NATIONAL SCIENCE FOUNDATION

Co - Organizer

Pathway to Knowledge Hub

2nd International Conference on Frontiers in Molecular Life Sciences

10th to 12th September 2014

Colombo, Sri Lanka

PROGRAMME AND ABSTRACT BOOK

IBMBB

Second International Conference on Frontiers in Molecular Life Sciences: Pathway to Knowledge Hub

Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB) of the University of Colombo
Sri Lanka

PROGRAMME AND ABSTRACT BOOK

CONTENTS

WELCOME MESSAGE

"Pathway to Knowledge Hub", the theme of the Second International Conference on Frontiers in Molecular Life Sciences, reflects the role that Molecular Life Sciences will play in creating knowledge based society in Sri Lanka. This conference organized to mark the 10th Anniversary of the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), University of Colombo will provide Sri Lankan students and scientists an opportunity to learn from World Authorities in the field. National Science Foundation has extended its fullest support as the co-organiser of this event.

We are extremely grateful to His Excellency President Mahinda Rajapakse for sparing his precious time to support us. The Key Note Speaker, Professor Ulf Pettersson, Senior Professor of Medical Genetics, University of Uppsala, Sweden, and the Plenary Speaker Prof. Iqbal Choudhary, Director, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan have long associations with Sri Lanka. Five symposia will also be held during the conference. Symposia lectures will be delivered by eminent researchers from India, Singapore, Sweden, United Kingdom and United States of America as well as from Sri Lanka. We are extremely grateful to these eminent speakers for sparing their valuable time, and even resources to be with us and share their knowledge and experience to make this conference a success.

Forty two research presentations will be made by students and scientists from Sri Lanka (from the IBMBB and from other Higher Educational Institutes), and from overseas. The conference will provide a forum for these researchers to interact with each other and with eminent resource persons to discuss their findings during the conference.

In addition to the main conference two pre-conference activities, a course on NMR Spectroscopy and a workshop on DNA Identification; and two post conference activities, namely a workshop on Stem Cells and a workshop on Next Generation Sequencing Data Analysis will also be held with the assistance of overseas resource persons.

On behalf of the IBMBB and the organising committee, we thank Minister of Higher Education, Secretary, Higher Education and other officials; Chairperson, Vice Chairman and other officials of the University Grants Commission; Vice Chancellor, University of Colombo and other officials; Chairman, Director General and other officials of the National Science Foundation, the co-organiser; all our sponsors, and sincerely hope that this conference will be intellectually stimulating and rewarding to all the participants. Guidance of the Founder Director/IBMBB, Emeritus Professor Eric H Karunanayake is highly appreciated.

Professor M H R Sheriff
Acting Director/IBMBB

Professor Kamani H Tennekoon
Chairperson/Organizing Committee

PROGRAMME

DAY 1 – 11th September 2014

Main Hall	Side Hall	
Key Note lecture: A new era in Life science has started Prof Ulf Pettersson, Senior Professor of Medical Genetics, University of Uppsala, Sweden		During Inauguration (9.00 to 11.30 h)
Oral presentations Molecular Medicine (OP 1- OP 5)	Oral presentations Immunology (OP 6- OP 10)	11.45 to 13.00 h
Lunch		
Symposium on **Human DNA identification**	Symposium on **Immunology**	14.00 to 16.00 h
Invited Lectures *Recent advances in forensic DNA analysis* *Prof. Marie Allen, University of Uppsala, Sweden* *Genetic landscape of India: relevance to health and disease in South Asia* *Dr. K Thangaraj, Centre for Cellular and Molecular Biology, Hyderabad, India* *Oral presentation* *Mitochondrial lineage of Sri Lankans* *Ranasinghe RACR, Tennekoon KH, Karunanayake EH, Lembring M, Allen M* *Industry Lecture* *Improving efficiencies in DNA typing workflow to achieve high quality data* *Dr. Nicholas Ng, Promega Corporation, Singapore*	*Invited Lectures:* *Therapeutic uses of monoclonal antibodies and nanobodies* *Prof. Sunil Premawansa, University of Colombo, Sri Lanka.* *Primary Immunodeficiency: Recent Advances* *Dr. Rajiva de Silva, Consultant Immunologist, Medical Research Institute, Sri Lanka* *How T cells work* *Dr. Dharshan De Silva, Director & Senior Scientist, Genetech Research Institute, Sri Lanka* *Immunopathogenesis of infectious diseases* *Dr. Shiroma Handunnetti, IBMBB, University of Colombo, Sri Lanka*	
Industry lecture - Mass Spectrometry Agilent		16.00 to 16.30 h
Posters at Poster Venue and Tea		16.30 to 17.45 h

DAY 2 – 12th September 2014

Main Hall	Side Hall	
Industry lecture Method Validation Agilent		08.45 to 9.15 h
Plenary: Drug Discovery Research: Lessons from Pakistan Prof Iqbal Choudhary, Director, HEJ Research Institute, Pakistan		09.15 to 10.00 h
Tea		10.00 to 10.30 h
Symposium on **Stem Cells** *Invited Lectures* *Promises and challenges of stem cells for regeneration* *Prof. Gerald Schatten, University of Pittsburgh, USA* *Blood stem cells: Its utility in discovery research and clinical applications* *Prof. Amittha Wickrama, University of Chicago, USA* *Human endometrial basalis epithelial progenitor cells: Implications in endometrial proliferative disease* *Dr. Dharani Hapangama, University of Liverpool, UK* *Human umbilical cord Wharton's jelly stem cells: Its unique properties and clinical applications* *Dr. Chui Yee Fong, National University Health System. Singapore*	Symposium on **GMOs and Tea** *Invited Lectures* *Risk assessment and the approval process for genetically modified plants in Europe* *Dr. Anders Falk, Scientific Officer in Plant Breeding and Gene Technology, Swedish Agricultural Board, Sweden* *Tea and Health* *Dr Tissa Amarakoon, University of Kelaniya, Sri Lanka* *Oral presentations* *Metabolomics of Sri Lankan tea germplasm* *Punyasiri PAN, Brasathe J, Kottawa–Arachchi JD, Ranatunga MAB, Abeysinghe ISB, Gunasekare MTK, Bandara BMR* *Application of genomic approaches to genetic conservation and improvement of tea: Achievements and future directions* *Mewan KM, Abeysinghe ISB*	10.30 to 12.30 h
Lunch		12.30 to 13.15 h
Oral presentations Medicinal Plants (OP 11 –OP 17)	Oral presentations Plant Molecular Biology (OP 18 – OP 23)	13.15 to 15.00 h
Industry lecture **The cell based approach**		15.00 to 15.30 h
Symposium on **Reproduction and Development** *Invited Lectures:* *New insights into endometriosis* *Prof. Asgi Fazleabas, Michigan State University, USA* *Genomics and proteomics of birth weight in healthy Sri Lankan newborns* *Prof. Kamani Tennekoon, IBMBB, University of Colombo, Sri Lanka*		15.30 to 16.30 h
Closing Ceremony		16.30 to 16.45 h
Tea		16.45 to 17. 30 h

INVITED SPEAKERS FROM OVERSEAS

Keynote Lecturer

Prof. Ulf Pettersson

Prof. Ulf Pettersson is a Senior Professor of Medical Genetics,University of Uppsala, Sweden. He was a former Vice Rector of University of Uppsala, Sweden. Many Sri Lankan Molecular Biologists were trained under Prof. Pettersson.

Plenary Lecturer

Prof. Iqbal Choudhry

Prof. Iqbal Choudhry is the Director of International Center for Chemical and Biological Sciences H.E.J. Research Institute of Chemistry, University of Karachi, Pakistan. He is a leading scientist and scholar in the field of organic chemistry.

Symposia Lecturers

Prof. Marie Allen

Prof. Marie Allen is a Professor in the Department of Immunology, Genetics and Pathology at the Science for Life Laboratory, University of Uppsala and a Forensic DNA expert to the Swedish Government.

Dr. K. Thangaraj

Dr. K. Thangaraj is a Senior Principal Scientist and Group Leader at the Centre for Cellular and Molecular Biology (CCMB), Hyderabad. He is the President of the Indian Society of Human Genetics and the founder of the Society for mitochondrial Research and Medicine.

Prof. Gerald P. Schatten

Prof. Gerald P. Schatten is the Professor of Cell Biology and Physiology, University of Pittsburgh School of Medicine in Pennsylvania. He is also the Director of the Pittsburgh Development Center, as well as Director of the Division of Developmental and Regenerative Medicine .

Prof. Amittha Wickrama

Prof. Amittha Wickrama currently directs the clinical stem cell manipulation and cell manufacturing facilities at the University of Chicago Hospital, USA. He also directs a course on "Stem Cells & Medicine" for medical students at the University of Chicago, USA.

Dr. Chui-yee

Dr. Chui-Yee Fong is currently Research Associate Professor in the Department of Obstetrics and Gynaecology of the Yong Loo Lin School of Medicine, National University of Singapore , National University Health System.

Dr. Dharani Hapangama

Dr. Dharani Hapangama is a Clinical Senior Lecturer and Consultant Gynaecologist Surgeon at the University of Liverpool and Liverpool Women's Hospital UK.

Dr. Anders B. Falk

Dr. Anders B. Falk was an Assistant Professor at the Department of Plant Biology, SLU and was empoyed at the Swedish Board of Agriculture for risk assessment and legislation on GMO, plant breeding and seed legislation issues. He is a Scientific Officer at the European Food Safety Authority and working on risk assessment of genetically modified plants.

