWARD - PUBLICATIONS/ROADSHOWS



PUBLICATION OF 1ST MTSF SELECTED SCIENCE EDUCATION AWARD WINNING ENTRIES (1994-1996)

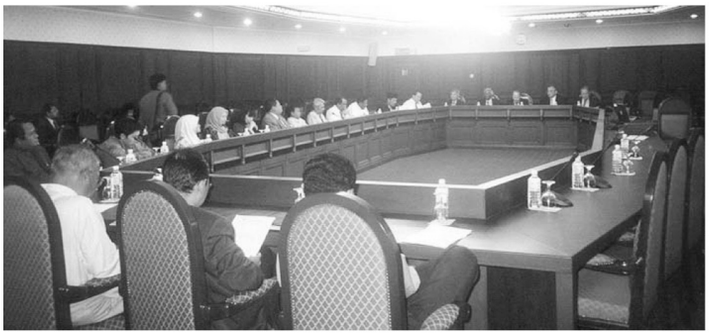
PUBLICATION OF 2ND MTSF SELECTED SCIENCE EDUCATION AWARD WINNING ENTRIES (1997-1999)

Roadshows

Demonstrations by winners of Science Education Awards during the roadshows

Universiti Malaya
6 October 2003

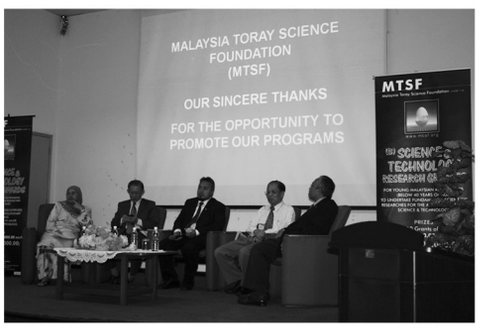
Institute for Medical Research
7 October 2003



Universiti Putra Malaysia
6 October 2003

Universiti Sains Malaysia
18 October 2004

AIMST University
18 October 2004

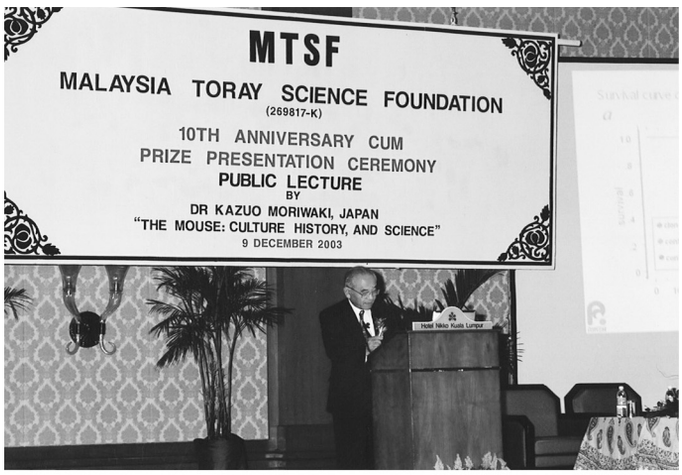


Universiti Kebangsaan Malaysia
6 April 2009

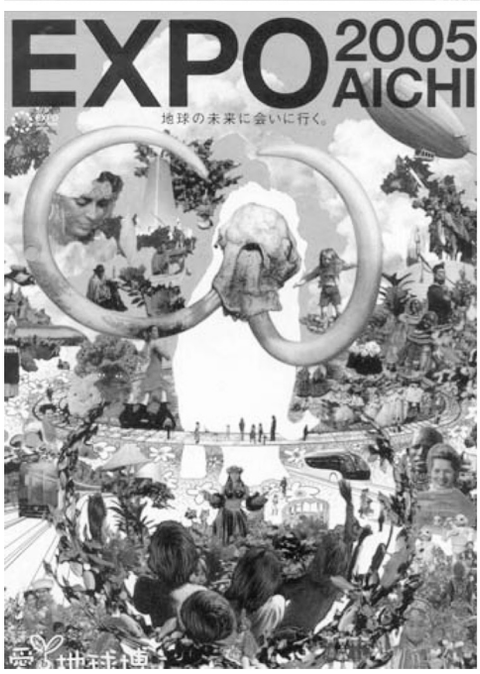
Universiti Tunku Abdul Rahman
7 April 2009

International Medical University
8 April 2009

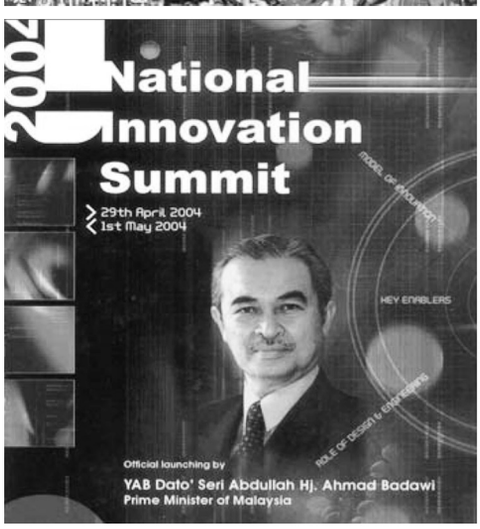
MTSF ACTIVITIES



Public Lecture held on 9 December 2003



Sponsored and Participated at the 2005
JAPAN AICHI- Children Science Exhibition in July 2005



2004
The Nobel Prize Centennial Exhibition

Participated in the "Creativity Science & Mathematics Education Expo 2004" at PWTC,
Kuala Lumpur – Nobel Prize Centennial Exhibition

南洋商報 要闻

刘贤镇警告不逐步减用氯氟化烃

中小工业必自食苦果

〔吉隆坡10日讯〕科学、工艺及环境部部长拿督刘贤镇披露，本地中小型工业对环球号召减用臭氧层破坏物质的反应不理想，尤其主要采用溶剂的中小型工业，反应更是冷淡。

他促请这些尚未参与行动的工业，善用《蒙特利尔议定书》属下多边基金之拨款，以便在国际完全禁用氯氟化烃（CFC）前，尽早做好应对准备。

他说，由於先进国家今年1月1日起已经禁用氯氟化烃，发展中国家的采用期限则在2010年结束，工业者若不在这段期间改用其他代替品，届时将无法在国际市场生存。

他指出，国际社会设立《蒙特利尔议定书》多边基金优惠，目的是鼓励和资助各国工业单位在1999年7月1日冻结使用前，逐量减用对臭氧层有害的氯氟化烃，并以其他化学物质代替。

