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UNIMAS

Special focus on

Biotechnology

UNIVERSITI MALAYSIA SARAWAK
UNIMAS

FOREWORD

Biotechnology is a set of enabling techniques for bringing about specific man-made changes in deoxyribonucleic acid (DNA), or genetic material, in plants, animals and microbial systems, leading to useful products and technologies.

Concluding her overview article on the human genome project, recombinant DNA technologies, biochips, biosensors, gene therapy etc., Dr Anna Treohan of the Woodrow Wilson Biology Institute wrote in 1993 "whatever the future of these particular ventures, it seems molecular biology and biotechnology will be important sciences of the coming century". True enough – in fact the universality of such vision had prompted the inclusion of Chapter 16 in Agenda 21, which acknowledges the promising roles of biotechnology in inter alia better health care, enhanced food security, improved supplies of potable water, effective detoxification of hazardous wastes, efficient industrial development processes for transforming raw materials, as well as sustainable methods of afforestation and reforestation.

Today, bio-engineering of crops, for example, has grown leaps and bounds and become billion-dollar industry worldwide. Acknowledging these potentials, the first thrust of the National Missions (2006-2020), as recently announced by our Prime Minister, has specifically identified biotechnology as one of the imperatives in driving and sustaining the economic growth of this country.

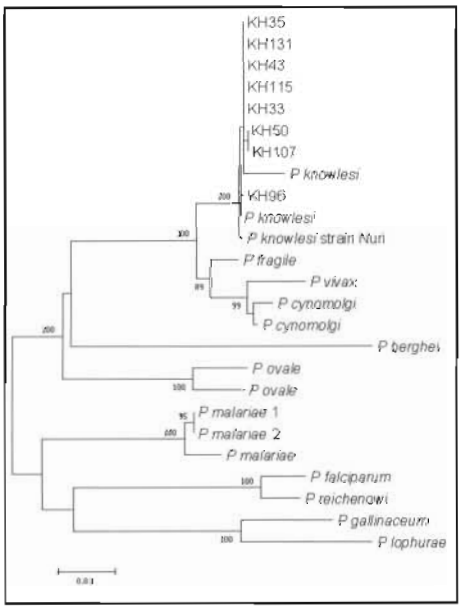
As for UNIMAS, our strength in biotechnology R&D is acknowledged by peers. Prominent biotechnological research at UNIMAS include epidemiological studies e.g. on dengue, JE, FMHD and malaria, molecular biogeographical studies (phylogenetics) and agriculture biotechnology, particularly of sago.

This volume of Research Update highlights some of the biotechnology based R&D at UNIMAS. It is hoped that some of the projects presented herein would invite interests for exchange of ideas, transfer of technology, partnership for further studies, or even ventures for joint product development and commercialization. Also for this particular issue, we acknowledge with thanks the editorial contribution by Assoc Prof Dr Edmund Sim.

Prof Dr Murtedza Mohamed
Deputy Vice Chancellor
(Research & Innovation)

Content

MOLECULAR EPIDEMIOLOGICAL, POPULATION GENETIC AND CLINICAL STUDIES ON *PLASMODIUM KNOWLESI* AND OTHER POTENTIALLY ZOONOTIC MALARIA PARASITES IN MALAYSIAN BORNEO

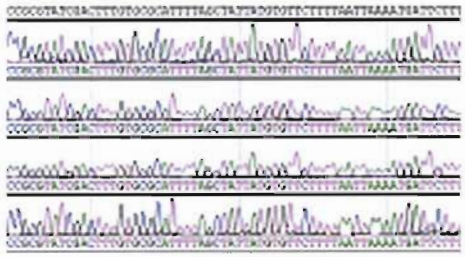


We have recently found that more than half of malaria patients in some hospitals in Sarawak, Malaysian Borneo, are infected with *Plasmodium knowlesi*. This malaria parasite, normally found in long-tailed and pig-tailed macaque monkeys in nature, is unique as it has a 24-hour erythrocytic cycle. One of the main aims of the project is to determine detailed clinical, haematological, biochemical and parasitological profiles of humans infected with *P. knowlesi*, and assess in vivo response to antimalarial treatment, as this information is currently lacking. A preliminary study has shown that 2 out of 25 monkeys from Sarawak were infected with P. knowlesi. The project also aims to examine the possibility of a parasite host switch and further characterise the epidemiology of *P. knowlesi* by sampling from human and non-human host populations and applying classical epidemiological, ecological and molecular approaches. The information obtained will aid the Sarawak State Health Department in the implementation of appropriate malaria prevention and control measures. Two new monkey malaria parasites have been discovered and partially characterised. We propose to fully characterise these parasites by molecular techniques, as there is a risk that these may also be transmitted to humans, and to identify and characterise additional new malaria parasites.

Researchers:
Balbir Singh, Janet Cox-Singh & David Conway.

Supporting grant:
Wellcome Trust, United Kingdom (RM 1.62 million)

MOLECULAR STUDIES ON HUMAN *PLASMODIUM KNOWLESI* INFECTIONS AND DEVELOPMENT OF IN VITRO MODELS OF *P. KNOWLESI* FOR INVASION, DRUG RESPONSE AND TRANSFECTION STUDIES



Human infections with *Plasmodium knowlesi*, a parasite normally found in nature in monkeys, are widespread in Sarawak and Sabah and also occur in Pahang. Human infections generally cause mild disease but recently there have been 4 deaths in Sarawak attributed to *P. knowlesi*. The fatal infections in Sarawak and infections in Pahang have been identified as *P. knowlesi* by PCR. One of the aims of the study is to confirm that these infections are due to *P. knowlesi* by detailed molecular characterization of the parasites and to phylogenetically compare parasites from different geographical areas. It is possible that *P. knowlesi* has been one of the primary causes of malaria-related deaths in Sarawak and in order to determine whether previous fatal malaria cases were due to P. knowlesi, archival blood films will be examined with the nested PCR malaria detection assay and parasites will be characterized by molecular methods. It is essential to provide in vitro models for studies on invasion, drug response and transfection but currently only *P. knowlesi* isolates cultured in rhesus monkey erythrocytes are available. Another aim of this study is to adapt new strains of *P. knowlesi* to long-term in vitro culture in human red blood cells.

Researchers:
Balbir Singh, Janet Cox-Singh, Hasan Abdul Rahman, Jamail Muhi & Angela Siner

Supporting grant:
UNIMAS Fundamental TopDown Grant (RM 120,000)

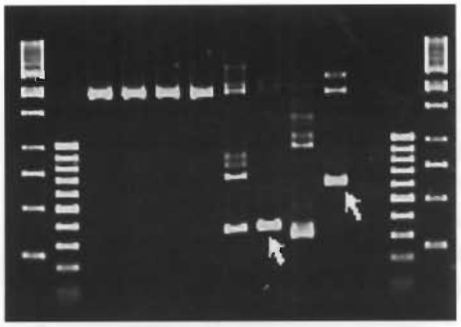
REGULATION OF G PROTEIN-COUPLED RECEPTORS BY THE STRESS INDUCIBLE 70 KDA HEAT SHOCK PROTEIN

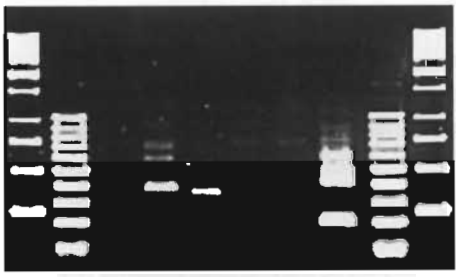
The family of G protein-coupled receptors (GPCR) are found across species from plants to humans, and constitutes the largest class of cell surface molecules in the mammalian genome. These receptors activate intracellular signal transducing proteins called G proteins. They are active in just about every organ system, and are potential therapeutic targets in areas including cancer, cardiac dysfunction, diabetes, central nervous system disorders, obesity, inflammation and pain. This receptor family is among the most heavily investigated drug targets in the pharmaceutical industry as it is the molecular target of more than half of currently approved prescription drugs. An increasing number of intracellular binding partners of this receptor family have been identified. There is evidence that heat shock proteins may interact and regulate cell surface receptors. One of the best studied, hsp70, is highly expressed after cell stress and helps to promote cell survival. This project aims to study the functional relationship between G protein coupled receptors and hsp70. The approaches taken will range from functional proteomic analysis at the subcellular level, to GPCR signaling in mammalian cells, and will involve techniques in molecular biology, receptor pharmacology (radioligand binding) and signaling in cultured cells. This research will further expand our understanding of signaling networks involving these receptors, which may reveal new molecular targets for regulation of these receptors.