Prof. Asgi Fazleadas

Prof. Asgi Fazleabas is Professor and Associate Chair for Research in the Department of Obstetrics, Gynecology and Reproductive Biology, Director for the Michigan State University Center for Women's Health and Co-Director of the Reproductive and Developmental Sciences Program.

Industry Lecturers

Dr. Nicholas Ng

Dr. Nicholas Ng is the General Manager for Promega Corporation (Singapore Branch). He oversees the commercial, logistics and support operations of the business in the East Asia, South Asia and South East Asia regions.

Dr. Karthik Narasimhan

Dr. Karthik Narasimhan is the field application specialist for Asia pasific for the promega corporation.

Dr. Mohammed Ishak bin Abu

Dr. Mohammed Ishak bin Abu is currently the Channel Partner Manager of Agilent Technologies responsible for several countries. He is familiar with cGMP, GLP, Quality Management System, Lean Management, Method Validation, Instrument Qualification and Change Control management.

Oral Presentations - 11th September 2014

Molecular Medicine

OP 01: Circulating levels of leptin and soluble leptin receptor (SLR) in a cohort of sporadic breast cancer patients
Rodrigo HACIK, Tennekoon KH, Karunanayake EH, Amarasinghe IY, De Silva GSK

***OP 02:* Prevalence of missense mutation c.865A>C in *BRCA2* gene in a cohort of Sri Lankan breast cancer patients**
De Silva S, Tennekoon KH, Karunanayake EH

OP 03: Association of *H19* rs 217727, *IGF-I* dinucleotide and *IGF-II-* Apa 1 polymorphisms with birth weight
Hewage AS, Jayanthiny P, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundera APDeS

OP 04: *GH1* gene sequence variants in a cohort of Sri Lankan children with short stature
Sundralingam T, De Silva S, Hewage AS, de Silva KSH, Tennekoon KH

OP 05: *In silico* characterization of a RNA binding protein of cattle filarial parasite *Setaria digitata*
Nagaratnam N, Karunanayake EH, Tennekoon KH, Samarakoon SR, Mayan K

Immunology

OP 06: Evaluation of leptospirosis diagnostic tests: microscopic agglutination test, IgM based ELISA and immunochromatography test
Niloofa MJR, Fernando TRGN, De Silva NL, Karunanayake L, Wickremesinghe H, Dikmadugoda N, Premawansa G, Wikramasinghe AR, De Silva HJ, Premawansa S, Rajapakse S, Handunetti SM

OP 07: Oxidative damage of lipids and proteins in leptospirosis
Fernando TRGN, Niloofa MJR, Maduranga S, Rodrigo C, Karunanayake L, De Silva HJ, Rajapakse S, Premawansa S, Handunnetti SM

OP 08: Existence of mammalian counterparts of flagellin recognition machinery in teleosts: evidence at molecular, genomic and transcriptional levels
Umasuthan N, Lee J

OP 09: Immunomodulatory activity of Link *Samahan*, a herbal formulation in Sri Lankan market
Ranaweera BVLR, Edward D, Handunnetti SM, Weerasena OVDSJ

OP 10: *In vitro* inhibition of nitric oxide production in rat peritoneal cells by Sri Lankan medicinal plant extracts
Rukshala BAD, Handunnetti SM, de Silva ED

Oral Presentations - 12th September 2014

Medicinal Plants

OP 11: Bioactive tetrahydro-protoberberine type alkaloids from plants of genus *Corydalis*
Adhikari A, Shrestha RL

OP 12: *In vitro* anti-hepatocarcinogenic properties of a mangrove plant *Scyphiphora hydrophyllacea*
Samarakoon SR, Chanthirika S, Tennekoon KH, Thabrew MI, Ediriweera PMK, de Silva ED

***OP 13:* Hexane extract of *Mangifera zeylanica* bark exhibits cytotoxic activity through induction of apoptosis in triple negative breast cancer cells (MDA-MB-231) and ovarian cancer cells (SKOV3)**
Ediriweera PMK, Tennekoon KH, Samarakoon SR, Thabrew MI, de Silva ED

OP 14: Effects of *Flueggea leucopyrus* (Willd.) decoction on HSP 70 expression and apoptosis in triple negative breast cancer cells (MDA-MB-231)
Mendis AS, Thabrew MI, Samarakoon SR, Tennekoon KH

OP 15: Antifilarial activity of *Curcuma zedoaria* against adult bovine *Setaria digitata*
Senathilake KS, Samarakoon SR, Karunanayake EH, Tennekoon KH

OP 16: Screening of selected medicinal plants for possible cytotoxic effects on breast cancer
Jayarathna DDP, Tennekoon KH, Samarakoon SR, Thabrew MI, Karunanayake EH, Shanmuganathan C, de Silva ED

OP 17: New antileishmanial sesquiterpene coumarins from *Ferula narthex* Boiss
Bashir S, Alam M, Adhikari A, Yousuf S, Ahmad B, Parveen S, Aman A, Choudhary MI

Plant Molecular Biology

OP 18: Diversity assessment in Sri Lankan aromatic rice using InDel and SSR markers
Kottearachchi NS, Steele K

OP 19: Construction of species specific suppression subtractive hybridization library from the endemic wild rice species *Oryza rhizomatis* and characterization of cDNA clones
Rajkumar G, Weerasena OVDSJ, Fernando K

OP 20: Differential expression of defense-related genes in moderately-resistant *Sinapis alba* and susceptible *Brassica juncea* upon Alternaria *brassicae* challenge or defense inducer treatment
Nayanakantha NMC, Rawat S, Ali S, Grover A

OP 21: Cystathionine-β-synthase domains containing OsCBSCBSPB4 protein from rice confers abiotic stress tolerance to transgenic tobacco plants
Ariyadasa TU, Pareek A, Sopory SK, Singla-Pareek SL

OP 22: The tea shot hole borer beetle (*Euwallacea fornicatus*) is a vector of the canker causing Fusarium spp of tea [*Camellia sinensis* (L.) O Kuntze] in Sri Lanka
Pradeepa NHL,Weerasena OVDSJ, Liyanarachchi CJ, Wijesundera RLC, Abeysinghe ISB

OP 23: Evolution of Jasmonic acid biosynthesis pathway from bryophytes to vascular plants
Bandara PKGSS, Takahashi K, Nabeta K

Poster presentations

Molecular Medicine

PP 01: Establishment of DNA extraction from paraffin embedded tissues and preliminary analysis of somatic mutation of *TP53* in breast cancer
Manoharan V, Tennekoon KH, Angunawela P, De Silva S

PP 02: Association of endometriosis and *p53* gene codon 72 polymorphism in a group of Sri Lankan women
Nanthaprakash T, Silva N, De Silva S, Senanayake H

PP 03: Extraction of DNA from old bones: optimization of methods and assessment of suitability for individual identification
Nagaratnam N, Karunanayake EH, Ranasinghe R

PP 04: Plasma free amino acid concentration in a group of apparently healthy adults
Kajaluxan R, Karunanayake EH, Punyasiri N

PP 05: Association between antibody-mediated platelet cytotoxicity and the severity of dengue infections in Sri Lanka
Bandara MNS, Handunnetti SM, Premawansa G, Loeb M, De Silva AD, Premawansa S

Immunology & Marine Molecular Biology

PP 06: Bioinformatic analysis of four members from Teleostean complement pathway with their immune response against bacterial infections
Godahewa GI, Bathige SDNK, Wickramaarachchi WDN, Jayasinghe JDHE, De Zoysa M, Lee J

PP 07: Molecular and biological characterization of two antimicrobial peptides, β defensin and piscidin, from rock bream *Oplegnathus fasciatus*
Mothishri MS, Umasuthan N, Thulasitha WS, Lee J

PP 08: Molecular characterization of two galectins from *Oplegnathus fasciatus*: Transcriptional responses against immune stimuli and biological activities
Thulasitha WS, Umasuthan N, Mothishri MS, Lee J

PP 09: Two paralogs of NF-κB inhibitor-alpha (*IκBα*) genes from rock bream (*Oplegnathus fasciatus*): Genomic organization and expressional analysis
Lee Y, Umasuthan N, Lee J

PP 10: Ligand-receptor system of teleost IL8 signalling: Immune responsive expression and genomic organization of *IL8*, *CXCR1* and *CXCR2* in rock bream
Umasuthan N, Thulasitha WS, Mothishri MS, Lee J

PP 11: Molecular characterization of two STAT1 isoforms from rock bream, *Oplegnathus fasciatus* and their immune responses upon viral infection
Bathige SDNK, Umasuthan N, Lee J

PP 12: Genomic arrangement of vertebrate *MAPKs* p38α and *p38β* is highly conserved: Identification, comparison and expression of two rock bream *(Oplegnathus fasciatus)* MAPKs
Umasuthan N, Bathige SDNK, Lee J

Medicinal Plants, Natural Products, Xenobiotics & Plant Molecular Biology

PP 13: Investigation of antimicrobial properties of *Areca concinna* extracts
de Silva WS, de Silva ED, Tennekoon KH

PP 14: *In vitro* genotoxicity evaluation of *Walidda antidysenterica* by comet assay
Baragamaarachchi RY, Weerasena OVDSJ, Handunnetti SM, Samarasekara R

PP 15: Antioxidant activity and total polyphenolic content of weedy herb *Commelina diffusa*
Perera HDSM, Samarasekera R, Handunnetti SM, Weerasena OVDSJ