除了溶剂，其他可对臭氧层构成破坏的化学物质包括有泡沫、气溶胶、卤化烃等。

他说，这也意味着我国在1999年7月1日后，每年只能进口不超过1995－1997年氯氟化烃的平均用量，2010年後则全面禁用。

“氢氯氟化烃（HCFC）也将在公元2016年开始逐步冻结使用，并於2040年全面禁用。”

他说，基金会已批出1千100万美元拨款，目前仅剩大约900万美元可申请，机会有限，本地工业应尽快通过环境局提出申请。

拿督刘贤镇是代表环境局颁发《马来西亚保护臭氧层荣誉》予本地11家公司后，向新闻界发表谈话。

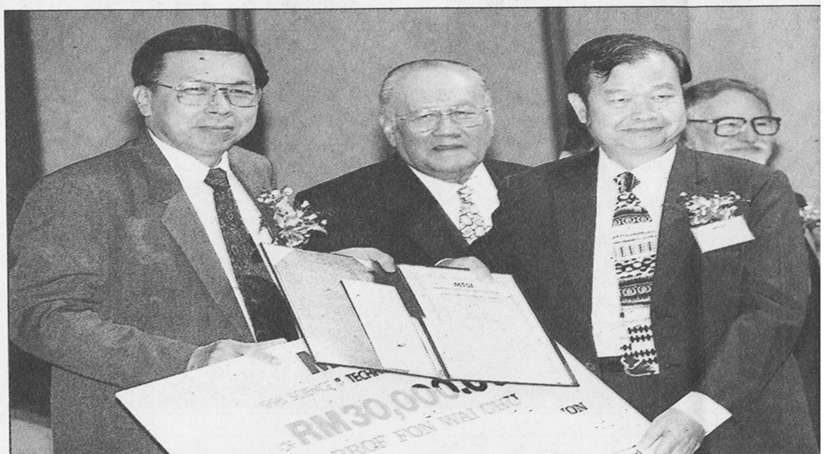
12公司保护臭氧层受表扬

共有12家公司获得本年度《马来西亚保护臭氧层荣衔》，分别是MAYA CHEMICAL INDUSTRIES，TENCO，RICWILL SDN BHD，BAN SENGLEE INDUSTRIES，AE TECHNOLOGY，P.U. MATE，WIDETECH SDN BHD，DENSO，STARFOAM PAPER PRODUCT，EVERSOFT FOAM INDUSTRIES，ALLIED FOAM INSULATION，NIAN AIK FOAM。

赞助商美国杜邦控股（马））有限公司东协区域经理王友福在会上也宣布把原本今年结束的3年赞助期，延长至1999年。

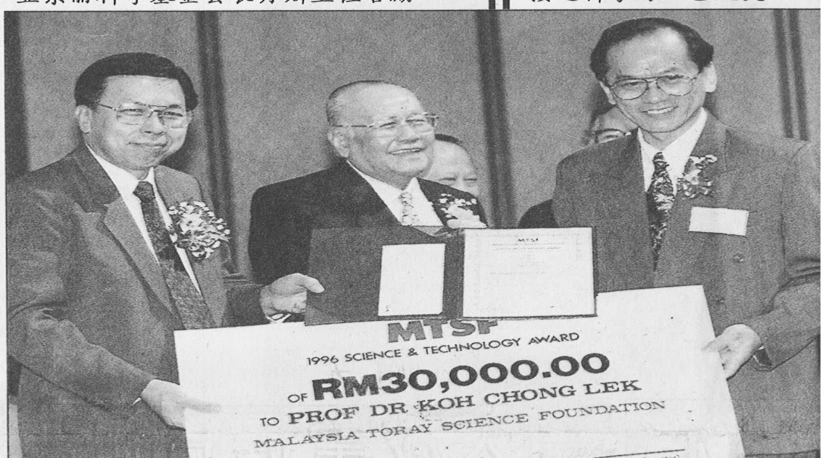
另外，部长也祝贺环境局上月在华盛顿，喜获联合国环境规划署颁发《1996年环境保护组织之US－EPA同温层臭氧荣誉》，为发展中国家争光。

他说，除了中小型工业在行动配合上稍见缓慢，其他工业进展顺畅；截至目前为止，我



⬆拿督刘贤镇颁发科学与工艺大奖予马大教授潘炜初（右），中为马来西亚东丽科学基金会长丹斯里佐哈励。

⬇马大教授许壮砀（右）从拿督刘贤镇手中接过科学与工艺大奖。



1996年科学与工艺大奖

〔吉隆坡10日

CONGRATULATIONS!

Recipients of 2007 MTSF Awards & Grants

Our heartiest congratulations to the following recipients who will be receiving their awards and grants, totalling RM425,000 at the 14th MTSF Prize Presentation Ceremony on 13 December 2007 at Hotel Nikko, Kuala Lumpur, officiated by the Honourable Minister of Science, Technology & Innovation.

Science & Technology Award (Total: RM60,000)

Prof Dr Harith Bin Ahmad, *Universiti Malaya*
Prof Dr Thong Kwai Lin, *Universiti Malaya*

Science & Technology Research Grant (Total: RM305,000)

Dr Ng Yong Foo and co-researcher, *Universiti Kebangsaan Malaysia*
Mr Daicus Anak Belabut, *Universiti Malaya*
Dr Chan Kok Gan, *Universiti Malaya*
Dr Tai Cheh Chin and co-researcher, *Universiti Malaya*
Dr Ha Sie Tiong and co-researchers, *Universiti Tunku Abdul Rahman*
Mr Mahenderan Appukutty and co-researcher, *Universiti Teknologi MARA*
Ms Lim King Ting and co-researchers, *Universiti Malaya*
Ms Wong Shew Fung and co-researchers, *International Medical University*
Mr Lee Lin Kiat and co-researcher, *Universiti Malaya*
Mr Tay Kheng Soo and co-researchers, *Universiti Malaya*
Ms Teh Ser Huy and co-researchers, *Universiti Malaya*
Mr Yam Mun Fei and co-researchers, *Universiti Sains Malaysia*
Ms Tiong Kai Hung and co-researchers, *International Medical University*

Science Education Award (Total: RM60,000)

WINNER PRIZES:
Mr Yeo Peck Cheng, *SMK Datuk Peter Mojuntin, Sabah*
Mr Jong Chung Hian, *QED Tuition Center, Sarawak*
Mdm Sia Peng Yee and co-applicant, *SMJK Seg Hwa, Johor*

RUNNER-UP PRIZES:
Mdm Lau Yoke Yin, *SMK Methodist Tanjong Malim, Perak*
Mr Ching Lee Hook, *Sek Men Sri Kuala Lumpur, Selangor*
Mdm Anisah Bt Omar, *Kolej Sultan Abdul Hamid, Kedah*
Mr Chong Cham Kong, *Sek Men Keb Saint Joseph (B), Johor*
Mr Yip Chi Kiong, *SMJK Chan Wa, Negeri Sembilan*
Mdm Elsie Mathew, *SMK Dato' Mohd Said, Negeri Sembilan*
Mr Woon Chee Yong, *Foon Yew High School, Johor*