Researcher:
William Lim

Supporting grant:
IRPA-EAR (09 02 09 1018 EA001)

THE ROLE OF THE APOPTOTIC PROCESS IN LEUKEMOGENESIS



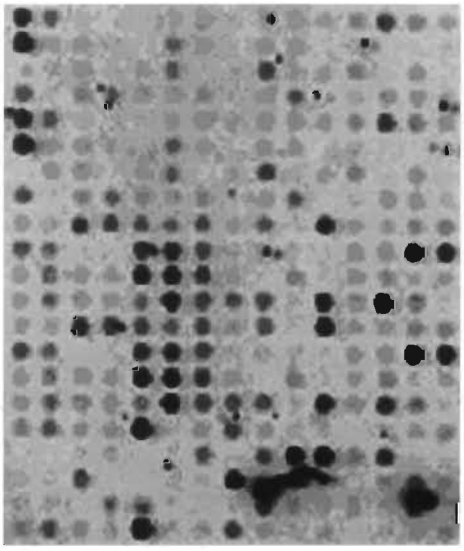


Chromosome translocation in leukaemia is a non-random event. Till date, many mechanisms have been proposed for the translocation process. More recently, the apoptotic nuclease (CAD) has been suggested to take part in the initial event of translocation, that is the chromosome breakage. This project focuses on the analysis of chromosome breaks induced by apoptosis. The genes of choice are the partner genes of the Mixed Lineage Luekemia (MLL) gene translocation. Reason being they are involved in de novo as well as in secondary leukaemia. When cells were induced to undergo apoptosis, the non-isotopic Southern Hybridisation technique detected a 600bp cleavage fragment in the AF-9 gene (the most common translocation partner of the MLL gene). To improve the sensitivity, a PCR-based method was employed. With the use of Inverse PCR, apoptosis-induced chromosome break within the MLL gene was reconfirmed. Two of the cleavage fragments were sequenced to identify the exact breakpoints. The IPCR product of the intact MLL gene was also sequenced. Upon optimization, the IPCR was used to analyse chromosome breaks within the AF-9 gene. A few breaks were detected and they fall within a region where patients' breakpoints were reported before. In conclusion, an IPCR method has successfully detected chromosome breaks within the MLL and the AF-9 genes in cells that were induced to undergo apoptosis. In view of the similar gene structure of the MLL and the AF-9 genes, similar mechanism involving the CAD could be applied in the translocation of the MLL and the AF-9 gene.

Researcher:
Sim Sai Peng

Supporting grant:
IRPA-EAR

MOLECULAR STUDIES ON DISEASE BIOMARKERS AND PATHOGENESIS OF CANCER: COLORECTAL CARCINOMA AS A MODEL

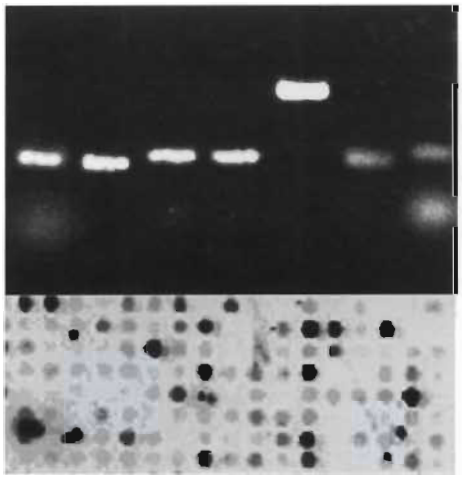


Colorectal carcinoma or cancer of the large intestine (colon cancer) has the incidence of 150 for every 100,000 persons in Malaysia. It is a major health concern as it now ranks second among the ten leading cause of cancers in this country. This cancer progress through a series of clinical and histopathological stages ranging from small benign tumours (adenomatous polyps) to malignant cancers (invasive carcinomas). To date, the molecular analysis of this cancer has revealed genetic alteration in genes of tumour suppressor, growth factor, cell cycle, and DNA repair functions. Despite this, there is still no information on the actual genetic mechanism(s) and biochemical pathways that pertains to the development and manifestation of colon cancer. Our study aims to use techniques in gene expression profiling of cancer tissues to understand the genetic mechanisms underlying tumour formation and progression in colon cancer. This involves the use of DNA microarray technology that allows the simultaneous and quantitative assay of thousands of expressed known human genes from cancer tissues and cells. Understanding the susceptibility genes of this disease and the pathways in which they exert their effects would be invaluable in the efforts of early detection strategies and therapy regimes.

Researchers:
Edmund Sim, Ivyna Bong, Pauline Balraj (IMR), Patricia Lim (MBDR), & Rahman Jamal (UKM)

Supporting grant:
NBD-MDBCC TopDown (06-05-01-003 BTK/ER/018)

CHROMOSOME FRAGILITY AND GENE EXPRESSION ANALYSIS OF LOCAL NASOPHARYNGEAL CARCINOMA (NPC) CASES

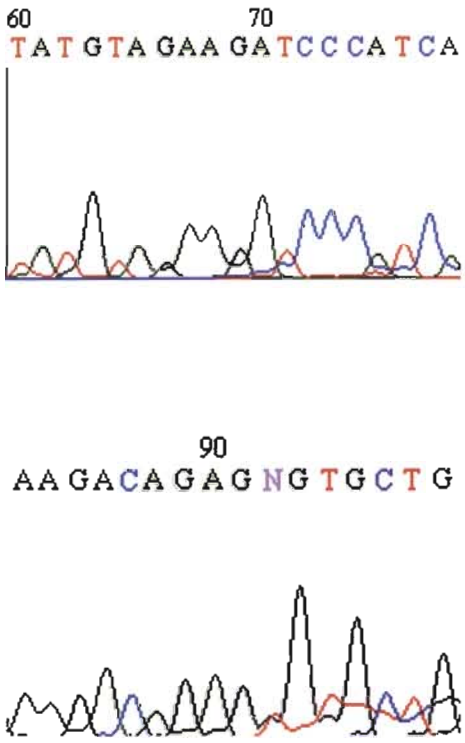


Nasopharyngeal carcinoma (NPC) represents epithelial cell cancer that generally arises from tumourigenic development of squamous cell at the lateral or posterosuperior walls of the nasopharynx. Globally, NPC has its highest incidence in South East Asia, and is more prevalent in population of Chinese heritage. The occurrence of NPC is multifactorial in origin and multigenic in mechanism. To date, the definitive susceptibility gene(s) of NPC has not been identified. Although a few candidates have surfaced recently, their roles as predisposition and progression-related factors have yet to be verified. In addition, although EBV association with NPC has been reported extensively, and that chromosomal addition/deletion have been widely detected in NPC cases, studies investigating the initial cause leading to the chromosomal anomalies, apoptosis and their association to EBV-related NPC are lacking. To solve the knowledge gap in NPC research, our studies focused on two aspects; (1) to delineate the susceptibility factors and genetic pathways of NPC carcinogenesis via gene mutation analysis and expression profile assays, and (2) to investigate the role of apoptotic event in chromosomal breakage in order to find the initial mechanism of chromosome deletion that leads to NPC. Combining the outcomes of mutation analysis, gene expression study, and mechanism of chromosomal anomalies, more insight and information can be contributed to the field of NPC research.

Researchers:
Edmund Sim, Sim Sai Peng, Alan Toh, Peter Yee, Joy Tan, & Tiong Thung Sing.