PP 16: Acetyl cholinesterase inhibitory and antioxidant activities of leaves of *Toona ciliata*
Samaradivakara SP, Samarasekera R, Handunetti SM, Weerasena OVDSJ

PP 17: Liquid chromatographic profiling of flavonols in Sri Lankan black tea and green tea
Jeganathan B, Punyasiri PAN, Madhujith WMT, Bandara BMR

PP 18: Inhibition of iNOS expression and enhancement of CD40 and CD40L expression by combined hot water extract of *Coriandrum sativum* and *Coscinium fenestratum*
Harasgama HDAJC, Handunnetti SM, Weerasena OVDSJ, Premakumara S

PP 19: Comparative study of the cytotoxic effects of microcistin-LR and crude cyanotoxin extract from Beira lake cyanobacteria on human embryonic kidney (HEK 293) cell line
Piyathilaka MAPC, Pathmalal MM, Tennekoon KH, Samarakoon SR, Chanthirika S, De Silva BGDNK

PP 20: Development of markers to identify blister blight resistance and susceptibility in tea *(Camellia sinensis L.)* cultivars.
Karunarathna KHT, Mewan KM, Punyasiri PAN, Weerasena OVDSJ, Edirisinghe ENU, Brasathe J, Abeysinghe ISB

Key Note Lecture

"A new era in life science research has started"

Ulf Pettersson, MD

Senior Professor of Medical Genetics
University of Uppsala
Sweden

Technology for reading DNA sequences paved the way for a remarkable progress in the medical biosciences. The double Nobel Prize winner, Fred Sanger, published his revolutionizing dideoxy sequencing method in 1977. This methodology combined with molecular cloning and PCR laid the foundation of molecular genetics enabling studies of the causes of disease at the DNA level. The next step in the scientific revolution was the automation of Sanger´s method, accomplished by Leroy Hood and coworkers in 1985. These capabilities made the complete sequencing of the human genome possible, which was an astonishing accomplishment completed ahead of the dead line.

After the turn of the century a series of inventions were made known as the Next Generation of Sequencing technologies. A mammalian genome can now be sequenced within days at a cost of a few thousand dollars. This opens a vast number of possibilities in medicine and biology which I will discuss in my lecture. I will also dwell on the new technologies that enable rapid and cost-effective diagnostics at both the DNA and the protein levels.

Plenary Lecture

Drug Discovery Research: Lessons from Pakistan*

M. Iqbal Choudhary, Sc.D

Director
International Center for Chemical and Biological Sciences
H.E.J. Research Institute of Chemistry and
Dr. Panjwani Center for Molecular Medicine and Drug Research
University of Karachi
Pakistan

Drug discovery and development is primarily an interdisciplinary process, right at the interface of chemistry and biology. Most of the exciting discoveries about the molecular basis of disease and treatment are made at the interface of these two disciplines. Boundaries of two disciplines are blurring as new fields such as genetic engineering, functional genomics, proteomics, metabolomics, computational medicinal chemistry, structure biology and system biology are emerging. Following two are examples of our work at the interface of chemistry and biology for the discovery of therapeutically important.

A rapid decline in research and development on new antibiotics coincides with increasing frequency of infections caused by multi-drug-resistant pathogens. The outbreak of methicillin-resistant *Staphylococcus aureus* (MRSA) occurred over fifty years ago is now widespread throughout the world. S. aureus is the most common bacterial pathogen, which cause skin, soft-tissue and endovascular infections as well as pneumonia, septic arthritis, endocarditis, osteomyelitis and sepsis. As the efficacy of currently available antibiotics is declining due to MDR, there is an urgent need to develop new approaches to meet this challenge. In the present study, we discovered several novel and potent inhibitors of MDR S. aureus (EMRSA-17, EMRSA-16, MRSA-252 and Pak clinical isolates) from natural and synthetic sources. Resistance-reversal studies at molecular level were carried out by employing flow cytometric and microscopic techniques. Synergistic and partial synergistic effects of these compounds in combination with antibiotics were investigated. This work has so far resulted in the identification of novel “helper molecules”, which can increase the efficacy of existing antibiotics to over 1000-fold in some cases.

Obesity is an emerging challenge to human well-being. Molecular cascade involve in obesity and associated disorders is not fully understood. Proliferation of adipocytes plays in important role in the on-set and progression of obesity. Understanding the phenomena of adipogenesis is of major importance as adipocyte dysfunction makes an important contribution to metabolic diseases due to obesity. Differentiation of preadipocytes to adipocytes not only results in increasing number of adipocytes but also provide a large pool of fat depots in adipose tissues. Thus one strategy to treat obesity is to reduce the adipocyte numbers and fat content through targeting the mature adipocytes by diverse molecular activities. Among different therapeutic interventions, the discovery of effective antiadipogenic compounds from various sources is considered to be a promising approach. Our recent research is focused on the study of the inhibitory effects of natural and synthetic compounds, such as steroids, flavonoids, terpenes, alkaloids and sulphonamide derivatives, on the proliferation of adipocytes, in a dose dependent manner, as well as to assess their effects on to the mature adipocytes. This study has resulted in the identification of several new inhibitors of adipogenesis process.

***Professor Atta-Ur-Rahman has contributed towards the scientific work**

SYMPOSIA INVITED LECTURES & ORAL PRESENTATIONS

Symposium on DNA Identification

Invited lecture 1

Recent advances in forensic DNA analysis

Marie Allen, PhD

Professor
Science for Life Laboratory
University of Uppsala
Sweden

DNA testing for individual identification has become routine in crime scene investigations. However, some samples are challenging as they have been subjected to harsh environments leading to degradation of DNA. Old samples are also extremely susceptible to modern DNA contamination and may contain inhibitors. We are exploring several new purification procedures and new PCR enzymes for an increased sensitivity and success rate in forensic DNA testing. Optimal protocols have allowed investigations of our past in our identifications of the remains of Nicolaus Copernicus (1473-1543) and Carin Göring (1888-1931).

Forensic analysis can be further improved by using Next Generation Sequencing (NGS) such as the benchtop MiSeq instrument. We have designed a novel forensic NGS marker panel that allows analysis of 10 STRs and the mtDNA genome. In addition are 386 SNPs on the autosomes, X- and Y-chromosomes analysed in the assay. Some SNPs were selected to provide information about visible characteristics as eye, hair and skin-colour as well as ancestry. The targets are enriched by HaloPlex probes that were selected using the SureDesign software (Agilent). The use of mtDNA, short SNP targets and a highly sensitive target enrichment method allows an optimal analysis. Degraded samples, mixed profiles as well as small input amounts can be analysed, all commonly seen challenges among samples.

In cases without a suspect, knowledge of the biological age of the person leaving an evidence sample at a crime scene can provide an investigative lead. We have identified a number of DNA methylation markers in the form of CpG sites that can be used for age prediction. Preliminary data show that methylation level change with age and we have seen a good correlation between observed age and predicted age. Next step is to develop a Forensic Epigenetic Age Signature test based on our best performing markers.

Symposium on DNA Identification

Invited lecture 2

Genetic landscape of India: Relevance to health and disease in South Asia

K. Thangaraj, PhD

Senior Principal Scientist
Centre for Cellular and Molecular Biology
Hyderabad
India

India represents one of the largest sources of human diversity, comprising of more than four and half thousand anthropologically well-defined populations. Since most of the Indian populations are maintaining very strict endogamy marriage practices, for the last thousands of years, genetic mutations introduced in every population remains populations-specific. Hence we have studied the genetic variation among large number of Indian populations to get an insight about their complex origin, health and genetic disease. Using the complete mitochondrial DNA (mtDNA) sequence information, we have demonstrated that the India inhabited by the descendents of the very first modern humans, who migrated out-of-Africa about 60,000 years ago. Subsequently using about a million SNPs, we have shown relatively small group of ancestors founded most Indian groups, which then remained largely isolated with limited gene flow for long periods of time. We also identified two main ancestral groups in India: an “Ancestral North Indian (ANI)”, which is distantly related to those in the Middle East, Central Asia, and Europe, and an “Ancestral South Indian (ASI)”, not related to groups outside India. Groups with only ASI ancestry may no longer exist in mainland India. Our results show that genetic patterns in Indian populations have been shaped by a long history of genetic isolation between different groups that predates the caste system in place in India during colonialism. Allele frequency differences between groups in India are larger than in Europe, reflecting strong founder effects whose signatures have been maintained for thousands of years owing to endogamy. We, therefore, predict that there will be an excess of recessive and complex diseases in India. During the presentation, I would focus on how the Indian genetics are different from rest of the world and what would be its implications in health and disease, not only in India but also in South Asian countries.