CONSOLATION PRIZES:
Mr Mahamad Bin Hj Hamid, *Sek Men Teknik Sg Petani 2, Kedah*
Ms Sim Ley Yee, *SMK Bandar Baru Seri Petaling, Kuala Lumpur*
Mr Lau Yong Fuei, *Sek Men Keb Convent Ipoh, Perak*
Mdm Ng Lai Na, *SMK (L) Bukit Bintang, Selangor*
Mr Tan Jui Yong, *SMJK Seg Hwa, Johor*
Mr Ling Toh Woon, *HELP University College, Kuala Lumpur*
Mr Sim Pow Kim

More MTSF Programmes are up for grabs in 2008

SCIENCE & TECHNOLOGY AWARD
For outstanding Malaysian scientists / researchers
2 awards of RM30,000 each (Total: RM60,000)

SCIENCE & TECHNOLOGY RESEARCH GRANT
For Malaysian researchers below 40 years old
5 to 10 grants up to RM60,000 each (Total: RM300,000)
(Including 3 to 5 grants awarded by Toray Science Foundation, Japan)

SCIENCE EDUCATION AWARD
For science educators and teachers of secondary schools and pre-university colleges
- 5 Winner prizes of RM6,000 to RM10,000 each;
- 5 Runner-up prizes of RM4,000 each; and
- 5 Consolation prizes of RM2,000 each (Total: RM70,000)

Interested? Kindly log on to our website: www.mtsf.org
or
Email: mtsf@toray.com.my
or
Call: 04-385 4151 / 390 8157
or
Fax: 04-390 8260
or
Mail: Malaysia Toray Science Foundation c/o Toray Malaysia Group / Penfabric Sdn Berhad
Block B, Prai Free Industrial Zone 1, 13600 Prai, Penang, Malaysia

PENYEBAR FIKIRAN RAKYAT
Utusan
MALAYSIA
SELASA 29 NOVEMBER 2005 / 27 SYAWAL 1426 www.utusan.com.my

Perkongsian pintar kerajaan dan swasta tambah pendapatan nega

KUALA LUMPUR 28 Nov. — Kerajaan akan mewujudkan perkongsian pintar dengan pihak swasta khususnya penyelidikan dan pembangunan (R&D) dalam bidang sains dan teknologi supaya sektor tersebut dapat dijadikan sumber pendapatan negara.

Menteri Sains, Teknologi dan Inovasi, Datuk Seri Dr. Jamaluddin Jarjis berkata, pendekatan itu diambil kerana peratusan produk yang dapat dipasarkan melalui bidang tersebut masih rendah iaitu antara lima hingga tujuh peratus.

Katanya, keadaan itu tidak membantu usaha negara mengembangkan ekonomi pengetahuan (k-ekonomi) pada masa akan datang.

"Produk-produk R&D sains dan teknologi yang dihasilkan oleh para penyelidik terutama di universiti mempunyai potensi tinggi untuk dipasarkan.

"Melalui perkongsian pintar yang dijalinkan, produk-produk berkenaan tidak lagi 'tersangkut' di dalam makmal atau hanya menjadi kertas kerja semata-mata tetapi dapat dikilangkan dan seterusnya mampu menjana pendapatan negara," katanya.

Beliau berkata demikian kepada pemberita selepas menyampaikan anugerah Yayasan Sains Toray Malaysia (MTSF) ke-12, di sini hari ini.

Turut hadir ialah Pengerusi MTSF, Tan Sri Law Hieng Ding; Naib Presiden Eksekutif Toray Industries Inc., Hiroaki Kobayashi dan wakil Duta Jepun ke Malaysia, Masaru Aniya.

Pada majlis itu, MTSF menyerahkan geran penyelidikan dan wang tunai bernilai keseluruhan lebih RM440,000 kepada penyelidik yang mencapai kecemerlangan dalam bidang masing-masing.

MTSF merupakan sebuah pertubuhan kebajikan yang ditubuhkan oleh Toray Industries

JAMALUDDIN JARJIS

Inc., dengan pembiayaan awal sebanyak RM4 juta pada 1993 bagi mempromosikan sains dan teknologi di Malaysia.

Menurut Jam kat-syarikat sw perlu memainka bantu kerajaan kasikan k-ekono ngemukakan i produk R&D sai yang boleh dipa

"Kajian boleh hak universiti s tis di situ, tetap kan idea perlu syarikat swasta kejayaan menja katanya.

Dalam perken tan, Jamaluddin kerajaan berset lam MTSF bagi bih banyak proj kan pada masa

Katanya, pel bertujuan men banyak syarika ruskan dana-da kan oleh kerajaa baik dari segi a

大馬東麗科學基金
開始接受申請

（北海 17 日讯）第九届马来西亚东丽科学基金已开始接收申请，欢迎大马的研究员、科学家、教育工作者提出申请。

该基金会秘书拿督李耀建在一项发布会上表示，是项基金于 1993 年成立，并将于本月 21 日在吉隆坡颁发第八届的奖项予得奖者。

他表示，该基金会正式注册，通过东丽公司拨款 400 万的基金，并希望能藉以提升大马的科学及工艺，其中函盖自然科学范围但不包括药剂研究扩数学。

拿督李耀建说，在目前全球化及竞争的趋势下，单只靠产品出产是不足以长久立足，因此，多元化及产品附加值的生产路线是势在必行的。所以，拿督李表示，有必要加强科学与工艺的发展的发展，方能推动更佳的科学与生产技能，达到减低成本的目标。

马来西亚东丽科学基金共分三种类，即科
工艺奖励金及科学教育奖项，资以奖励杰出
员。

科学及工艺奖项每一年只录取二位名额，
3 万令吉的奖金。委员会将对申请者的研究背
请者需由受承认的单位或大学推荐，获得录取
一项的面试。第二种类的科学及研究奖励金供
研究员申请。委员会每一年将录取 5 至 10 位
30 万的奖励金。

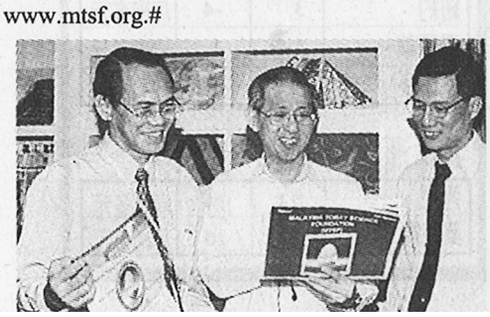
申请者也需通过受承认的单位或大学提出
查及面试，最后才由基金会委员通过。

第三组的科学教育奖项申请供开予国内中
项奖项共设有 15 个奖项，奖金数目共达 7 万令
此奖项的设立，是希望加以鼓励教师们创
者不单不限于报告或计划书，也可将现实、可
兴趣的作品、讲座与教学技能呈上。