Supporting grant:
National TopDown IRPA-PR (08-06-02-09-1020 PR0054/05-02)

MOLECULAR STUDIES ON THE ROLE OF TUMOUR SUPPRESSOR GENES DURING TUMOURIGENESIS OF PROSTATE ADENOCARCINOMA USING *IN VIVO* GENE ACTIVITY ASSAY AND CELL LINE SYSTEMS AS STRATEGIES



Prostate adenocarcinoma (PAC) is a malignant tumour that arises from the glandular epithelium of the peripheral zone of the prostate. Globally, it has the highest incidence of any malignancy and is the second cause of cancer-related deaths in the male population of industrialized countries. Based on current available record in the Sarawak Cancer Registry and Statistics (1996 – SGH), PAC ranks 7th in the list of most common cancer afflicting male population of Sarawak. PAC is suspected to be on the increase, as amongst other causes, high fat diet has been implicated as a risk factor. To date the actual predisposition gene(s) of PAC remains unknown although numerous suspects have been suggested, such as the tumour suppressor genes of p53 and p63. Our research aims to unravel the in vivo (endogenous) gene activities behind the occurrence of PAC. The methods of cell line manipulation, genetic engineering, transgenic technology, and DNA microarray will be used for the purpose of this study. More interestingly, we will also aim to create and induce prostate-derived cell lines to form cancer-like clones for gene expression profiling analysis. Our study will enable us to gather important information on the gene activities that predispose, and affect carcinogenesis of PAC.

Researchers:
Edmund Sim, Su Ngouk Ngie, & Ong Teng Aik

Supporting grant:
IRPA-EAR (09-02-09-1012-EA001)

MOLECULAR ANALYSIS OF THE STARCH BIOSYNTHESIS PATHWAY IN SAGO PALM

The starch biosynthesis in sago pathway involves four major enzymes, namely ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), starch-branching enzyme (SBE) and starch-debranching enzyme (SDE). AGP forms ADP-glucose from glucose 1-phosphate. SS adds ADP-glucose to the elongation end of an a-(1-4)-linked glucose chain, whereas SDE cuts a(1-4)-links and rejoin them as a(1-6) branches that are subsequently trimmed by DBE to yield short chains for further synthetic extension. A study aimed at determining the genetic and biochemical mechanisms involved in starch biosynthesis in the sago palm has been initiated. The main objective is to isolate and characterize the genes that code for the four key enzymes mentioned above. The research work involves the application of molecular and biotechnological techniques including PCR, gene screening, cloning and DNA sequencing. To date a near-complete 1755-base pair cDNA sequence of granule-bound SS (GBSS) and partial cDNA sequences specific for soluble SS (824-bp), AGPase (400-bp), SBE (1200-bp) and SDE (480-bp) enzymes have been obtained. The near complete GBSS cDNA codes for 505 amino acids and shares high homology with GBSS genes from several crop plants including rice, maize and wheat. Sequence comparison showed that it is truncated at the 5'-end. PCR-screening of genomic library using specific primers derived from this sequence led to the identification of a 3,865-bp genomic locus for this gene. The structural organization and complete nucleotide sequence of this locus has been determined. It contains 12 exons and 11 introns but the significance of this structure in its expression and regulation is still unknown. A complete analysis of the structure, organization and regulation of the above genes would lead to a full understanding of the starch biosynthesis process in the sago plant. This in turn would enable genetic modifications to be carried out to produce new sago varieties with higher yields or those that can synthesize modified forms of starch for various applications.

Researchers:
Mohd Azib Salleh, Mohd Hasnain Hussain, Hairul Azman, Jennifer Lau Siew Kee, Bala ak Jamel and Hwang Siaw San.

Supporting grants:
MALAYSIA TORAY SCIENCE FOUNDATION Research Grant (2001-2003); UNIMAS Fundamental Research Grants Nos. 234/2000(25) & 01(113)487/2004(224).

STARCH BIOSYNTHESIS PATHWAY: SCREENING AND CHARACTERIZATION OF GENE ENCODING ISOFORMS OF STARCH SYNTHASE ENZYMES IN SAGO PALM (*METROXYLON SAGU* ROTTB.)

This is one of the projects in sago biotechnology in Unimas.The aim of this specific study is to fully identify various isoforms of starch synthase enzyme, one of the core enzymes involved in starch biosynthesis, that is present in sago,. This project will attempt to characterise them based on the type of isoform in their respective group and further find out the availability of other isoforms in each group. This will help towards complete elucidation of sago starch biosynthesis pathway. Previous research on sago has indicated the presence of GBSS and soluble starch synthase. Nevertheless, it is still unclear regarding the numbers of isoforms for these respective enzymes (GBSS and soluble starch synthase) that are present in sago plant. Based on the information of starch synthase isoforms from other plant species, it is known that there are various isoforms for each group. However, the number and type of different isoforms for each group could only be ascertained once this study is completed. The objectives of this study are to determine and establish numbers and types of different isoforms of starch synthase enzymes, and to obtain and characterize the full length of open reading frames (ORF) for all isoforms of starch synthase genes.

Researcher:
Mohd. Hasnain Mohd Hussain

Supporting grant:
Unimas Fundamental Research Grant

DEVELOPMENT OF A DEFINED TISSUE CULTURE MEDIUM FOR SOMATIC EMBRYOGENESIS AND PLANT REGENERATION IN SAGO PALM (*METROXYLON SAGU* ROTTB.).

In plant biotechnology, a successful technique in tissue culture is important to establish new lines of plants varieties after the manipulation of genes of interest in the plant. The main part of a successful methodological for tissue culture lies in the setting up of optimal medium for culturing the explants. Attempts have been made to micropropagate M. sagu using tissue culture technique but the success rate for the regeneration of explants into viable plant was very low compared to other established plants such as tobacco and banana. This rate is not suitable for use in biotechnology as the number of repetitions that is required to obtain the intended clone would be high. In view of this, there is a need to establish optimal and 'in-house' tissue culture protocols in order to have strong research in sago, especially for use in molecular biology work such as cloning of genes with new characteristics into the plant. The new approach in formulating tissue culture media is based on the chemical content of seed for the specific plants. Basically, seeds store minerals and food reserves essential for the seedling until the seedling becomes self-sufficient. It has shown that the composition of minerals and organic substances in proportions similar to those found in the seed composition has provided an optimum tissue culture medium for micropropagation of hazelnut plant. Therefore, if similar approach is taken it is highly likely that medium specific for tissue culture of M. sagu could be developed.

Researcher:
Mohd. Hasnain Mohd. Hussain

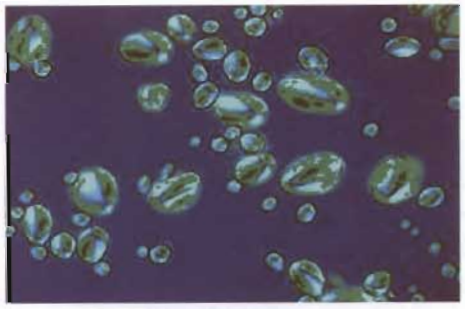
Supporting grant:
Unimas Fundamental Research Grant

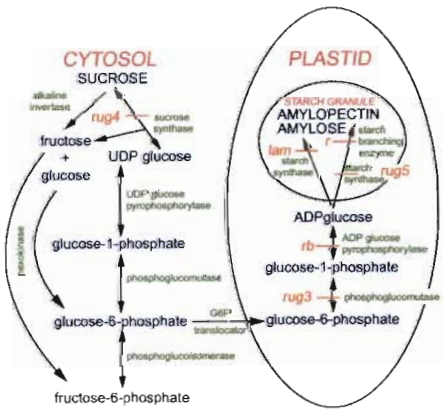
ISOLATION AND CHARACTERISATION OF *LEAFY*- AND *CONSTANS*-LIKE GENES IN SAGO PALM

Sago palm is a plant in the genus Metroxylon. The name metroxylon is derived from Greek words; 'metra' means "pith" and 'xylon' means "xylem". Sago palm is a "once-flowering" plant where during vegetative stage carbohydrates are accumulated in the plants. Following the final reproductive stage where the plant's food reserves are expended for the production of inflorescence, flowers and then fruits and the palm dies. Sago palm takes a long time to mature and subsequently produce flower that can take between 10-14 years. Traditionally, planters have used the flowering stage as an indicator for logging to occur. This is because the starch content is increased and modified just before the flowering stage. The characterisation of genes and factors that are involved in the initial phase of the flowering process could give an indication on how the factors interact. Researches in the plant model, *Arabidopsis thaliana*, have shown that the flowering process involves the transformation from a vegetative form (apical meristem) to a floral form (floral meristem). The transformation can take place through three pathways; gibberellin, autonomous and photoperiod pathways. CONSTANS (CO) is adaptive gene that is involved in the photoperiod pathway and involved in the timing of flowering. CO acts as a transcriptional activator and is upregulated during initiation of flowering stage, either directly or indirectly, activating the floral meristem identity gene LEAFY. LEAFY is a gene that is directly involved in the transcription activation of the Floral Homeotic Gene Group that determines the development of inflorescence. Preliminary works have been initiated in UNIMAS that involved PCR screening of various degenerate primers based on the LEAFY and LEAFY-like genes. We have managed to generate a reproducible fragment specific to LEAFY genes in sago palm. Further funding for the project is being sought. The overall purpose of this research is to look if there is any interaction between the onset of flowering and the starch accumulation in sago palm.