Symposium on DNA Identification

Oral presentation

Mitochondrial lineage of Sri Lankans

Ranasinghe RACR[1], Tennekoon KH[1], Karunanayake EH[1], Lembring M[2], Allen M[2]

[1]IBMBB, University of Colombo, Sri Lanka
[2]Science for Life Laboratory, University of Uppsala, Sweden

Historical records indicate significant human migration beginning more than 2600 years ago from mainland India to Sri Lanka which was already inhabited by several tribes. In order to investigate maternal lineage relationships between contemporary Sri Lankans, we analysed mitochondrial hypervariable regions I and II and selected coding region polymorphisms in maternally unrelated individuals from six ethnic groups [i.e.: Sinhalese (N=60), Sri Lankan Tamil (N=30), Muslim (N=30), Malay (N=30), Indian Tamil (M=22) and Vedda (N=30)]. Hypervariable regions were directly sequenced and the coding region polymorphisms analysed by PCR-RFLP and confirmed by sequencing. We observed 135 unique haplotypes and 22 shared haplotypes. In multivariate and phylogenetic analyses Vedda people clearly separated from the other ethnic groups. Sri Lankan Tamils showed a closer genetic affiliation to the Sinhalese than to the Indian Tamils. The N macro-haplogroup was the most common (63.33%) among Veddas and the M macro-haplogroup the most prevalent among other groups (53.33% to 86.36%). South Asian (Indian) haplogroups contributed 65%, 66.7%, 63.3%, 90%, 86.7% and 95.4%to the mthaplogroups in Sinhalese, Sri Lankan Tamils, Veddas, Malays, Muslims and Indian Tamils, respectively. Moreover, considerable presence of West Eurasian haplogroups was evident among Sinhalese (25%), Vedda (23.3%) and Sri Lankan Tamils (20%). Our data confirm a distinct maternal lineage in Vedda people supporting the historical view that they are descendents of the early inhabitants of the country. Furthermore, higher prevalence of West Eurasian mthaplogroups among the ethnic groups with longer histories of settlement in the country with an extremely low prevalence or total absence among the recent migrants suggest that the island was also populated by women carrying West Eurasian mthaplogroups in earlier times.

This work was supported by National Research Council Grant No: 09-20, Sida/Secretariat for Research Cooperation Grant for Molecular Biology and Biotechnology and constitutes part of the PhD studies of RACRR.

Symposium on DNA Identification

Industry Lecture

Improving efficiencies in DNA typing workflow to achieve high quality data

Nicholas Ng, PhD

General Manager
Promega Corporation
Singapore

A principal goal of the modern DNA Typing Lab is to improve efficiencies in the DNA Typing Workflow to achieve high quality data for interpretation. Success factors for optimizing the STR workflow include

- Novel extraction chemistries including preprocessing buffers for diverse samples and the utility of extraction using extractors versus robotic platforms in throughput handling.
- Next generation STR systems – autosomal and Y –STR; impact of expanded STR markers for casework analysis; the incorporation of direct amplification and fast PCR chemistries for streamlining casework and database work
- Challenging samples – success rates; degradation versus inhibition

Symposium on Immunology

Invited lecture 1

Therapeutic uses of monoclonal antibodies and nanobodies

Sunil Premawansa, PhD

Professor in Zoology
Faculty of Science
University of Colombo
Sri Lanka

The discovery of the production of monoclonal antibodies (Mabs) through Hybridoma technology that won the Nobel Prize in 1984 is recognized as a major breakthrough in the field of biomedical research. Since then, other novel procedures such as Phage display have also been developed to produce Mabs. With the invention of Mabs production, its promising applications were witnessed specifically in the fields of immunodiagnosis, antigen purification and antibody therapy. Advantages were also highlighted in the use of Mabs therapy over conventional chemotherapy. Humanized Mabs too were later developed to minimize the development of allergic reactions to Mabs using transgenic animals and using genetic engineering processes. Vital uses of these Mabs were indicated in treatments such as the prevention of blood clots in patients undergoing cardiac procedures and patients with lymphocyte leukemias. Mabs have also been developed to treat patients with breast cancer and autoimmune diseases. Versatility of using Mabs can also be obtained when radioactive payloads are conjugated to the Mabs in specific targeted killing of cancer cells to retard tumor growth. Toxins too are conjugated to Mabs to make immunotoxins to treat leukemias and lymphomas. Another promising Mabs treatment is involved with the production of anti-cytokine Mabs to prevent inflammatory diseases. Understanding the potential uses of Mabs, mass production of Mabs has even been developed with genetically engineered animals and plants. In further advancement of Mabs therapy, antibody fragments such as Fabs have been developed. A novel way of using only the comparatively tiny variable segments of an antibody known as nanobodies is another revolutionary area of using Mabs. Thus, the applications of Mabs in the field of immunology and immunotherapy are very much evident and expanding.

Symposium on Immunology

Invited lecture 2

Primary Immune Deficiency: Recent Advances

Rajiva de Silva, MD

Consultant Immunologist
Department of Immunology
Medical Research Institute
Sri Lanka

Primary immune deficiencies (PID) are due to genetic defects in the immune system. The prevalence of PID may be as high as 1:2000 in the US. The International Union of Immunological Societies (IUIS) has classified PID into 9 groups. These include combined immune deficiencies (where both T and B lymphocytes are affected), predominantly antibody deficiencies, defects of phagocytes and complement deficiencies.

Thirty new PID have been described between 2011 and 2014. One such disease is Interferon-stimulated gene 15 deficiency, and it is the most recent identified cause of Mendelian susceptibility to mycobacterium disease (MSMD). MSMD is due to 9 genetic diseases which interrupt cross talk between macrophages and T cells/NK cells. This leads to local or disseminated disease with poorly pathogenic environmental mycobacteria and salmonella.

IL 21 is a cytokine that acts via the IL-21R/γc/JAK3/STAT3 pathway leading to B cell maturation. New insights into functioning of this pathway has highlighted the role of IL21 in certain PID, namely hyper IgE syndrome, severe combined immune deficiencies due to JAK3 and γc deficiency, and the newly identified loss-of-function mutations in the IL-21R gene.

This presentation will give a broad overview of Mendelian susceptibility to mycobacterial disease and IL21 related defects.

Symposium on Immunology

Invited lecture 3

How T cells work

Aruna Dharshan De Silva, PhD

Director & Senior Scientist
Genetech Research Institute
Colombo
Sri Lanka

T cells play a major role in the destruction of cells that harbor intracellular invaders, such as viruses. Antibodies (from B cells) play a key role in clearing pathogens from the blood & extra cellular spaces, but T cells are able to identify cells with pathogens hiding inside the cells. T cells undergo a thorough "education" process in which they are taught to recognize foreign pathogen infected cells, while leaving healthy cells alone. Following their development in the thymus they can be separated into two main classes, CD4 and CD8 T cells. While CD8 T cells all become cytotoxic T lymphocytes with the ability to kill cells, CD4 cells differentiate into four main types of T helper cells that promote distinct types of responses against different infections. These are known as T_H1, T_H2, T_H17 & Follicular helper cells (T_{FH}). We have been studying the T cell response against dengue infections at Genetech in collaboration with US investigators. Originally it was suspected that T cell responses induced by a primary dengue infection with one serotype causes a less effective response upon secondary infection with a different serotype predisposing individuals to severe disease. Surprisingly our recent data showed that even though skewing of responses toward primary infecting viruses was detected, this was not associated with impairment of responses either qualitatively or quantitatively. Furthermore, we demonstrated higher magnitude and more polyfunctional responses for HLA alleles associated with decreased susceptibility to severe disease, suggesting that a vigorous response by multifunctional CD8+ T cells is associated with protection from dengue virus disease.

Symposium on Immunology

Invited lecture 4

Immunopathogenesis of infectious diseases

Shiroma Handunnetti, PhD

Institute of Biochemistry, Molecular Biology & Biotechnology
University of Colombo
Sri Lanka

Although there are significant advances in treatment and management of infectious diseases, the morbidity and mortality resulting from these viral, bacterial and parasitic infections still remain a challenge. Sri Lanka has successfully controlled parasitic diseases such as malaria & filariasis, however other viral and bacterial diseases such as dengue and leptosirosis have emerged.

Immune response to infections includes activation of a complex network that involves different types of immune cells and soluble factors which are targeted for elimination of the pathogen. Most often, the immune response is over expressed and damages the host causing the immunopathology and even death in some patients. Although there are numerous hypotheses, the exact molecular mechanisms of immunopathogenesis of many infectious diseases are not clearly understood. Our ongoing studies on lepospirosis and dengue suggest that activation of innate immune responses leading to overproduction of reactive oxygen and nitrogen species results in oxidative stress in the host. Such findings would be useful for developing biomarkers for early detection of infections that may develop into severe conditions and also for developing or testing agents that would block immunopathogenesis.

Recent mutagenesis studies using *Leptospira interrogans* have identified several virulent factors including LPS, Loa22 and Ompa-proteins. Further, the absence of virulence factor homologues among the known proteins, suggests that *Leptospira* possesses unique virulence mechanisms. In dengue virus infection, the aberrant immune responses impair the clearance of virus, result in overproduction of cytokines and abnormal production of autoantibodies. This aberrant immune response generate anti-NS1 antibodies that cross-react with platelets and endothelial cells. Interaction of these anti-platelet or anti-endothelial cell autoantibodies is considered to be involved in immunopathogenesis and in clinical manifestation of thrombocytopenia and endothelial cell dysfunction. Findings from our preliminary *in vitro* studies support this hypothesis of anti-platelet antibodies causing platelet cytotoxicity and destruction of platelets.

Symposium on Stem Cells

Invited lecture 1

Promises and challenges of stem cells for regeneration

Gerald Schatten, PhD

Director, Pittsburgh Development Center
Professor & Vice-Chair of Obstetrics, Gynaecology & Reproductive Sciences
Professor of Cell Biology and Physiology
& Bioengineering
Director, Division of Developmental & Regenerative Medicine
University of Pittsburgh School of Medicine
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The 2012 Nobel Prizes to Sir John Gurdon and Shinya Yamanaka for their seminal work in nuclear transfer and induced pluripotency, coupled with the 2010 Nobel Prize to Bob Edwards for pioneering *in vitro* Fertilization in humans, attests to the contemporary importance of stem cells and regenerative medicine for assisted reproductive technology (ART). This lecture will consider pluripotent stem cells, their differentiation into both sperm and oocytes, as well as their role of PSCs in cancers and epigenetics.