基金会提供五项的奖项，每个奖金介于 5
份每份 4 千令吉，安慰奖五份每份 2 千令吉。

委员会将审查表格申请及面试，再由委员
马来西亚东丽科学基金每三年也将汇集其
合成书出版，再赠送至全国各中学，做为教学
该基金会已将各项奖项的申请表格及通知
专学府，申请截止日期是今年 5 月 31 日。任
校单位索取申请表格，或与基金会负责单位耳
纲页中下载表格。任何询问，欢迎与以下单位

MALAYSIA TORAY SCIENCE FOU
PEN-GROUP/PENFABRIC SDN BHD，BLOCI
TRIAL ZONE 1，13600 PRAI PENANG，
3854151，传真：04-3908260，电邮 MTSF@
www.mtsf.org.#



NEWS NEW STRAITS TIM

Japan's Toray Industries supports local science drive

By Marina Emmanuel
marinae@nstp.com.my

JAPANESE chemical conglomerate Toray Industries Inc will continue to help Malaysia's efforts in creating a science and technology culture via its local arm, the Toray Pen-Group of Companies.

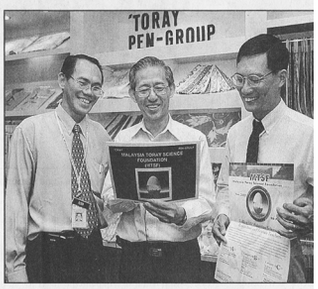
The group, through the Malaysia Toray Science Foundation it established nine years ago, has reiterated its investment presence in the country by helping Malaysia gain a competitive edge through value-added manufacturing services.

"The future of any nation is dependent on its ability to create, attract and retain the right brains," Toray Pen-Group executive director/general manager Datuk O.K. Lee said at the company's Prai Industrial Estate premises in mainland Penang recently.

The Malaysia Toray Science Foundation, through a RM4 million endowment by Toray Industries Inc, operates through awards, grants and operating expenditure totalling RM600,000 each year.

A total of 216 Malaysians have benefited from the foundation which has spent close to RM5 million since 1993.

"The current economic downturn in Japan is not permanent," said Lee, "and we see the need for science and technology to be entrenched in the local culture."



ALL SMILES ... Lee (centre) with the 2001 MTSF Science Education Award winners Dr Ivan Douglas Filmer, Jr (left) and Ooi Gim Ewe (right).

invested in Malaysia in 1973, comprises seven companies with capital investments totalling RM3 billion.

Lee, who is also the MTSF secretary, said winners of the foundation's Science and Technology Award, Science and Technology Re- projects.

"To contribute to the creation the future prosperity of Malaysia is essential that we build a f foundation for scientific and te nological progress."

"To achieve this objective, we

WELL DONE...Bhul Vindar Kaur receiving her prize from guest of honour, Science Technology and Environment Minister Datuk Law Hieng Deng, while MTSF chairman Tan Sri Mohd Khir Johari looks on.

Awards for top science teachers

By SIMRIT KAUR

DESPITE having been in the profession for over 30 years, Cecily Mary Peters of SMK Assunta, Selangor has not lost her zest for teaching and her love for Mathematics.

"To me teaching Maths is not a job, it's a hobby," said Peters, whose enthusiasm when imparting the subject was justly rewarded recently.

She was one of four recipients of the Malaysian Toray Science Foundation's (MTSF) Science Education Award. Peters won for two entries on Average Speed and Polyhedron.

The awards recognise workable, creative and innovative ideas which enhance the teaching of science and technology in schools.

"My winning entries were activities that I do in class every year, especially by teaching average velocity using balloons.

dron was a project that I did last year with my Form Three students."

"The children had fun doing it and it was also economical as they used cardboard boxes to make it."

Peters said that the award, worth RM5,000 was the highlight of her teaching career, and would spur her to continue make learning Maths fun.

"I owe it to my students, without them, I wouldn't have been able to carry out the ideas in the classroom."

One of the runner-ups Bhul Vindar Kaur of SMK St Cecilia in Sabah, who was also a consolation prize winner last year, believes that competitions like MTSF are important.

"As head of science in my school, I encourage my teachers to take part in competitions like these if they have creative ideas."

The other winners for the Science Education Award were Tan Ming Tang, Maktab Per-

Ching Lee Hock, Sek Men Sri Kuala Lumpur, Selangor, and Sadiah Baharom of USM's matriculation centre in Penang.

There were also eight runner-up and five consolation prizes.

Prof Dr Khairul Anuar Abdullah of Universiti Malaya and Prof Dr Chuah Hean Teik of the Multimedia University were named the joint recipients of the Science and Technology Award.

Researchers and academics from five institutions — Universiti Malaya, Universiti Sains Malaysia, Universiti Kebangsaan Malaysia, Universiti Putra Malaysia, and The Institute for Medical Research — with a total of 10 research projects in all, received grants worth RM300,000 from MTSF.

The MTSF was established in 1993 through a RM4mil endowment by Toray Industries Inc, Japan and rewards outstanding achievements as well as promotes fundamental research in

MONDAY, JANUARY 21, 2002 NATIONAL

www.mtsf.org

MTSF
Malaysia Toray Science Foundation
(269817-K)

Established in 1993 through a RM4 million initial endowment, MTSF is a premier privately-funded charitable organisation with the primary objective of advancing science and technology in Malaysia.

Invitation to Research Report Session By Past MTSF Grant Recipients
Date : Tuesday, 22 January 2002
Time : 9.00 am
Venue : Institut Pengajian Siswazah & Penyelidikan (IPSP)
Universiti Malaya, Kuala Lumpur
FREE ADMISSION
For enquiry, please call Ms Wong,
UM Tel: 03-7967 4525

RECIPIENTS OF 2001 MTSF AWARDS & GRANTS

Congratulations to the following recipients of RM365,000 in awards and grants:

SCIENCE & TECHNOLOGY AWARD (Total: RM60,000)

Dr Choo Yuen May	*Malaysian Palm Oil Board*
Dr Tee E Siong	*Institute for Medical Research*

SCIENCE & TECHNOLOGY RESEARCH GRANT (Total: RM235,000)

Ms Lim Chui Hun and co-researcher	*Universiti Sains Malaysia*
Mr Tan Chin Jye and co-researcher	*Universiti Malaya*
Ms Tai Yan Chin and co-researcher	*Universiti Malaya*
Dr Hairul Azman @ Amir Hamzah Bin Roslan and co-researchers	*Universiti Malaysia Sarawak*