Researchers:
Hairul Azman Roslan, & Mohd Hasnain Mohd Hussain

ISOLATION OF THE LARGE SUB-UNIT OF THE ADP-GLUCOSE PYROPHOSPHORYLASE GENE



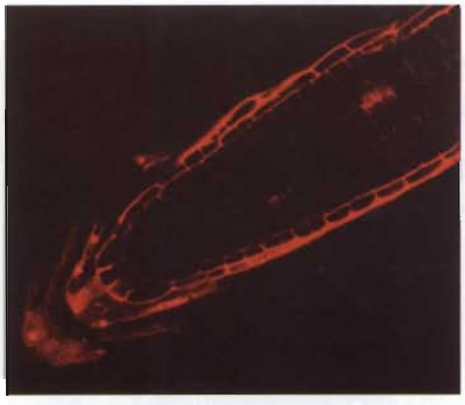


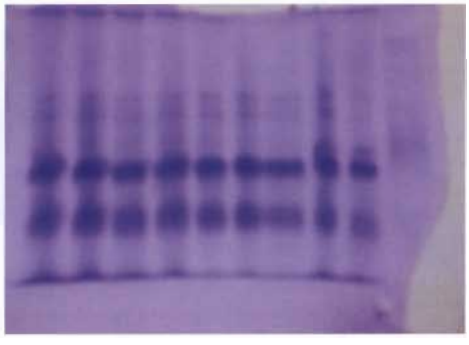
The sago palm (*Metroxylon sagu*) can be found in abundance in the freshwater swamps of Southeast Asia. In the state of Sarawak, this palm is grown as a starch crop for its ability to produce between two to five tons of dry starch per hectare in the wild and ten to twenty-five tons per hectare in cultivated areas. Sago is popularly grown as a commercial crop on smallholdings in Sarawak, exporting over 50 000 tons of air-dried flour a year. Apart from starch being used as food, sago starch can be used as adhesives in paper, textiles and plywood, or as stabilizers in pharmaceuticals. New uses and ideas for sago include in biodegradable plastics, biopolymer plastics, high fructose syrup and ethanol. Starch consist of glucose polymers and abundant in the plant kingdom. The site of accumulation varies such as in the plastids and also in the storage organs. Starch is made of 2 polysaccharide namely the amylose and amylopectin. Amylose is a simple polysaccharide, linear in form. Meanwhile amylopectin is highly complex consisting of branched polysaccharides. Various enzymes are involved in the biosynthesis of starch such as ADP-glucose pyrophosphorylase (AGPase), starch synthase, starch branching and debranching enzymes. AGPase is one of the key enzymes in the biosynthesis of starch in higher plants. In plants, this enzyme is heterotetrameric composing of two small and two large subunits. The AGPase enzyme is the first committed step that catalyses the formation of ADP-glucose from glucose-1-phosphate in the starch biosynthetic pathway. Similarly in sago, this enzyme plays an important role in the flux of starch production. It has been suggested that there are at least three types of AGPase polypeptides: one small subunit that is found in both the photosynthetic and non-photosynthetic tissues; and two large subunits that is found exclusively in each non-photosynthetic and photosynthetic tissues. In this project, the initiating genes involved in starch synthesis, the AGPase, will be isolated. Currently the mRNA from leaves and pith cells are being extracted. The cDNA will then be synthesised by the use of polymerase chain reaction (PCR). The genomic and cDNA sequences will be determined to further understand the biosynthesis of starch in sago palm.

Researchers:
Hairul Azman Roslan, Mohd Azib Salleh,
& Patricia Chong Ing Pei

Supporting grant:
UNIMAS Fundamental Research Grant 01(84)/424/2004(161)

IDENTIFICATION AND CHARACTERISATION OF WATERLOG TOLERANT GENES IN SAGO PALM





Flooding is a worldwide phenomenon in wetland and river areas. Excess water in the soil could produce anoxic soil condition. This will in turn produce either a complete or partial submergence of roots. Subsequently the roots suffer hypoxia or anoxia. Most plants are obligate aerobes and needs constant supply of oxygen for function. Disruption of this supply could result in reduced phloem transport and depletion of carbohydrates in roots, decrease in stomatal aperture that regulates photosynthesis, wilting of leaves, reduced mitochondria integrity and other anaerobic responses. Plants are resilient and have evolved to overcome the flooding phenomenon. Various adaptation, physically and physiologically, are activated. Proteins are produced in response to the low- or no- oxygen conditions to the roots. The physiological ability of plants to convert its metabolism into a low- or no- oxygen condition enables it to tolerate waterlogging. The main ability of tolerance is to switch to an ethanolic fermentation in the roots. In an aerobic condition, sugars are broken down to form pyruvate and used in the tricarboxylic acid cycle (TCA) to produce 4 ATP, 10 NADH and 2 FADH2. The product is then used for respiration to produce 34 ATP, 10 NAD+ and 2 FAD with each oxygen molecule. Sago palms in Sarawak can be found on mainly swampy/waterlogged areas. These plants seemed to be able and have evolved a system in which to overcome the anoxic/hypoxic conditions of the roots. Previous researches have shown that plants are able to adapt the low-/no- oxygen conditions by switching its metabolism of pyruvate to ethanolic pathways. During alcoholic fermentation, Adh seemed to be one of the main enzymes produced with a high concentration and production of Adh corresponds to ethanol production in flood-tolerant and intolerant plants. Currently we have managed to isolate proteins that are present in waterlogged and non-waterlogged roots, leaves and pith cells. Characterisation of Adh isozyme was undertaken using polyacrylamide gel electrophoresis. This project will identify and characterise the number of Adh loci that is present in sago palm and subsequently isolate the genes and factors regulating Adh expression.

Researchers:
Hairul Azman Roslan, & Yasotha Sundaraj

Supporting grant:
UNIMAS Fundamental Research Grant 01(84)/424/2004(161)