Pluripotent stem cells from humans (hPSCs), including human embryonic stem cells (hESCs) and by induced pluripotency (hiPSCs) captivate medical attention due to their unique properties of unlimited self-renewal and differentiation. ESC lines have only been established robustly and investigated intensively in mice (mESCs) and more recently in humans after derivations from fertilized-blastocysts and after induced pluripotency (iPSCs). Scientists around the World are now asking whether these cells might treat or even cure juvenile diabetes with insulin secreting β-islet cells responsive to circulating glucose; cerebral palsy treatments with neuroprogenitors to repair white matter injuries due to premature births; heart muscle repair with cardiomyocytes; spinal cord regeneration with peripheral motor neurons; multiple sclerosis with neuroprogenitor cells or astrocytes for Schwann cell; Parkinson's disease using dopaminergic neurons; amyotrophic lateral sclerosis with neuronal lineages; reduction or replacement of whole organ transplantation by single cell transplantation of hepatocytes for diseased livers; renal cells in place of kidney transplants, and many others.

Symposium on Stem Cells

Invited lecture 2

Blood stem cells: Its utility in discovery research and clinical applications

Amittha Wickrama, PhD

Associate Professor
Department of Medicine - Section of Hematology/Oncology
University of Chicago
USA

Blood stem cells are the origin of all our circulating blood cell types. It is responsible for generating all immune cells as well as red blood cells and platelets in vertebrates. Bone marrow is the primary site of blood cell production in mammals during the post natal period. Many theories have been proposed with respect to the mechanisms underlying stem cell self-renewal and their ability to regulate commitment to a particular cell lineage in a balanced manner without depleting the pool of quiescent stem cells during the life span of the organism. Experimental evidence gathered over the last fifty years strongly suggest that regulation of normal hematopoiesis is influenced by both humeral factors such as hormones and cytokines together with dynamic mechanical forces that exist within the bone marrow microenvironment. These elements act to regulate the gene transcriptional program at the stem cell level leading to down regulation of the stem cell and multi-lineage genes and at the same time up-regulating lineage specific genes. Most recent studies have uncovered a strong epigenetic influence in modulating lineage specification and subsequent differentiation into a particular type of blood cells.

When normal hematopoiesis is disrupted as is the case in leukemia and other myeloproliferative and dysplastic conditions balance between various blood cell types are disrupted. It is now widely accepted that disruptions in the gene programs occur at the early stem cell/progenitor level with respect to all types of blood malignancies as oppose to the late stages in the differentiation program. A stem cell transplant to replace disease stem/progenitor cells with healthy cells are a true and tried approach to cure many malignancies including inborn genetic diseases such as thalassemia and rare metabolic enzyme deficiencies. In these cases allogeneic bone marrow derived stem cells as well as umbilical cord blood stem cells have proven to be extremely useful. An overview of current status of basic research and clinical applications of blood stem cells will be presented.

Symposium on Stem Cells

Invited lecture 3

Human endometrial basalis epithelial progenitor cells: Implications in endometrial proliferative disease

Dharani Hapangama, MD

Senior Lecturer and Consultant in Obstetrics & Gynaecology
University of Liverpool
UK

Endometrial development and function in menstruating upper order primates (including humans) is complex compared with most other mammals. The human endometrium is stratified into two functional layers; the transient superficial stratum functionalis and the permanent deeper stratum basalis adjacent to the myometrium. The germinal layer of the endometrium where the stem cells reside is, postulated to be the stratum basalis. We have recently characterised the subpopulation of basalis epithelial cells that have the ability to generate endometrial functionalis gland-like structures and a monolayer similar to luminal epithelium in vitro 3D culture. Further characteristics of these germinal epithelial cells include higher expression of "stemness" genes, longer mean telomere lengths and telomerase activity. Since benign endometrial proliferative diseases endometriosis and endometrial cancer are postulated to originate from these basalis epithelial cells, further examination of the basalis epithelial cells to identify a primitive stem cell is warranted. Data on the potency of these cells and discussion of the implication of that in the pathogenesis of endometrial diseases are included.

Symposium on Stem Cells

Invited lecture 4

Human umbilical cord Wharton's jelly stem cells: Its unique properties and clinical applications

Chui Yee Fong, PhD

Research Associate Professor
Department of Obstetrics and Gynaecology
Yong Loo Lin School of Medicine
National University Health System
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Stem cell therapy offers incredible promise as a novel treatment for many diseases, especially incurable diseases. Stem cells extracted from the Wharton's Jelly compartment (hWJSCs) of the human umbilical cord have been garnering tremendous interest as they offer great clinical utility because of their unique properties. hWJSCs share similar CD signatures, and differentiation capabilities as human bone marrow stem cells (hBMMSCs) but they have unique properties of their own. These include their non-controversial status, their ability to be harvested in abundance from the largest compartment of the umbilical cord, and their homogenous stem cell population. Additionally, hWJSCs are very proliferative, hypo immunogenic, multipotent, and they remain stable even after undergoing numerous passages. hWJSCs are also non-tumorigenic and they have tumericidal effects on several cancer cells. Unlike hBMMSCs, they do not form tumor/carcinoma-associated fibroblast (TAF/CAF) in the presence of a tumor environment.

Extensive pre-clinical studies have shown that hWJSCs are effective in treating diseases like heart failure, Parkinson's, traumatic brain injury, cutaneous wound healing and cancer. To date, there had been several clinical trials conducted in Australia, China, Taiwan, and the USA to exploit hWJSCs' properties. These trials aimed to treat liver failure, autism, spinocerebellar ataxia, multiple system atrophy, HIV, myocardial infarction and type I diabetes mellitus to name a few. There are already several FDA approved clinical trials with published data to show their safety and efficacy.

Symposium on Genetically Modified Organisms (GMOs) and Tea

Invited lecture 1

Risk assessment and the approval process for genetically modified plants in Europe

Anders Falk, PhD

Scientific Officer in Plant Breeding and Gene Technology
Swedish Agricultural Board
Sweden

Plant breeding has a fundamental importance for increasing crop yield, quality of the harvested crop and the resulting food and animal feed. Plant breeding was done rather unconsciously for about 10000 years starting with the initial domestication of crop plants until about year 1900 when Mendel's work was rediscovered. Since then plant breeding has been done in a more systematic way mainly relying on new combination of germplasm through deliberate sexual crossing. Since about 1990 new plant breeding techniques have appeared, resulting in plants that may be considered as genetically modified organisms (GMO or GM plants) if "the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination" (EU directive 2001/18). In this category, only the insect-tolerant GM maize MON810 is currently being grown commercially in Europe. The acceptance of GM crops and GM food has been slow because of food, feed and environmental safety concerns. Farmers have been reluctant to embrace the new technology due to intellectual property issues and labelling requirements (EU regulation 1830/2003) that have led to co-existence problems between the different cropping systems. In addition, cropping systems called ecological or organic farming take pride in not using GM technology at all in the production, further complicating the issue of co-existence. Among the general public, genetic modification is sometimes perceived as not being natural although a proper definition of the concept of naturalness seems to be lacking. The concerns of the general public are sometimes taken advantage of politically leading to a slow regulatory process resulting in considerable obstructions in international trade.

The EU legislation (EU regulation 1829/2003) requires all GMO to be risk assessed before approval. The risk assessment procedure is described in Directive 2001/18 and in Guidance Documents from EFSA (The European Food Safety Authority). The risk assessment is performed by EFSA together with authorities from the EU member states. Risk is assessed with respect to food and feed and environmental safety. The food safety assessment requires a 90-day animal feeding trial. The environmental risk assessment may require toxicity testing on organisms existing in the environment that will receive the GM plant. So far, no risk for food or feed safety but some environmental risks have been identified with GM plants. For instance, herbicide-tolerant GM plants allow high herbicide intensities which may lead to herbicide-tolerant weeds. Likewise, use of insect-resistant GM plants may lead to selection of tolerance in insect populations. In addition, non-target organisms may be affected by the GM plant. Environmental risks must be considered in the context of general environmental impact of agriculture and handled as part of a risk management strategy.

Plant breeders have in recent years developed more sophisticated ways of plant breeding that may or may not result in a GMO. Such techniques include oligonucleotide-directed mutagenesis, reverse mutagenesis screens, advanced marker-assisted selection, zinc-finger nuclease technology and combinations of techniques that may make it possible to control recombination

Symposium on GMOs and Tea

Invited lecture 2

Tea and Health

Tissa Amarakoon, PhD

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Department of Chemistry
University of Kelaniya
Sri Lanka

Tea has been consumed regularly since 2737 BC, the time that the habit of tea drinking started, by a large number of individuals living in different regions of the world. Today, it is the most consumed beverage after water and therefore it could have a great impact on human health. Although known as a healthy beverage since 2737 BC, proper scientific studies on tea and human health were started only in late 1970s and large number of studies had been carried out in recent years.

According to the results of these studies, tea does not provide significant quantities of nutrients to the daily intake as more than 99% of a brewed tea cup is water and only a very small quantity of tea solids are extracted into the brew. Therefore, most of the reported beneficial effects are due to bioactive constituents of tea where only very small quantities are required for a significant effect. Different types of polyphenolic compounds, caffeine and theanine have been identified as the important bioactive constituents in tea. Antioxidant activity of the polyphenols has been identified as the major beneficial effect. However, these polyphenols could interfere with many biological processes in the body through many other mechanisms. Caffeine is the well known stimulant found in tea while theanine, which is an amino acid, could have a relaxing effect on the brain.