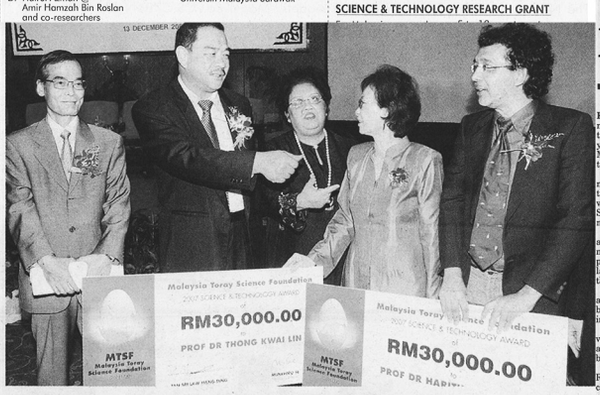
APPLICATION FOR 2002 MTSF PROGRAMMES

Your Brilliant Idea Could Win You An Award or A Research Grant

PROGRAMMES PRIZES

SCIENCE & TECHNOLOGY AWARD
For outstanding Malaysian scientists/researchers — *2 awards of RM30,000 each (Total: RM60,000)*

SCIENCE & TECHNOLOGY RESEARCH GRANT



(From left) Japanese Ambassador Masahiko Horie, Datuk Kong Cho Ha and Universiti Malaya's vice chancellor Datuk Rafiah Salim congratulating Prof Dr Thong Kwai Lin and Prof Dr Harith Ahmad.

SUNDAY STAR December 22 2002 EDUCATION NEWS

Awards for teachers and researchers

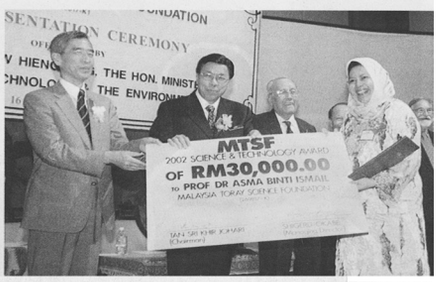
MALAYSIAN researchers should tap the country's resources for research on areas beneficial to the local community instead of mimicking western innovations, said Universiti Sains Malaysia's Medical Innovations and Technology Department (MITB) director Prof Dr Asma Ismail.

"If we decide to embark on research on areas such as HIV/AIDs for example, Malaysian scientists will be 20 years behind our Western counterparts," she said.

Prof Dr Asma, who is a recipient of Malaysian Toray Science Foundation (MTSF) Science and Technology Award, said local scientists should get involved in fields such cholera, typhoid and malaria as they are relevant to this part of the world.

"Research should not be done for the sake of research as discoveries made by Malaysian scientists should be turned into practical applications," she told reporters at the 9th MTSF Awards presentation ceremony here last week.

She said although the infrastructure and envi-



NEW STRAITS TIMES
NATION
WEDNESDAY, DECEMBER 10, 2003 7

Bio-entrepreneur incubator at UPM

■ By Syed Izzaddin
news@nst.com.my

KUALA LUMPUR, Tues. — The Government will set up a bio-entrepreneur incubator at Universiti Putra Malaysia by next year, Science, Technology and Environment Minister Datuk Seri Law Hieng Deng said today.

"We need such centres to develop biotechnology and assist future bio-entrepreneurs to translate their researches into business ventures," he said at the Malaysia Toray Science Foundation prize presentation ceremony at a hotel here.

He said fields related to biotechnology such as health, crop development and nanotechnology (study of the use of extremely small particles in life) was growing fast and Malaysia must move fast to take advantage of the growth.

"The centre will provide all the training and assistance to either scientists wanting to be entrepreneurs or businessmen starting out in biotechnology."

Earlier, in his speech, he said more investments were needed to set up research and development centres as the economic benefits were substantial.

"The ministry has been allocated a fund of RM1.5 billion for this reason and the fund can be tapped by the private sector through programmes like the Research in Priority

PROUD WINNERS: Lee (left) and Zarita with their mock cheque.

cipients.

The winners of this year's Science and Technology award were Institute for Medical Research head of medical entomology divi-

Each received RM30,000.

Dr Lee won for his research on insect-borne diseases while Dr Zarita for her work in accelerated learning method.

3 dana R&D galak inovasi produk

KUALA LUMPUR: Kementerian Sains, Teknologi dan Inovasi mewujudkan tiga dana bagi tujuan penyelidikan dan pembangunan (R&D) ke arah pembentukan generasi berpengetahuan dan inovasi produk.

meningkatkan pengetahuan pekerja industri teknologi negara.

"Melalui skim ini, kementerian mensasarkan 50 penyelidikan dihasilkan daripada 10,000 pekerja menjelang 2010 berbanding hanya 18 penyelidikan daripada 10,000 pekerja

y 14 December 2006

ASMAN IBRAHIM / BERITA HARIAN

PENERIMA ANUGERAH: (Dari kiri) Prof Dr Roslan, Ng Woon Shien dan M Hariandra meneliti sijil kemenangan anugerah Yayasan Sains Toray Malaysia semalam.

N4 Nation
THE STAR

ike teacher like pupil

M don
ires
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mentor

LUMPUR: When he
tle boy, Prof Dr Roslan
dor had always want-
e a mechanical engi-

ver, this ambition
l when he met physics
Yip Chi Kiong when he
6 and studying at
Teknik Tuanku Jaafar
Sembilan.
ven 24, convinced him
e a career in physics
terday, the professor
niversiti Kebangsaan
a's School of Applied
won the Science and
ogy Award at the 13th
a Toray Science
ion (MTSF) prize pre-
n ceremony here.
ward was for his work
-temperature super-
or research which he
in 1989 for his doctor-
ertation.
o make the event even
emorable, Yip was also
at the event to receive
ince Education Award,
now teaching at SMK
Ya in Negri Sembilan,
nsured for coming up
ectronic impulse mod-
e human nervous sys-

olan, who shared his
and RM60,000 prize
with Health Ministry

PROUD MOMENT: Yip (left) showing immense satisfaction at the success of his former student Dr Roslan at the Malaysia Toray Scienc Foundation prize presentation ceremony yesterday.

consultant clinical virologist Prof Dr Chua Kaw Bing, said he was surprised when he saw Yip get up on stage.

"He is a very good teacher and I started to develop my interest in the subject because of his teaching," said Dr Roslan, now 44.

"He was my inspiration to go on this path and I would not be

As for Yip, he said he was not surprised over Dr Roslan's achievement, despite it being a situation where the student has overtaken the master.