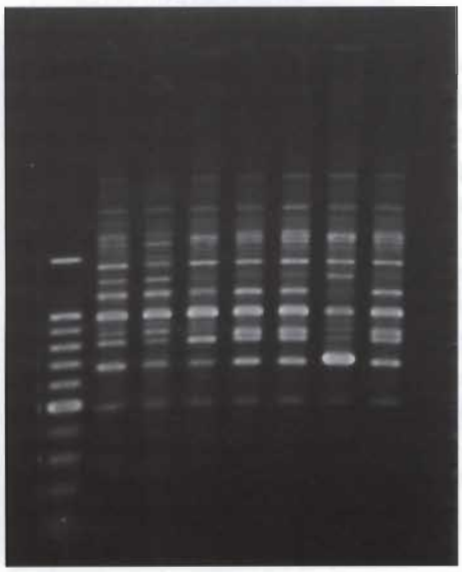
LARGE-SCALE PRODUCTION AND PURIFICATION OF L-LACTIC ACID FROM SAGO STARCH

The emergence of new markets for application of lactic acid such as biodegradable thermoplastics together with the more traditional industries such as tanning of leather, pharmaceuticals, food and beverages and cosmetics (skin care, toiletries, hair care products) have created an impetus for the lactate industries to grow into a larger scale. Large-scale fermentation processes has always been plagued by expensive substrates which poses a serious hindrance in promoting lactic acid industries. It is therefore imperative for such processes to utilize renewable and alternative sources to reduce production costs. Previous research have shown the possibilities of using natural rubber serum powder (NRSP) or natural rubber serum concentrate (NRSC) as an alternative to yeast extract. Locally, sago starch can be used as the cheap substrate for lactate production. Sago palm, which grows in swamp areas inhabitable for most other crops is also the world's highest starch producer, at 25t/ha/year. Sago starch can be developed either as a substrate for production of glucose or this can be fermented to generate L-lactic acid. At 98% recovery, hydrolysis of sago starch into glucose is a sensible alternative since glucose (US$0.34/kg) fetch a higher price to sago starch (US$0.20/kg), and the conversion process cost only about US$10/t. Fermentation studies have been carried out to achieve maximum lactate production by *Lactococcus lactis* IO-1 on enzymatically hydrolyzed sago starch producing 0.96g/g lactate, a 96% conversion of glucose into lactic acid. At US$60/litre, conversion to lactic acid will certainly add further value to sago starch. In our laboratory, lactic acid is purified using powdered activated charcoal in glass columns layered with glass wool. Lactate recovery is over 98% with excellent removal of color (99%), glucose (100%) and protein (98%). Conclusively, we have shown the economic feasibility of converting sago starch to glucose and subsequently to lactic acid at almost 1:1 ratio as the new value added product from the sago industry of Sarawak.

Researchers:
Kopli Bujang (Unimas), Ayaaki Ishizaki (New Century Fermentation Research), Yoshiyuki Nomura (Sojo University, Kumamoto, Japan); J.M. Lopez-Real (Imperial College, University of London)

Supporting grant:
New Energy and Industrial Technology Development Organization (NEDO) L18403 FO7; IRPA 09 02 09 1024 EA001; IRPA 08-02-09-1023 EA001

GENETIC ASSESSMENT OF PEPPER GERMPLASM USING DNA MARKERS FOR IMPROVEMENT AND VARIETY IDENTIFICATION



Pepper (*Piper nigrum* L.) is one of the most important commodities to Sarawak. It ranks second in the State's export earnings after oil palm. Sarawak is the main pepper producing state in Malaysia, contributing about 98% of the country's total production. The main areas are concentrated in the central and south-western parts of the State; i.e. in the Sarikei, Kuching, Samarahan and Sri Aman Divisions. Currently, there are about 45,000 farm families involved in pepper cultivation. The pepper variety identification in most of the breeding programs is mainly based on the morphological characters or markers such as leaf area, leaf shape, spike length, number of spikes per lateral branch and etc. In 1995, the descriptor lists based on morphological characters have been developed by IPGRI and used to characterize some of the pepper varieties in India, Indonesia and Malaysia. However, these morphological markers are largely subjected to environmental conditions and human judgement. For instance, the variety Kuching (from Sarawak) is known as 'Singapura' in Sri Lanka and Brazil. Nevertheless, these limitations can be eliminated by the use of DNA-based marker technologies. They are developmentally stable and not influenced by the environmental factors. Therefore, this project is aimed at determining the genetic relatedness of various accessions of pepper and other Piper species maintained at Agricultural Research Centre (ARC), Semongok using DNA based-markers, e.g. RAPDs, DAMD-PCR and SCARs, and also to establish the DNA based-marker systems specific for pepper hybrid identification. By developing the DNA-based marker systems that detect differences in DNA sequences between varieties, highly specific marker profiles can be developed for each variety and used for marker-assisted selection (MAS) and variety identification, which will provide protection of breeders' right. To date, these marker systems have been widely used to produce species-specific molecular markers for identification, validation of plant materials, and marker-assisted selection in wheat, rice, and other commercial crop species.

Researchers:
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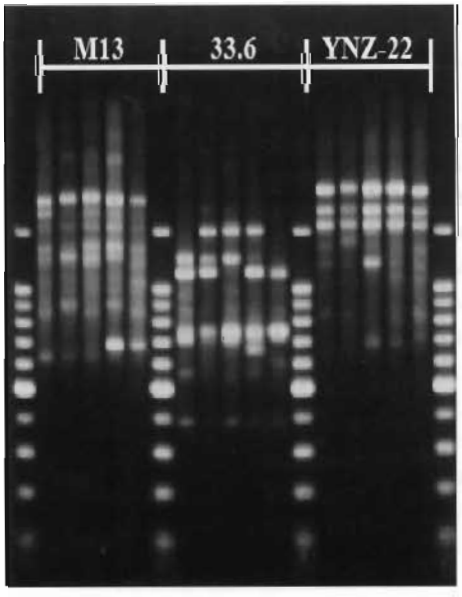
Supporting grant:
UNIMAS Short-term Research Grant: 01(79)/410/2003(147)

MOLECULAR TECHNIQUES FOR BELIAN CONSERVATION



Belian or its popular name, Borneo Ironwood, is the most famous and well-known durable hardwood timber tree of Borneo, which comes from the family Lauraceae. There are two species of Belian, i.e. Eusideroxylon zwageri and *Potoxylon malagangai*. Both species have a very close similarity in their vegetative characters except the wood structure. *P. malagangai* has lighter colour of wood and lower durability compared to *E. zwageri*. Thus, it is not easy to distinguish the two species based on their vegetative characters when in the field. To date, little genetic information is available on these valuable timber species, and therefore the belian must be studied thoroughly using molecular techniques to identify genetic variation within and among belian populations in order to conserve the rapidly declining belian populations in Sarawak. The characterization of genetic variation is central to the conservation of genetic diversity in natural or domesticated populations. Populations with little genetic variation are more vulnerable to the arrival of new pests or diseases, pollution, changes in climate and habitat destruction due to human activities or other catastrophic events. The inability to adapt to changing conditions greatly increases the risk of extinction. Moreover, the belian has been counted as one of the endangered species in Sarawak. The objectives of this study are to genotyping belian via PCR-based molecular marker techniques, and to develop species-specific genetic markers (sequence characterised amplified regions, SCARs) for identification of belian. The identification of individuals at species level constitutes one of the first basics in any effective conservation programme. Besides these, the genetic diversity of belian populations will also be determined using molecular marker techniques such as isoenzymes, Restriction Fragment Length Polymorphisms via PCR (PCR-RFLPs), Random Amplified Polymorphic DNAs (RAPDs), Directed Amplification of Minisatellite-region DNA (DAMD), Inter-Simple Sequence Repeats (ISSRs) and Simple Sequence Repeats (SSRs). The choice of molecular markers is largely dependent on the level of polymorphism to be detected and the genomic coverage of molecular marker.

Researchers:
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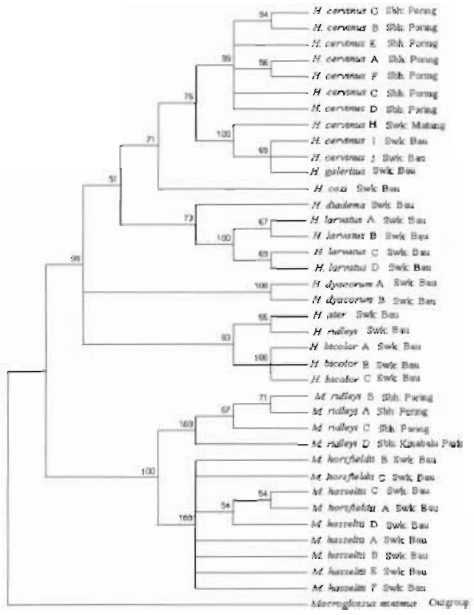
Supporting grant:
UNIMAS Short-term Research Grant