Numerous animal model and epidemiological studies also have been carried out to find the effect of tea consumption on common degenerative diseases. Attention had been mostly focused on heart disease, cancer and diabetes and the results indicate that tea consumption could reduce the risk of these diseases. Recent studies also have shown that tea may have beneficial effects on Alzheimer's disease.

Tea has been time-tested by large populations over millennia and found to be a safe beverage. Further, recent scientific studies have indicated additional health benefits. Therefore, it could be concluded that tea is a beverage suitable for regular consumption.

Symposium on GMOs and Tea

Oral presentation

Metabolomics of Sri Lankan tea germplasm

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Metabolomics is the scientific study of chemical processes involving metabolites. Therefore metabolomics is very useful in identifying differences in chemical constituents in plants and could be effectively used to classify plants based on concentration and types of metabolites. Tea Plant *(Camellia sinensis, L.)* is renowned for its biochemical constituents, which play an indispensable role in defining the quality of the final product and in conferring pest and disease resistance in the plant. Although much of the previous work has been focused in studying single metabolite variation of tea cultivars, limited work has been documented on the measurement of a broad range of metabolites. Therefore an investigation was carried out to profile Catechins (Epicatechin, Epigallocatehin, Epicatechin gallate and Epigallocatechin gallate), methylxanthines (Caffeine and Theo bromine), Flavonols glycosides (as aglycones: Quercetin, Myricetin and Kaempferol) using HPLC with photodiode array detection. In addition amino acids (L-Theanine and other amino acids) were quantified by online derivatization followed by HPLC with fluorescence detection. Tender leaves of 87 germplasm accessions C.sinensis and 6 non–beverage type cultivars, collected from the ex situ field gene bank of Tea Research Institute of Sri Lanka (TRI), Talawakelle were used for metabolite profiling. Results show that there is a wide variation in the amounts of all the metabolites tested in *C. sinensis* cultivars in the Sri Lankan Tea Germplasm. The accessions used to produce high quality black tea had high amounts of dihydroxylated catechins, Epicatechin and Epicatechin gallate as well as high ratios of dihydroxylated to trihydroxylated catechins. Metabolic profiles can be effectively used in selecting tea cultivars of desired quality for propagation and in tea breeding programme to generate progenies with wide variations.

Symposium on GMOs and Tea

Oral presentation

Application of genomic approaches to genetic conservation and improvement of tea: Achievements and future directions

Mewan KM, Abeysinghe ISB
Tea Research Institute of Sri Lanka (TRISL)

Conventional breeding has provided excellent resources for the development of new tea cultivars and novel germplasm. Regardless of the value of the past contributions, classical breeding alone will not provide adequate breakthroughs to improve yield and quality, to solve the complex problems with respect to biotic and abiotic stresses and to develop/enhance tolerance capacities of tea cultivars. In the modern era, plant improvement seems vulnerable and inadequate without the contributions of the new tools of molecular biology and genomics. Genomics provides novel, innovative, integrative approaches to overcome and to enhance the efficiency and effectiveness of conventional plant improvement program irrespective of species of interest.

For tea, which is predominantly an out-breeding woody perennial, genetic improvement through conventional approaches is relatively ineffective, slow and costly. As a potential tool to enhance process, TRISL has integrated molecular biology to supplement the conventional program. To date, considerable progress has been achieved in key areas such as assessing genetic diversity, isolation and characterization EST and genomic SSRs & construction of SSR primers, construction of genetic maps, and application of genomics approaches to understand the role of polyphenols in disease resistance in tea are discussed in this paper.

Towards effective conservation and utilization of tea genetic resources pre-requisites for sustainability and for increasing productivity, RAPD, AFLP and SSR markers were successfully applied to assess genetic diversity and relationship of improved tea cultivars. The study was further extended to the entire tea germplasm and also to the old seedling tea populations which is suspected as a source with a potential to enrich narrowed tea genetic pool.

Of the various marker systems available, SSRs have proven their power and efficiency as a potential tool in many areas. The major limitation for the application of SSRs in tea is the lack of sufficient number of primers. To aid this deficiency, we isolated and characterized SSRs from tea expressed sequenced tags (EST) and enriched genomic libraries. A total 305 genomic and 192 EST-SSR primer-pairs were developed and effectiveness of these markers to construct a genetic map and for genetic diversity studies is also evaluated.

Among these tools, as for many other important woody perennial crops, construction of detailed genetic linkage maps is the fundamental first step towards cultivar improvement through marker assisted breeding. Although a practical genetic map is not available for tea, the potential of maker assisted selection is clear and strong. In 2007, we developed the first EST- and genomic SSRs based genetic linkage map of tea. Further attempts are in progress towards developing QTL maps for economically important traits.

Furthermore, to understand the role of flavonoids in disease tolerance in tea, a genomics based approach was used and a comprehensive set of ESTs were derived from tea cDNA libraries. The results generated from this study led to identification and characterization of several key components in flavonoid pathway which may be potentially used to improve disease resistance in tea.

Industry Lecture

Deducing the mechanism of toxicity- The cell based approach

Karthik Narasimhan, PhD

Field Application Specialist for the Asia Pasic
Promega Corporation

Drug discovery is a highly knowledge oriented, rapidly advancing field. The process of drug discovery involves multiple stages beginning with basic research to identify targets, validation of the targets, screening of candidates, identification of leads and lead optimisation. Understanding the necessity for sensitive and robust assays to increase the success rate, compatible technologies to streamline workflows, and simple solutions to enable efficiency and time reductions, Promega has developed a wide spectrum of bioluminescent assays for the various processes involved in drug discovery. High-throughput screening assays and multiplexed viability and cytotoxicity analysis to screen for candidates; mechanistic assays to understand the MOA of therapeutic candidates and to map the signalling regulation that they orchestrate, kinase assays to screen for possible inhibitors of kinase activity, powerful tools for ADME-Tux screens, technologies to assess the cell health, etc offer the researchers with a bouquet of highly sensitive and easy-to-use tools to support the drug discovery workflow. With a strong pipeline of new technologies, we also offer customisable solutions to suit specific needs.

Symposium on Reproduction and Development

Invited lecture 1

New insights into endometriosis

Asgi T. Fazleabas, PhD

Professor and Associate Chair
Department of Obstetrics, Gynecology and Reproductive Biology
Director, Center for Women's Health Research
Michigan State University
USA

Endometriosis, the presence of endometrial glands and stromal outside of the uterine cavity, is one of the most common causes of chronic pelvic pain and infertility affecting 1 in 10 women of reproductive age. The incidence increases up to 30-50% in patients with infertility. Several theories have been proposed to explain the etiology of endometriosis but the most widely accepted hypothesis is Sampson's theory of retrograde menstruation, in which fragments of menstrual endometrium are refluxed through the fallopian tubes into the peritoneal cavity. Although retrograde menstruation occurs in 70-90% of women, endometriosis is only diagnosed in 10% of this population. While the existence of this disease has been known for more than one hundred years, our current knowledge of the pathogenesis of spontaneous evolution and the pathophysiology of the related infertility remains unclear mostly because diagnosis takes between 8-11 years. Thus, because ethical and practical considerations limit studies in the human, relevant animal models are needed. Menstruating primates develop spontaneous endometriosis, developing lesions that are histologically identical to the human disease and over the past several years it has become evident that the baboon is the most appropriate model to study the pathophysiology of endometriosis. In this model intraperitoneal injection of menstrual tissue mimics the normal physiological process of retrograde menstruation and permits the study of disease progression from the initial onset since this induction recapitulates the human disease. These extensive studies have clearly demonstrated that the presence of ectopic lesions dramatically alters the eutopic environment resulting in rapid changes in micro RNA expression which have functional consequences in the pathogenesis of endometriosis. Lesion development is also continuous and is associated with the aberrant expression of cell fate markers, metastatic inducer proteins and nerve fibers. The ability to study the eutopic and ectopic tissues right at the onset of the disease provides new insights for diagnosis and therapy.

Symposium on Reproduction and Development

Invited lecture 2

Genomics and proteomics of birth weight in healthy Sri Lankan newborns

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Professor of Molecular Life Sciences
Institute of Biochemistry, Molecular Biology Biotechnology
University of Colombo
Sri Lanka

Low birth weight is known to predispose individuals to several non-communicable diseases in later adult life. Improvement in maternal nutrition has not been able to reduce the prevalence of low birth weight beyond a certain level, perhaps in view of the genetic determinants of birth weight. Insulin-like growth factor *(IGF)* genes *IGF-I* and *IGF-II, H19* gene which codes for a non coding RNA and regulate IGF-II action, adipocyte and placental hormone leptin have been implicated in the regulation of birth weight. Reports in the literature are inconsistent and there were no data for Sri Lankans or native South Asians. We examined the association of three dinucleotide repeat polymorphisms of *IGF-I*, Apa-1 polymorphism of *IGF-II,* three SNPs of *H19* (rs2067051, rs217727, rs2839703) with birth weight in a cohort of mother newborn pairs. IGF-1, IGF binding protein-1 which modulate IGF-I bioavailability, leptin and soluble leptin receptor level which modulate leptin bioavailability were also studied. Mothers had uncomplicated, singleton, term pregnancies and the newborns were apparently healthy. Newborn and maternal intron 2 CT repeat polymorphism of *IGF-I* gene showed a significant effect on birth weight and several other birth indices. However these effects were limited to primiparous pregnancies. Maternal, but not newborn *H19*/rs217727 TT genotype was also associated with a significantly higher birth weight, but this effect was not modulated by parity. Effects of *IGF-I*/ intron 2 CT repeat polymorphism and H19 rs217727 TT genotypes on birth weight were independent. Cord blood IGFBP-1 showed a significant negative association with birth weight whereas cord blood leptin showed significant positive association with birth weight. These effects were also independent of each other. Thus the IGF system and the leptin system are independent modulators of birth weight even among newborns from healthy normal pregnancies.