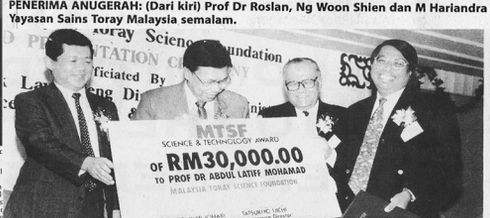
"I am very happy to see one of my students doing so well. He was one of the students who made good use of the opportunities given to him at Sekolah Teknik Tuanku Jaafar," he said.

In his speech for the occasion, Science, Technology and Innovation Minister Datuk Seri Dr Jamaludin Jarjis said the Government was constantly reviewing its science and technology policies in keeping with the rapid changes in the world.

His speech was read out by the ministry's Science, Technology and Innovation Division deputy-director Sakil Kusmi.

He said that with globalisation and the growth of knowledge-intensive industries, Malaysia needed to build strong foundations in science, technology and innovation.

MTSF was established in 1993 with an initial endowment of RM4mil given by Toray Industries Inc, Japan.



REWARDING EFFORT ... Abdul Latiff (far right) showing off the RM30,000 mock cheque which he received from the foundation for his work on "Plant Taxonomy, Ethnobotany and Plant Diversity". With him are (from left) Tetsuro Shiguchi, the minister and Deputy Chief-of-Mission of the Japanese Embassy; Law and foundation chairman Tan Sri Khir Johari

Need to revise education system to emphasise science, technology

KUALA LUMPUR, Thurs. — Malaysia has to constantly revise, upgrade and restructure its education system so that greater emphasis on science and technology can be injected from time to time.

Science, Technology and Environment Minister Datuk Law Hieng Ding said the school curriculum, from primary to pre-university levels, had been and would be modified whenever neces-

who could introduce fresh and innovative young minds into the creation of knowledge, thereby educating the next generation of scientists and engineers.

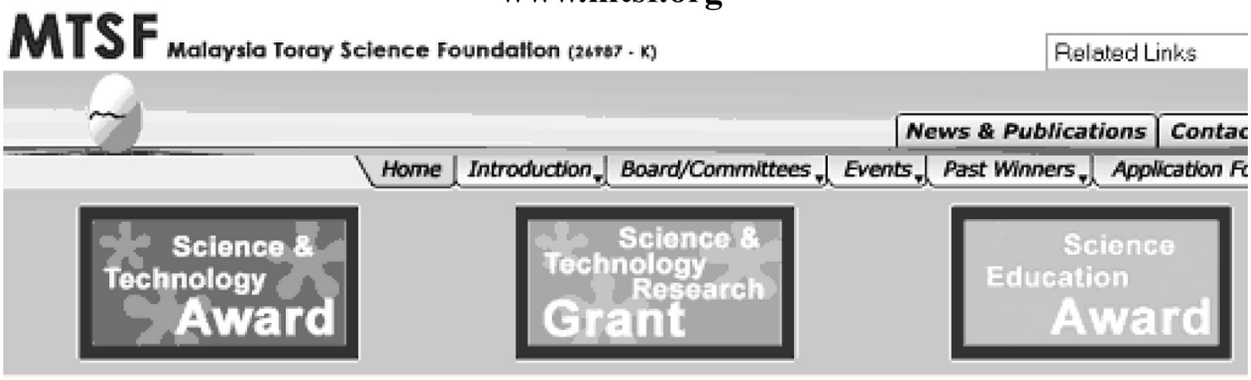
Law said that in the Sixth Malaysia Plan, which will end this year, RM600 million had been allocated for public research programmes.

"These public fundings, together with the private sector's investment in the research and development

MTSF SUPPORTERS

Name	Location	Main Business
Toray Industries, Inc.	**Tokyo, Japan**	Established in 1926, Toray is Japan's largest manufacturer of synthetic fibres and textiles, high-performance films and engineering plastics. Toray is a world leader in the development and production of carbon fibre and other advanced composite materials. Building on its extraordinary technological strength, Toray has diversified into chemicals, pharmaceuticals, medical supplies, electronic materials, housing and construction materials.
Toray Science Foundation	**Chiba, Japan**	Established in 1960, through a Yen 1,000 million endowment by Toray Industries, Inc. to contribute to the progress of science by supporting basic research in science and technology.
Toray Malaysia Group	**Penang**	
Penfibre Sdn. Berhad		Polyester Staple Fibres and Polyester Film "Lumirror"
Penfabric Sdn. Berhad		**Penfabric Mill 1** (Spinning of Yarn)
		Penfabric Mill 2 (Weaving of Grey Fabrics)
		Penfabric Mill 3 (Yarn-Dyed Woven Fabrics & Sewing Thread Yarn)
		Penfabric Mill 4 & HQ (Dyeing, Printing & Finishing of Fabrics)
Toray Plastics (Malaysia) Sdn. Berhad		ABS Resins
Toray BASF PBT Resin Sdn. Bhd.	**Pahang**	PBT Resins

www.mtsf.org



www.toray.com

www.ttsf.com

www.itsf.com

MTSF
MALAYSIA TORAY SCIENCE FOUNDATION
(269817-K)

SCIENCE & TECHNOLOGY AWARD

INFORMATION ON NOMINATION FOR SCIENCE & TECHNOLOGY AWARD

1. Qualification of Nominee

   Malaysian citizen who resides in Malaysia and has excelled in an area of study relating to natural sciences, excluding mathematics and clinical medicine, who :

   a) has made a major scientific discovery or discoveries which contributed to the enhancement of scientific knowledge,

   b) has made an original, revolutionary and important invention, or

   c) has successfully solved a major technological problem with an economically viable solution.

2. Science & Technology Award

   Two (2) awards are conferred every year and each consists of :
   i) a Certificate of Award, and
   ii) a cash prize of RM30,000.

3. Nominating University/Institution

   Nominations must be submitted by an authorised representative of a scientific or professional institution/organization or a university in Malaysia of which the nominee is a member.

4. Nomination Procedure

   Complete the Nomination Form, which is obtainable from MTSF or from the MTSF website, and return the same to MTSF at the mailing address stated therein.

5. Deadline

The completed Forms must reach the Foundation by 31 May of each year. Late entry and incomplete forms will not be processed.

6. Selection Committee and Method of Selection

The winners shall be selected by the Selection Committee and approved by the Board of the Foundation. For the list of the Selection Committee Members, please visit our website.

Shortlisted candidates will be invited to attend an interview by September of each year. The decision of the Selection Committee and the Board of the Foundation is final and no correspondence regarding the decision will be entertained.

7. Presentation of the Award

The presentation ceremony shall be held not later than the end of January of the following year.