PHYLOGENETIC RELATIONSHIP AND STATUS OF *HIPPOSIDEROS* AND *MYOTIS* BASED ON MTDNA 16S RRNA SEQUENCES IN BORNEO



Insectivorous bats (suborder Microchiroptera) or microbats are also known as 'echolocation bat' because they develop the ability to use echolocation to navigate and to find food. Microbats are also well known as significant predator of nocturnal insect and act as natural biocontrol of pest. Despite their contribution and importance to the ecosystems, phylogenetic studies among the microbats species and their place in the mammalian tree of life have been until recently, poorly studied. Bornean insectivorous bats (suborder Microchiroptera) comprise seven families within which consist of 75 species. To date, classification of Bornean microchiroptera has been based on morphological characterization, and has not been established via molecular systematic . We have previously reported on an updated distribution and abundance of microchiroptera based on field survey performed in six forest regions in the Malaysian Borneo states of Sabah and Sarawak. Our reports revealed the widespread distribution of *Hipposideros dyacorum* and *Myotis ridleyi*. In this study, we demonstrated the successful utilization of 16S mitochondrial ribosomal ribonucleic acid (mt rRNA) in the delineation of phylogenetic status of the genus *Hipposideros* and *Myotis* found in Sabah and Sarawak. Our findings clarified and revalidated six taxonomic status of the genus *Hipposideros*. The previously ambiguous inclusion of *H. galeritus* into the *H. cervinus* taxon was re-demonstrated. Within the Myotis clade our data indicates close genetic relationship between *M. horsfieldii* and *M. hasseltii*, and an independent grouping of *M. ridleyi*. Although the specific inter-relationship among the genus studied remains partially resolved, our findings present novel molecular phylogenetic status of two Bornean microchiropteran taxa. This will represent important baseline reference for future studies on the precise phylogenetic relationship of Bornean insectivorous bats.

Researchers:
Imelda Vivian Paul, Edmund Sim & Mohd. Tajuddin Abdullah

Supporting grant:
UNIMAS Short-term Research grant

EVOLUTIONARY BIOLOGY, ECOLOGY AND PHYLOGENETIC RELATIONSHIPS OF FRUIT BATS USING DNA SEQUENCES AND MORPHOMETRIC ANALYSIS

Armed with the idea that *Cynopterus brachyotis* (Malaysian short-nosed fruit bat) is a cryptic species (i.e. it looks alike but has enough major differences), we started a research on this species in 1995. We hypothesized that since after the last ice age, about 10,000 years ago when Borneo Island was separated from the Asian Mainland due to the inundation and expansion of the South China Sea, the *Cynopterus brachyotis* populations in Borneo have evolved because there was no genetic exchange with those populations in the Asian Mainland. We used both the mitochondrial DNA cytochrome b gene and body measurements to detect any changes in the Cynopterus brachyotis populations from Borneo, Peninsular Malaysia and Thailand. Pooling the data into geographical regions failed to produce the results we expected. Instead, we found that by re-analysing the data and taking into account some ecological factors, we discovered there were significant differences in the *Cynopterus brachyotis* populations due to ecological habitats. The smaller *Cynopterus brachyotis* lives in the dense tropical rain forest while the larger *Cynopterus brachyotis* is found in the open areas. Thus, there is a new species yet to be described from Borneo due to its major differences in the ecology, morphological characters and genetics. Our recent findings are very important for biological science in three ways. Firstly, by using advanced DNA biotechnology and multivariate statistical tools, we were able to discover new species and create an effective inventory of the high biological diversity in Borneo's tropical rain forest. Secondly, any ecological study must be based on correctly identified species of animals so that the cryptic ones do not mask the actual results of the study leading to incorrect conclusions. Thirdly, our results could generate another evolutionary hypothesis that the small-bodied *Cynopterus brachyotis* evolved in order to adapt to greater agility and maneuverings in cluttered or dense vegetations of the forest, while the large-bodied species have evolved for fast and powerful flight to protect against nocturnal predators.

Researchers:
Mohd Tajuddin Abdullah, Yuzine Esa, Awang Ahmad Sallehin, Andy Kho Han Guan, Jayaraj Vijaya Kumaran, Siti Nurlydia Sazali, Fong Poi Har, Jeffrine Rovie Ryan, Besar Ketol, Faisal Ali Anwarali Khan & Ratnawati Hazali.

Supporting grant:
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MITOCHONDRIAL DNA PHYLOGEOGRAPHIC DIFFERENTIATION AMONG POPULATIONS OF WHITE-NEST SWIFTLET (*AERODRAMUS FUCIPHAGUS*) IN MALAYSIA

The edible bird-nest builder, white-nest swiftlet (Aerodramus fuciphagus), has received much attention in Malaysia over past few decades. In recent years, swiftlet farming has become a lucrative industry. The aims of this study are: (a) to elucidate the taxonomic problems within the species; and (b) to investigate the patterns and structure of the relationships among the populations of this species in Peninsular Malaysia, Sarawak and Sumatra. The populations included in this study were Middle Baram, Sibu, Sitiawan, Selangor, Kuantan, Endau-Rompin and Sumatra. Based on phylogenetic analyses of the 558 bp of the mitochondrial DNA cytochrome b and 462 bp of the control region, there were two major clades with a deep divergence among all the white-nest swiftlet populations studied. Clade II was closely related to the Aerodramus fuciphagus vestitus while Clade I was closely related to A. f. germani. The grouping of different clades was inconsistent with the geographical distribution. However, Clade II did not include individuals from the east coast of Peninsular Malaysia, while Clade I did not include individuals from Middle Baram. The possible Pleistocene refugia located between the the mainland Asia and the Borneo Island was suggested based on the distribution of the haplotypes. Further investigation by using longer DNA sequence and bigger sample size is needed to confirm whether the Clade I and Clade II could be classified into different subspecies. Both cytochrome b and control region have shown their potential in the phylogeographic study of the white-nest swiftlet.

Researchers:
Mustafa Abdul Rahman and Goh Wei Lim

Supporting grant:
Unimas Fundamental Research Grant: 01(121)/510/2005(09)

PATTERNS OF VARIATION IN THE MOUNTAIN BLACKEYE (*CHLOROCHARIS EMILIAE*) IN BORNEO

Mountain blackeye (*Chlorocharis emiliae*), an endemic species to Borneo, is commonly found above 1,600 m above sea level. The known populations of this montane resident include Mount Kinabalu and Mount Trus Madi in Sabah; Mount Mulu, Mount Murud, Tama Abo Range and Pueh Range in Sarawak; Maga mountains between Sabah and Sarawak border; and Mount Nyiut, in Kalimantan, Indonesia. This species is divided into four sub-species; *C. e. emiliae, C. e. trinitiae, C. e. fusciceps* and *C. e. moultoni*. The aim of this study was to investigate the patterns of variation among isolated populations of the mountain blackeye in Borneo by analyzing selected morphological characters and molecular data. The numeric analytical results of morphological characters between the mountain blackeyes populations in Borneo revealed that, the bill length trait showed highly significant character. Results from the molecular analysis showed that Mount Mulu and Mount Murud populations are genetically close to each other. The results of this study support the taxanomic classification that the two populations belong to the same sub-species, *C. e. moultoni*. The sub-species emiliae in Mount Kinabalu population has substantially diverged from the sub-species moultoni of the Mount Mulu and Mount Murud populations. In conclusion, the study has demonstrated that Mount Kinabalu population had probably been isolated from Mount Mulu and Mount Murud populations for a longer period. This study provides a platform for future research on evolutionary theory within biogeographically distributed species in the Southeast Asian region.

Researchers:
Mustafa Abdul Rahman & Dency Flenny Augustine Gawin

Supporting grant:
Unimas Fundamental Research Grant: 248/2001(07)

PATTERNS OF GENETIC VARIATIONS IN BIRDS (FAMILY: NECTARINIIDAE) IN SOUTHEAST ASIA

The theory of plate tectonics has revolutionised the understanding of the geological and biogeographic processes in the Southeast Asian region. These concepts of continental movements which were developed about 30 years have been used with much success to reassemble in general terms the palaeogeographical history of parts of Southeast Asia. Vicariant events associated with basin formation and collision events between formerly separate continental and microcontinental fragments offer potential explanations for patterns of differentiation among regional biotas. Using Mitochondrial DNA Atpase6 region of the little spiderhunter (*Arachnothera longirostra*), the results revealed that the rate of sequence divergence between little spiderhunter subspecies is concordant with the historical land connections and separations during glaciation periods. The sequence analysis revealed that subspecies *A. I. flammifera* (Mindanao) has diverged from the other two subspecies, *A. I. longirostra* (Peninsular Malaysia and Borneo) and A. I. dilutor (Palawan) approximate to that of the other species (*A. crassirostris and A. affinis*). The rate of divergence among the subspecies of the little spiderhunter followed that of the connections and separations of land masses in this region during glaciation.