RESEARCH PAPERS
ORAL & POSTER PRESENTATIONS

Research papers – Oral Presentations

Molecular Medicine

OP 01:

Circulating levels of leptin and soluble leptin receptor (SLR) in a cohort of sporadic breast cancer patients

Rodrigo HACIK [1], Tennekoon KH[1], Karunanayake EH[1], Amarasinghe IY[2], De Silva GSK[2]

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Reports on the association of adipocyte hormone leptin with breast cancer are inconsistent. Most studies have not considered soluble leptin receptor (SLR) levels which affect bio-availability of leptin. We examined possible association of circulating levels of leptin, SLR and bio-available leptin (ie: free leptin index) with breast cancer. A matched-pairs study was conducted on women with sporadic breast cancer (N=45) and healthy controls (N=45) matched for age and body mass index (BMI). Patients were recruited at the first diagnosis. Blood samples were collected before treatment. Plasma leptin & SLR concentrations were measured using enzyme-immunoassays. Leptin, leptin/BMI, SLR and the free leptin index (leptin/SLR) were compared between the two groups using Wilcoxan signed rank test. Plasma leptin levels [patients vs controls: geometric mean (95% CI) 34.4 (27.14, 43.59) vs 25.11 (20.79, 30.33) ng/mL; P=0.0002], leptin/BMI [patients vs controls: geometric mean (95% CI) 1.413 (1.138, 1.754) vs 1.033 (0.8804, 1.211); P=0.0002] and the free leptin index [patients vs controls: geometric mean (95% CI) 1.495 (1.141, 1.960) vs 1.008 (0.7825, 1.300); P=0.0006] were significantly higher and SLR levels were significantly lower [patients vs controls: geometric mean (95% CI) 23.01 (20.74, 25.53) vs 24.90 (22.36, 27.74) ng/mL; P=0.0302] in the patients. Leptin gene -2548 G/A polymorphism was not significantly different between patients and controls when analysed in a subset of matched pairs. Leptin appears to exert a positive association and SLR a negative association with breast cancer. Higher leptin levels even when normalized to BMI seen in the patients suggest that in breast cancer, adipocytes have an enhanced capacity to secrete leptin.

This work was supported by National Research Council Grant NRC 11-018 and constitutes part of PhD studies of HAICKR.

OP 02:

Prevalence of missense mutation c.865A>C in BRCA2 gene in a cohort of Sri Lankan breast cancer patients

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Germ-line mutations in breast cancer susceptibility *(BRCA)* 1 and 2 genes are known to predispose women to a higher risk of developing breast cancer. We have already reported several pathogenic and possibly pathogenic mutations in both *BRCA1* and *BRCA2* genes in breast cancer patients. Recently one missense mutation, previously considered as non pathogenic *(c.865A>C/ BRCA2)* has been reclassified as pathogenic for breast cancer following further advance studies by other authors. To report prevalence of pathogenic mutation *c.865A>C in BRCA2* gene in a cohort of Sri Lankan breast cancer patients we reanalyzed data from patients and controls previously studied by us for *BRCA* mutations. Nucleotide sequence data were available for *BRCA2* for a total of 111 patients (familial breast cancer: N = 57; sporadic breast cancer: N = 54) and 20 healthy controls (no personal or family history of breast/any other cancer). Exon 10 of *BRCA2* gene had been directly sequenced following polymerase chain reaction amplification of target DNA. Eight familial breast cancer patients and 3 sporadic breast cancer patients carried c.865A>C mutation giving a prevalence of 14% and 6% respectively for familial and sporadic breast cancer patients. None of the controls carried this mutation. Taken together with pathogenic mutations of *BRCA1//2* we have previously reported from Sri Lanka, c.865A>C mutation in *BRCA2* gene is the commonest so far detected in this cohort of Sri Lankan breast cancer patients. Its prevalence among familial breast cancer was more than twice that in sporadic breast cancer.

This work was supported by Sida/Secretariat for Research Cooperation Grant for Molecular Biology and Biotechnology.

OP 03:

Association of H19 rs217727, IGF-I dinucleotide and IGF-II- Apa 1 polymorphisms with birth weight

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We investigated the possible association of *H19* rs217727 TT genotype and *IGF-II* Apa 1 polymorphism with birth weight in healthy Sinhalese mother-newborn pairs taking into account our previous reports on the association of *IGF-I* polymorphism. *H19* (N=173) and *IGF-II* (N=133) polymorphisms were determined using polymerase chain reaction amplification and restriction fragment length polymorphism. Results were confirmed by primer extension-based SNP genotyping. Multiple regression analysis was carried out using different models to account for maternal *H19* rs217727 TT genotype, IGF-I intron 2 CT repeat polymorphism (either maternal or newborn 189 allele) and *IGF-II* Apa I polymorphism (either maternal or newborn AA genotype). All polymorphisms were in Hardy Weinberg equilibrium among mothers and newborns. A model (model 1) using maternal *H19* and *IGF-II* Apa-1 genotypes, IGF-I intron 2 CT 189 allele, newborn gender and parity explained 11.43% of the variation in birth weight. Maternal *H19* genotype (P=0.0038) and *IGF-I* allele (P=0.0175) were independently associated with birth weight. A model that replaced maternal allele/genotype with newborn *IGF-I* allele/ *IGF-II* genotype explained 18.4% of the birth weight variation. Maternal *H19* rs217727 TT genotype (P=0.0058) and *IGF-I* intron 2 CT 189 allele (P=0.0002) were independently associated with birth weight. *IGF-II* Apa-1 polymorphism, parity and newborn gender did not show a significant effect on birth weight in this cohort. Thus maternal *H19* rs217727 TT genotype and *IGF-I* intron 2 CT 189 allele (maternal or newborn) appear to be independently associated with a higher birth weight in healthy full term newborns.

This work was supported by Sida/Secretariat for Research Cooperation Grant for Molecular Biology and Biotechnology, National Research Council Grant 05-28 and constitutes part of PhD studies of ASH.

OP 04:

***GH1* gene sequence variants in a cohort of Sri Lankan children with short stature**

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Growth hormone deficiency results mainly from genetic defects of growth hormone 1 *(GH1)* gene or growth hormone releasing hormone receptor *(GHRH-R)* gene. The codon 72 mutation of *GHRH-R* gene leading to a premature stop codon resulting in a truncated non-functional receptor is reported to be common in the Indian subcontinent. We previously studied a cohort of short stature children (N=64) with clinically confirmed GH deficiency and observed this mutation to be less common in Sri Lanka, especially among the Sinhalese. In a subset of 20 children negative for *GHRH-R* codon 72 mutation we sequenced the *GH1* gene in the present study. We observed several nucleotide variants (a novel coding region variation 375G>A in exon 2, and reported variations rs6171, rs6172, rs2665802, rs41295043, rs9282699, CS030386). Of these, rs6171 occurred in three and rs2665802 in 6 patients. Other variants occurred in one patient each. 375G>A, a missense mutation that changes the amino acid Alanine to Threonine is deleterious as predicted by the SIFT score. Intron 2 CS030386 is a splice acceptor variant. Affected patients were heterozygous for these two variants. rs2665802, a functional polymorphism in intron 4 is associated with reduced GH expression. One individual was heterozygous for rs9282699 and rs6172, associated with a tendency for shorter stature. No pathological significance is reported to date for the other variants observed. Exon 2, 375G>A, CS030386, rs2665802, rs9282699, rs6172 variants in the *GH1* gene may contribute to GH deficiency and thus to short stature in this cohort.

This work was supported by National Science Foundation Sri Lanka (Grant No: RG/2011/BT/03).

OP 05:

In silico* characterization of a RNA binding protein of cattle filarial parasite *Setaria digitata

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Human lymphatic filariasis (HLF) is a neglected tropical disease which threatens nearly 1.4 billion people in 73 countries worldwide. *Wuchereria bancrofti* is the major causative agent of HLF and it closely resembles cattle filarial parasite *Setaria digitata*. Due to difficulties in procuring *W. bancrofti* parasite material, *S. digitata* cDNA library has been constructed to identify novel drug targets against HLF. In this study, a 549 bp long cDNA termed *sdrbp (S. digitata* RNA binding protein) has been sequenced and characterized *in silico*. The isolated cDNA contains an Open Reading Frame (ORF) of 249 bp and its conceptual translation predicts a protein of 82 amino acids (sdRBP). sdRBP shows an amino acid identity of 54% with the RNA recognition motif (RRM) of the 64 kda subunit of human cleavage stimulation factor (CstF-64). CstF-64 plays an important role in pre-mRNA polyadenylation by interacting with a specific GU-rich downstream sequence element. The structure of sdRBP obtained by homology modeling adopts a typical β1α1β2β3α2β4 topology commonly found in RRM containing proteins. The three dimensional structure of the protein was validated by superimposition tools and Ramachandran plot analysis. Molecular docking studies of sdRBP with different RNA molecules revealed that sdRBP has greater binding affinity to GU-rich RNA. However, as indicated by homology modeling, the sdRBP lacks two N- and C-terminal helices that are unique for RRM domain of human CstF-64. Thus, this study paves the way for functional analysis of RNA binding proteins of S. *digitata* and their evaluation as new drug targets against HLF.