Notes :

a) The Award can be given to a single person or a group of Malaysian nationality. If the nomination is for a group, the group will be treated as one nominee. It is the main nominee's responsibility to share the award money with his/her co-nominees.
b) Please write, phone or fax to MTSF at the address/contact numbers stated below for clarification and/or for additional forms.
c) This Award is not taxable in the hands of the recipient.
d) The Foundation reserves the right to vary the number and/or amount of the awards at its absolute discretion without prior notice.

Mailing Address/Inquiries to :
Penang Office of the MTSF, c/o Penfabric Sdn Berhad
Prai Free Industrial Zone 1, 13600 Prai, Penang.
Tel : (04) 3854151/3908157 Fax: (04) 3908260
Email : mtsf@toray.com.my
Website : www.mtsf.org

MTSF
MALAYSIA TORAY SCIENCE FOUNDATION
(269817-K)

SCIENCE & TECHNOLOGY AWARD

YEAR :	

NOMINATION FORM

To : PENANG OFFICE OF MTSF
C/O PENFABRIC SDN BERHAD
BLOCK B, PRAI FREE INDUSTRIAL ZONE 1
13600 PRAI, PENANG Tel : (04) 3854151/3908157 Fax: (04) 3908260

(Read the Information before completing the form. This form should be typed or printed. Use a separate form for each nomination. Photocopy of this form is allowed. **ALL NOMINATION FORMS AND ENCLOSURES SHOULD BE SUBMITTED IN DUPLICATE. PLEASE USE BINDER CLIPS, DO NOT STAPLE OR BIND).**

Particulars of Nominating University/Institution :

Name of University/ Institution : _____

Name of Authorized Representative (Title/ Present Position) : _____

Address : _____

Telephone : _____ Fax : _____

Email (if any) : _____

I hereby nominate _____ as candidate for the Science & Technology Award.

_____ _____
Authorized Signature & rubber stamp Date

Particulars of Nominee/Candidate :

Full Name : _____
(Prof/Assoc Prof/Dr/Mr/Ms – *delete those titles not applicable*)

PHOTO
(compulsory)

Chinese character (if applicable) : _____

Date of Birth : _____ Age : _____

Place of Birth : _____

Identity Card No : (New) _____ (Old) _____

Nationality : _____ Sex : Male _____ Female _____

Academic/Professional
Qualification (full details) :

_____

Present Position/ :
Occupation

_____

Official/ :
Correspondence Address

_____

Tel : _____ Fax : _____

Home Address :

_____

Tel : _____ Fax : _____

Email (if any) : _____

_____ _____
Signature of Nominee/Candidate Date

Field(s) of Specialization :

Basis of Nomination (Statement of Achievements – not more than 300 words)

This nomination is for : ☐ individual effort
(*please tick*) ☐ group effort

The members in the Group (limited to 4 members) are :
(Members of the group must be Malaysians)

Name & NRIC No. (with titles : Prof/Assoc Prof/Dr/Mr/Ms, *if applicable*)

1) 3)

2) 4)

Please state previous awards won by you/group members, if any :

Title of Award :
Year :
Awarding Body :
Amount : RM

Title of Award :
Year :
Awarding Body :
Amount : RM

Description of achievements and contributions in science, engineering and/or technology :

Publications (excluding conferences, seminars, workshops) :
(In the following order : Author, Year, Title of Publication, Title of Journal, Volume Number and Page Number. ISI Impact Factor.) COPIES NOT REQUIRED.

Resume/CV of Nominee/Candidate :
(State all outstanding professional honours and scientific awards, excluding scholarships, research grants, and travel fellowships. Please use additional A4 paper if space is insufficient. Maximum of 4 additional pages only).

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MALAYSIA TORAY SCIENCE FOUNDATION
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SCIENCE & TECHNOLOGY RESEARCH GRANT

INFORMATION ON APPLICATION FOR SCIENCE & TECHNOLOGY RESEARCH GRANT

1. Qualification of Applicant
   Energetic and creative researcher, Malaysian citizen aged below 40, residing in Malaysia, engaged in a basic fundamental scientific research field excluding mathematics and clinical medicine at a research facility in Malaysia, whose work has the potential to contribute greatly to the advancement of science and technology.

2. Research Grant
   The Foundation will provide up to about RM300,000 for research grants per year, comprising 5 to 10 grants of not more than RM60,000 each (including 3 to 5 grants from Toray Science Foundation, Japan worth about Yen5 million in total).

   The Foundation reserves the right to vary the number and amount of the grants at anytime at its absolute discretion without prior notice.

3. Recommending University/Institution
   Recommendation must be submitted by an authorised representative of a scientific or professional institution or a university of which the applicant is a member.

4. Application Procedure
   Complete the Application Form which is obtainable from MTSF or from the MTSF website and return the same to MTSF at the mailing address stated therein.

5. Deadline
   The completed Application Form must reach the Foundation on or before 31 May of each year. Late entry and incomplete forms will not be processed.

6. Method of Selection
   There are 3 stages in the selection process :

1st Stage	:	Shortlisting of applicants by the Selection Committee through the review of the Application Forms.
2nd Stage	:	Interview of shortlisted applicants by the Selection Committee.
3rd Stage	:	Approval of the successful applicants by the Board of the Foundation.

For applicants who have advanced to the 2nd stage, the shortlisted applicant or a member representing the research group will be interviewed by September of every year.

The decision of the Foundation is final and no correspondence regarding the decision will be entertained.

7. Selection Committee
The awardees shall be selected by the Selection Committee and approved by the Board of the Foundation. For the list of the Selection Committee Members, please visit our website.

8. Presentation of Research Grant
The presentation ceremony shall be held not later than the end of January of the following year.

9. Disbursement Procedure
The grant is to be disbursed through the nominating university/institution in accordance with the procedure set by the Foundation.

Notes :

a) This research grant is to be utilized in accordance with the proposed use of the research grant as stated in the application form only. If the recipient desires to use the grant for a different research project, prior written consent from the Foundation must be obtained. The recipient's university/institution is responsible for all financial transactions pertaining thereto.

b) The grant should be fully utilized according to the approved schedule. Any unused balance of the awarded amount will have to be refunded to the Foundation upon completion of the research or expiry of the approved schedule, whichever is earlier. Prior written approval of the Foundation must be obtained for any extension to the scheduled completion of the project.

c) The recipient is required to complete and sign the General Grant Conditions that stipulate the general terms and conditions of the grant.

d) Each year thereafter and until the research is concluded, each recipient of the Grant is required to submit an annual report, together with the up-to-date account of disbursement and utilization of funds, jointly certified by the University/Institution and the recipient. The annual report, covering January to December of a particular year should reach the Foundation by mid-March of the following year. Upon conclusion of the whole project, a final report and the statement of account must be submitted to the Foundation within 3 months from the date of such conclusion.

e) At the conclusion of the project, the recipient may be required to present his/her research work/paper at the Research Report Sessions that are arranged by MTSF from time to time.

f) In line with the Foundation's plans to publish and distribute a booklet containing the recipients' completed research work, the recipients may be required to help in this endeavour.

g) Please write, phone or fax to MTSF at the address/contact numbers stated below for clarification and/or for additional forms, or from the MTSF website.

h) This grant is not taxable in the hands of the recipient.