Researcher:
Mustafa Abdul Rahman

Supporting grant:
Unimas Fundamental Research Grant: 230/2000(21)

MOLECULAR PHYLOGENETIC ANALYSIS OF THE WHITE-CROWNED FORKTAIL *ENICURUS LESCHENAULTI* IN BORNEO

Comparison of 1017 nucleotides of mitochondrial ND2 and ND3 DNA sequences of 26 individuals of White-crowned Forktail (*Enicurus leschenaulti*) from S.E. Asia revealed multiple evolutionary lineages within Borneo. Montane birds were genetically homogeneous across localities, but diverged by more than 4.5% from all lowland individuals. Lowland birds formed two distinct clades, one comprised individuals from northern Borneo and the other included individuals from western Borneo, as well as Sumatra and Peninsular Malaysia. Individuals examined from Java and mainland Asia fell outside these clades and their exact relationships were not resolved. These findings have biogeographic, taxonomic, and conservation implications. They indicate another example of montane and north Bornean endemicity, support the separation of the montane and lowland species, and define areas of conservation interest.

Researchers:
Robert G. Moyle, Menno Schilthuizen, Mustafa Abdul Rahman & Frederick H. Sheldon

Supporting Grant:
Unimas Fundamental Research Grant: 248/2001(07)

A MOLECULAR PHYLOGENY OF THE PENINSULAR MALAYSIA FROG GENUS *RANA* (ANURA: RANIDAE) AS INFERRED BY PARTIAL 16S RIBOSOMAL MITOCHONDRIAL DNA

Members of genus *Rana* are very prominent and widespread feature of the Malaysian frog fauna. The relationships among these taxa are not well documented despite having great potential in providing information on historical biogeography. This study investigated phylogenetic relationship within genus *Rana* from protected areas of Peninsular Malaysia, Tasik Chini, Pahang, Kuala Koh, Kelantan and Temenggor, Perak. Since the genus *Rana* is widely distributed in the Oriental regions and the species are not likely to be isolated from other parts of Malaysia, tissue samples from Malaysian Borneo (Sarawak) were also used in this study. A 600bp fragment of the 16S rRNA gene was used to infer a molecular phylogeny. The sequences show high levels of variable sites (332 sites), parsimony informative sites (315) and amino acid replacements (96 sites) indicating that the gene is a good marker to infer a phylogeny of species within Malaysian Rana. We recognize five species group within the *Rana*; the *raniceps* group, the *erythraea* group; the *signata* group, the *luctuosa* group, the *baramica* group and the *hosii* group. Our results show that *nicobariensis* falls into the *erythraea* group; species that is associated with human activities. This is important, as it will not support the current placement of *nicobariensis* to other genus of *Ferjervarya*. Our study provides baseline information for future studies of phylogenies based on other techniques such as behavioral characters (advertisement call) or morphological characteristics.

Researchers:
Ramlah Zainudin, Mustafa Abdul Rahman, Norhayati Ahmad, Badrul Munir Mohd Zain & Shukor Mohd Nor

Supporting grant:
Unimas Fundamental Research Grant: 01(124)513(05)(12)

THE IMPACT OF INTRODUCED SPECIES (NON-NATIVE & EXOTIC) ON THE GENETIC DIVERSITY OF NATIVE FRESHWATER FISHES IN MALAYSIA

This study examines the impact of introduced freshwater fish species (non-native and exotic) on several native freshwater fishes in Malaysia using ecological and molecular genetics approaches. Native fish species have greatly been reduced in numbers throughout the world, or even destroyed/extinct, partly as a result of non-native and/or exotic species been introduced into their native habitats. Introduced species, are seen as competing or preying on native species or destroying their habitats. Introduced species (or subspecies), however, can generate another kind of extinction, a genetic extinction by hybridization and introgression with native species. Many exotic species imported or brought has dominated the fish culture industry in our country. Some of these species have been released into natural habitat such as rivers and lakes or into man-made paddy fields, mining pools or dams and, unfortunately, we don't have any information on their effects on our native freshwater fauna. Some of the cultured fish may escaped or accidentally been released into the natural habitat as a result of flooded during monsoon seasons. In addition, habitat modification resulted from human disturbance (deforestation, construction of hydroelectric dams etc) can also break down reproductive isolation between native species, with subsequent mixing of gene pools and potential loss of genotypically distinct populations. This study aim to determine the impact of introduced species in Malaysian freshwater systems on the ecology, genetic diversity and integrity of several native freshwater fishes. The project output will provide information for conservation management plan to protect Malaysian freshwater fauna from further devastation through fish introduction.

Researchers:
Yuzine Esa & Khairul Adha A. Rahim

Supporting grant:
ASEAN, Regional Centre for Biodiversity Conservation (ARCBC) grant (RE-MYS-002)

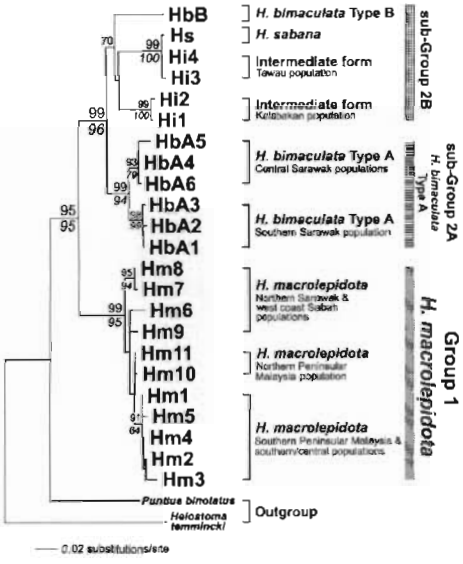
GENETIC ANALYSIS OF THE IMPACT OF INTRODUCED SPECIES (NON-NATIVE AND EXOTIC) ON NATIVE FRESHWATER FISHES IN MALAYSIA

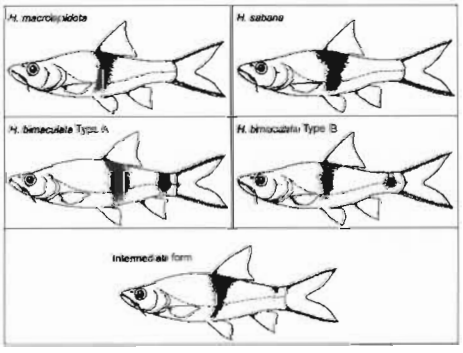
The project applied molecular genetics techniques in conjunction with ecological studies to examine the impact of several introduced freshwater fishes such as *Oreochromis niloticus* (Nile tilapia), *Barbonymus gonionotus* (Lampam jawa), and *Helostoma temminckii* (Tebakang or Biawan) on the genetic variability and ecology of native freshwater species. Through molecular approaches (sequencing of mitochondrial genes), the project investigated the systematic relationship (hence taxonomic status) between closely related introduced (in most case non-native) species and native/indigenous counterpart. Subsequently, population structure analyses were done to determine and compare the level of genetic diversity between introduced versus native species. The project also identified a few genetic markers useful for species discrimination between introduced and native species. The project results showed that introduced species exhibited comparables (sometimes higher) genetic diversity compared with native species, although they had recently been introduced in a particular area. Ecological studies showed that introduced species shared macro habitat and diet with native species (competition for foods and breeding grounds). No indication of hybridization (direct genetic impact) was observed between introduced and native species occurred in sympatry (e.g. between *Barbonymus schwanenfeldii* and *Barbonymus gonionotus*). Overall, all introduced species studied in the project have adapted well to the local environment and even dominated the particular habitat where the have been introduced (i.e. *Helostoma temminckii* in blackwater areas in Sarawak). The current ecological (food habit and macro habitat studies) and molecular (genetic diversity) analysis both pointed towards the potential of the introduced species to create negative impact (indirect genetic impact) on the viability of native/indigenous species. Although the genetic impact was not categorized as a serious threat but long term impact must be avoided by implementing appropriate management plan for both introduced as well as native species.

Researchers:
Yuzine Esa & Khairul Adha A. Rahim

Supporting grant:
ARCBC grant (RE-MYS-002)

PHYLOGENETIC ANALYSIS OF FRESHWATER FISHES OF THE GENUS HAMPALA (CYPRINIDAE) IN BORNEO





Fish of the genus *Hampala* (Cyprinidae) is an interesting candidate for phylogeographic study of Borneo Island. The taxonomy and systematic status of species within the genus Hampala is still problematic with current classification rely solely on classical method such as morphological and behavioural characters. Indeed, *Hampala* shows much geographic variation in coloration and fin ray counts. Morphological study has identified two species in Borneo; the common *Hampala macrolepidota*; which can be found throughout SouthEast Asia and a Bornean endemic *Hampala bimaculata*. Interestingly in North Borneo, Inger and Chin (1962) characterize and recognized three subspecies based on morphology; *H. macrolepidota bimaculata* in the west-coast region, *H. macrolepidota sabana* in the Labuk-Segama region, with an intermediate form (undescribed subspecies) in the Tawau region. Phylogenetic analysis revealed the reciprocally monophyletic status of *Hampala macrolepidota* from the other *Hampala* forms, thus strongly supporting its status as a distinct species. Phylogenetic analysis also discovered the existence of two *Hampala bimaculata* lineages endemic to Borneo: (1) a newly identified species from southern and central part of Sarawak assigned as *Hampala bimaculata* Type A and (2) the previously described *Hampala bimaculata* from northern Sarawak and west coast of Sabah assigned as *Hampala bimaculata* Type B. However, the status of Hampala sabana and the intermediate form could not be elucidated yet. Our results suggest that the intermediate form from the Tawau population is actually a subpopulation of *Hampala sabana* while the highly divergent intermediate form from Kalabakan could represent a cryptic species. Our results also suggest that the speciation of all *Hampala* forms could have occurred as early as the Pliocene period. The sharing of *H. macrolepidota* haplotypes in the southern Peninsular Malaysia and the southern/central Sarawak samples (Hm1 and Hm2) reflected the recent disconnection of the two regions, during the last Pleistocene periods. Overall, the partial sequencing of the cytochrome b mitochondrial DNA region was useful for resolving the phylogenetic relationships among *Hampala* fishes in Malaysia.

Researchers:
Yuzine Esa & Jeffrine Rovie Japning

Supporting Grant:
Unimas Short Term Grant 245/2001(4)

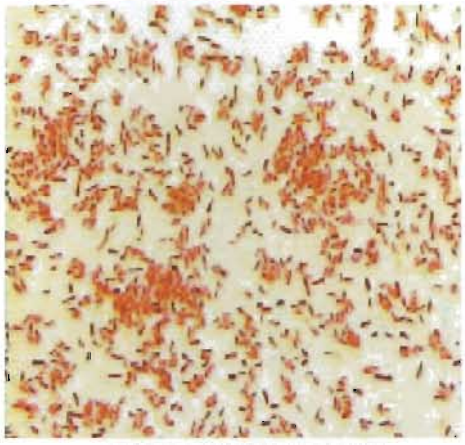
GENETIC DIVERSITY AND IDENTIFICATION OF MOLECULAR MARKERS IN THE ENDANGERED *TOR* FISH (CYPRINIDAE) IN SARAWAK

The genus *Tor* (Gray) belongs to the family Cyprinidae (subfamily Cyprininae). There are currently three described species in the genus based on non-genetic classification; *Tor tambroides* (Bleeker), *Tor douronensis* (Valenciennes) and *Tor tambra* (Valenciennes). However, morphological identification of Tor are difficult and sometimes unreliable, due to their high variations in coloration and local names, thus making molecular approaches very important for rapid genetic identification of species in the genus. Environmental disasters (i.e. river pollutions, deforestation, watershed erosion etc) had led to the rapid destruction of their natural habitat. In addition, overfishing of the fishes had greatly reduced their population size. Their distributions are now limited to the upper streams and protected areas of Peninsular Malaysia and Borneo. Thus, realizing the importance of the genus as food and recreational fishes, and given their limited distributions, a comprehensive study on their systematic and population genetic structure, are highly required in order to determine their genetic relationships and levels of genetic variations in all existing species of the genus in Malaysia. Phylogenetic study will be able to assign the genus into conservation unit: Management Unit (MU) for genetically distinct populations within the same species, and Evolutionary Significant Unit (ESU) for genetically distinct/ reciprocally monophyletic taxa. Hence, this study will shed light on the validity of current taxonomic and systematic classifications of the genus from the genetic viewpoint. Population genetics study will elucidate the contemporary genetic structure of the genus through studies of gene flow and levels of genetic variations. Overall, the study will aim to provide baseline genetic information useful for management authorities to develop conservation/management plan for protection of the genus. Our preliminary genetic analysis shows that three *Tor* froms following their local names (Semah from Sarawak, Belian from Sabah, and Kelah from Peninsular Malaysia) formed distinct monophyletic clusters between them. The Belian fish was genetically more closely related to the Semah (3.2-6.1% sequence divergence) compared to the genetic differences between Belian and Kelah fish (6.6-7.1%) and between Semah and Kelah (6.2-8.9%). More molecular results are underway to further elucidate the systematic relationships and population structure of *Tor* fishes in Malaysia.

Researchers:
Yuzine Esa & Khairul Adha A. Rahim

Supporting grant:
Unimas Short Term Grant 01(80)411/2003(148)

MOLECULAR DETECTION AND DIFFERENT OF *E.COLI* 0157:H7 FROM BEEF IN SARAWAK



Lactose-fermenting colonies of E. coli on MacConkey agar

Escherichia coli 0157 H7 : H7 has emerged as an important food-borne pathogens of animal origin and is associated to be the leading cause of haemorrhagic colitis in human. In Malaysia, E coli 0157 : H7 strains possessing important virulence traits were isolated from some retailed beef in West Malaysia. However, no study has been reported on occurrence of *E. coli* 0157:H7 in Sarawak. This project attempts to determine the existence of these bacteria and to investigate the variation between strains from beef marketed in Sarawak and Sabah.

Beef samples marketed in Sarawak and Sabah, East Malaysia, were investigated for the presence of *E. coli* O157. Identification was based on morphological and biochemical tests, followed by immunological test. On the basis of morphological and biochemical tests, 106 presumptive *E. coli* O157 isolates were isolated. However, none of the strains was confirmed as *E. coli* O157 or O157:H7 by multiplex PCR, although 14 strains exhibited weakly positive results for O157 antigen by latex agglutination test. One isolate harboured *slt-I* and *slt-II* genes while 5 isolates harboured $fliC_{h7}$ gene only. None of the strains possessed *rfbE* gene.

Multiplex PCR was shown to be a more reliable confirmation tool for E. coli O157 and O157:H7 compared to the latex agglutination test. Molecular subtyping by pulsed-field gel electrophoresis (PFGE) was performed on 51 confirmed *E. coli* isolates. Digestion of chromosomal DNA from these *E. coli* isolates with restriction endonuclease XbaI (5'- TCTAGA -3'), followed by PFGE, produced 45 restriction endonuclease digestion profiles (REDPs) of 10 to 18 bands. A large variety of PFGE patterns among non-STEC isolates were observed, demonstrating a high E. coli diversity in the beef marketed in East Malaysia. PFGE was shown to possess high discriminatory power in typing pathogenic and non-pathogenic *E. coli* strains, and useful in studying possible clonal relationship among strains.

Researchers:
Assoc. Prof. Dr Kasing Apun, Mr. Micky Vincent, Prof. Mohd. Azib Salleh and Dr Edmund Sim

Supporting grant:
Unimas Short-term grant (Project number : 243/2001(3))

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