Mailing Address/Inquiries to :
Penang Office of the MTSF, c/o Penfabric Sdn Berhad
Prai Free Industrial Zone 1, 13600 Prai, Penang.
Tel : (04) 3854151/3908157 Fax: (04) 3908260
Email : mtsf@toray.com.my
Website : www.mtsf.org

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Particulars of Recommending University/Institution :

Name of University/ Institution : _____

Name of Authorized Representative (Title/ Present Position) : _____

Address : _____

Telephone : _____ Fax : _____

Email (if any) : _____

I hereby recommend _____ as applicant for the Science & Technology Research Grant.

_____ _____

Authorized Signature & rubber stamp | Date

Particulars of Main Researcher :

Full Name : _____
(Prof/Assoc Prof/Dr/Mr/Ms – delete those titles not applicable)

PHOTO
(compulosory)

Chinese character (if applicable) : _____

Date of Birth : _____ Age : _____

Place of Birth : _____

Identity Card No : (New) _____ (Old) _____

Nationality : _____ Sex : Male _____ Female _____

Academic/Professional
Qualification (full details) :

_____

Present Position/
Occupation :

_____

Official Address :

_____

Tel : _____ Fax : _____

Home Address :

_____

Tel : _____ Fax : _____

Email (if any) : _____

_____ _____

Signature of Main Researcher Date

Particulars of Co-Researcher(s) (limited to two members) :
(Members of the group must be Malaysians)

Full Name : _____
(Title : Prof/Assoc Prof/Dr/Mr/Ms – delete those not applicable)

Date of Birth : _____ Age : ____ Chinese character (if applicable) : _____

Present Position : _____

NRIC No : _____ Sex : Male _____ Female _____

Name of University/ Institution :

_____

Correspondence Address :

_____

Tel : _____ Fax : _____

Email (if any) :

Full Name : _____
(Title : Prof/Assoc Prof/Dr/Mr/Ms – delete those not applicable)

Date of Birth : _____ Age : ____ Chinese character (if applicable) : _____

Present Position : _____

NRIC No : _____ Sex : Male _____ Female _____

Name of University/ Institution :

_____

Correspondence Address :

_____

Tel : _____ Fax : _____

Email (if any) :

Title of the Research Project :
Purpose of Research (specify objective and/or statement of hypothesis in about 300 to 400 words. Please avoid using additional page).
Desired amount of Research Grant : RM _____
Schedule for usage of Grant : _____ (Commencement Year) _____ (Completion Year)

1. Outline of Research Plan (Write specifically. Indicate where this research is positioned within the whole research design, if any).

2. Proposed Use of the Research Grant :
(**1. Cost of equipment 2. Consumables and 3. Other Expenditure**. Please separate the expenses and **state details of each expense**. Please show the estimated total cost of the project. If the total cost is more than the desired amount of research grant, please explain how you intend to cover the difference. Please state in specific details the main equipment used. "Other Expenditure" should not include the salary of research assistants or travel expenses for attending conferences, seminars, etc).

1. Cost of Equipment RM_____
2. Consumables RM_____
3. Other Expenditure RM_____
4. Total RM_____

3. Please state briefly your past research experience and achievements.

4. Please state the current status of related research in Malaysia and abroad. Please state concretely the unique features of your research plan.

5. Please state whether you have received any grant/subsidy during the past years:

a) this particular research project or part thereof from any foundation/ministry
Yes ( ) / No ( ) If Yes, When : _____
Source : _____
RM : _____

b) any of your other research projects from any foundation/ministry
Yes ( ) / No ( ) If Yes, When : _____
Source : _____
RM : _____

6. Biodata of the applicant, including the co-researcher(s).
   (Please use additional A4 paper if space is insufficient. Maximum of 4 additional pages for each).

7. Publications (excluding conferences, seminars, workshops) :
(In the following order : Author, Year, Title of Publication, Title of Journal, Volume Number and Page Number.) COPIES NOT REQUIRED.

ACKNOWLEDGEMENT

MTSF would like to put on record our sincere thanks and appreciation to the following agencies and members who have contributed to the success of the Foundation since its inception :

Chairmen
- The late YBhg. Tan Sri Khir Johari (July 1993 - September 2003)
- YM Ungku A. Aziz (September 2003 - June 2004)

Managing Directors
- Mr. Kazuo Tomiita (July 1993 - June 1995)
- Mr. Tatsukiyo Ijichi (June 1995 - June 1997)
- YBhg Dato' Tsuyoshi Watanabe (June 1997 - June 1999)
- Mr. Junichi Suwa (June 1999 - July 2001)
- Mr. Eiji Doi (July 2001 - July 2002) and (June 2005 – June 2006)
- Mr. Shigeru Okabe (July 2002 - June 2005)

Directors
- The late YBhg. Tan Sri Khir Johari (July 1993 – September 2003)
- YM Ungku A. Aziz (July 1993 – June 2004)
- Mr. Masatake Kashiwagi (July 1993 – August 1993)
- Mr. Bunzo Adachi (July 1993 - July 1996)
- Mr. Seishiro Inoue (July 1996 - June 1999)
- Mr. Naohiko Kusakari (June 1999 - June 2002)
- Mr. Katsumasa Nakano (July 2005 - June 2008)

Committee Members
- YM Ungku A. Aziz , Chairman of Examination Committee (January 1994 – June 2004)
- YM Dr. Tengku Mohd. Azzman Shariffadeen (January 1994 - December 1996)
- The late Hj. Mohd. Khairuddin Bin Hj. Ashaari (January 1994 - April 1999)
- Mr. Ibrahim B. Md. Noh (August 1999 – December 2000)
- Professor Sim Wong Kooi (January 1994 – December 2001)
- YBhg. Dato' Professor Emeritus Mohd Sham Mohd Sani (January 1994 - December 2005)
- YBhg. Dato' Professor Emeritus Tan Wang Seng (January 1997 - December 2005)
- YBhg. Dato' V.G. Kumar Das (January 1994 - December 2007)

Collaborators/Supporters
- Ministry of Science, Technology & Innovation
- Ministry of Education, State Education Departments, Secondary Schools,
- Universities and Institutions

and to all those who have contributed in one way or another to the success of the Foundation.