This work was supported by IPICS Grant for Molecular Biology.

Immunology

OP 06:

Evaluation of leptospirosis diagnostic tests: microscopic agglutination test, IgM based ELISA and immunochromatography test

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Leptospirosis is diagnosed on clinical grounds, and confirmed by laboratory testing by the microscopic agglutination test (MAT). There are rapid immunodiagnostic tests that detect IgM antibodies against Leptospira. Their sensitivity, specificity and applicability have not been systematically evaluated against MAT in Sri Lanka. Objective of this study was to compare the two rapid immune-diagnostic tests, ie, IgM by ELISA (Virion-Serion) and IgM based immunochromatographic test (Leptocheck-WB test [Zephyr Biomedicals, India]) against MAT. Clinically suspected leptospirosis patients (n=829) were recruited from National Hospital of Sri Lanka, Base Hospital Homagama and Colombo North Teaching Hospital during the period June 2012 to December 2013. All patients were screened with MAT, IgM ELISA and Leptocheck-WB tests. MAT titer of ≥400, anti-leptospiral IgM >20 IU/ml and presence of a red color band in the test window are considered as positive for MAT, IgM ELISA and Leptocheck-WB test respectively. Patients with alternative diagnosis (n=29) were excluded. Data analysis was performed using two methods, i) considering MAT as reference standard and ii) using Bayesian latent class model analysis which considers each test as imperfect. Positivity of MAT, IgM ELISA and Leptocheck-WB test were 33.4%, 40.5% and 47.4% respectively. The sensitivity and specificity of IgM ELISA respectively 88.4% and 83.5% were higher than for Leptocheck-WB test (85.8% & 71.9%) when MAT was used as reference standard. Bayesian latent class model analysis revealed lowest sensitivity and highest specificity for MAT (77.1% & 98.5%) whereas both parameters were high for IgM ELISA (90.6% & 96%) compared to Leptocheck-WB test (87.4% & 81.7%). The observed high sensitivity and specificity in both analyses make IgM ELISA a suitable test for early diagnosis of leptospirosis whereas Leptocheck WB test is suitable as rapid immune-diagnosis for resource poor settings.

This work was supported by NSF grant NSF/RG/2011/HS/19 and constitutes part of PhD studies of MJRN.

OP 07:

Oxidative damage of lipids and proteins in leptospirosis

Fernando TRGN[1], Niloofa MJR[1], Maduranga S[2], Rodrigo C[2], Karunanayake L[3], De Silva HJ[4], Rajapakse S[2], Premawansa S[5], Handunetti SM[1]

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In pathological conditions, overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) results oxidative stress. Lipid peroxides (LP) and protein carbonyls (PC) are formed by oxidative degradation of lipids and proteins causing cell damage disrupting normal cell functions in severe illnesses. Serum anti oxidant capacity (AOC) is an indirect measure of ROS production which indicates the potential for overall protection against oxidative damage. This study assesses the level of oxidative stress in leptospirosis patients using non-repairable metabolites of protein and lipid damage and to identify the potential prognostic markers in leptospirosis. Laboratory confirmed leptospirosis patients were clinically characterized as severe (SL) and mild (ML) patients (n=40/group). Using standard laboratory methods, serum LP, PC, AOC, bilirubin and uric acid (UA) levels were measured in patients and compared with sera of age, gender matched healthy controls (HC) (n=30). Leptospirosis patients had significantly high serum LP, PC levels and low AOC levels compared to HC ($p<0.005$). Using serum PC cutoff value of 6.21 nmoles/mg protein the test had a specificity of 83% and a sensitivity of 80% ($p<0.001$) and serum AOC cutoff value of 89.5 µM/mg protein, the test had 75% of specificity of and 77% of sensitivity ($p<0.001$) in detecting a case of leptospirosis confirming that both may be used as biochemical markers to identify asymptomatic leptospirosis cases. Serum PC, AOC and uric acid levels were significantly higher in SL patients compared to both ML and HC ($p<0.05$) Using a serum UA cutoff value of 5.42mg/dL, the test had 82.5% of specificity and 62.5% of sensitivity in detecting a case of SL ($p<0.001$) and UA may be used as a potential prognostic indicator for developing severe leptospirosis.

This work was supported by National Research Council grant 12 – 77 constitutes part of PhD studies of TRGNF.

OP 08:

Existence of mammalian counterparts of flagellin recognition machinery in teleosts: evidence at molecular, genomic and transcriptional levels

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Rock bream (RB; *Oplegnathus fasciatus*) is an economically important fish in East Asian aquaculture whose farming has recently encountered serious pathogenic threats. Towards developing a disease prevention scheme, we are exploring the innate immune components of RB. Toll-like receptors (TLRs) are evolutionarily conserved pattern recognition receptors sensing pathogens. In the course of characterizing the TLRs of RB, we identified TLR5, which recognizes bacterial flagellin and transduces the signal, as well as a few important downstream molecules including MyD88, IRAK4 and TRAF6. The number of exons in *RBTLR5* (5), *RBMyD88* (5), *RBIRAK4* (11) and *RBTRAF6* (7) was determined by means of a genomic BAC library. Comprehensive genomic analysis underpinned the genomic evolutionary aspects of each of these components. 5'-flanking region of all of these genes possessed immune relevant cis-regulatory elements. RBTLR5 had an ecto-(22 LRR motifs), transmembrane region and an endo-domain (TIR motif). RBMyD88 was composed of a death domain (DD) and a TIR domain. RBIRAK4 contained a DD and a kinase domain. Whereas, RBTRAF6 possessed a zinc finger RING-type profile, 2 zinc finger TRAF-type profiles, a coiled-coil region and a MATH domain. These domain architectures of their deduced proteins complied with the respective known orthologs. The spatial distribution of their mRNAs was examined in eleven RB tissues using qPCR and a broader expression was detected. While *RBTLR5, RBMyD88* and *RBIRAK4* transcripts were highly expressed in liver, RBTRAF6 mRNA level was highest in blood cells, followed by liver. We injected ultrapure flagellin into fish and examine the in vivo modulation of these genes in tissues including head kidney (HK), spleen (SP), liver (LV), gills (GL), blood cells (BC), intestine (IT) and kidney (KD) post-injection (p.i.). Dramatic up-regulation of *RBTLR5* (GL, HK, IT), *RBMyD88* (HK, GL), *RBIRAK4* (IT, LV, HK) and *RBTRAF6* (HK, IT, GL) was noticed at different time points (3-24 h) p.i. in a tissue-dependent manner. Our findings provide genomic and expressional insights of 4 selected components of flagellin-recognition and signalling pathway, and this is the first documentation of a set of teleostean TLR5 signalling components at the genomic level.

OP 09:

Immunomodulatory activity of Link *Samahan*, a herbal formulation in Sri Lankan market

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Link *Samahan* is a product of Link Natural Products (Pvt) Limited. It contains extracts of fourteen different medicinal plants and is commonly used for prophylaxis against cold and cold related symptoms in Sri Lanka. The aim of this study was to investigate immunomodulatory activity of Link Samahan mainly focusing on its anti-inflammatory activity. Anti-inflammatory activity was determined in vivo by assessing inhibition of rat paw edema, production of reactive oxygen species (ROS) - superoxide ions (O2-) and reactive nitrogen species (RNS) - nitric oxide (NO) by rats and inducible nitric oxide synthase (iNOS) gene expression in rats using RT-PCR. In rat paw edema assay, Link Samahan showed a continuously increasing inhibition in rat paw edema compared to the biphasic pattern exhibited by the reference drug, Indomethacin. The inhibition at the 1st and 3rd -5th hours were comparable (47% vs 45% and 84-77% vs 73-75% respectively, $p>0.05$). However, during early phase, at the 2nd hour Link Samahan exhibited a significantly higher inhibition compared to indomethacin (67% vs 29%; $p=0.013$). Link Samahan treatment significantly inhibited NO and O2- production in rat peritoneal cells (50.5±9.4%; $p=0.017$; 37.9±10.8%; $p=0.041$ respectively). In the same group of rats, Link Samahan treatment inhibited iNOS gene expression in comparison to expression of a house housekeeping gene, GAPDH which was normal, thus exhibiting specific inhibition of NO production. There was no effect of Link Samahan on endothelial and neuronal NOS (eNOS and nNOS) gene expression which are constitutive forms of NOS. In conclusion, this study showed that Link Samahan possesses potent anti-inflammatory activity. Therefore, this study demonstrates a contributory factor in the immunomodulatory activity of Link Samahan and scientifically validates its claimed use for prophylaxis against cold and cold related symptoms.

This study was supported by IBMBB and constitutes part of MSc studies of BVLRR.

OP 10:

***In Vitro* Inhibition of nitric oxide production in rat peritoneal cells by Sri Lankan medicinal plant extracts**

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[2]Department of Chemistry, University of Colombo, Sri Lanka.

Nitric oxide (NO) produced in large amounts by inducible nitric oxide synthase (iNOS) is a known factor for inflammatory diseases. Thus, inhibition of NO production via iNOS is expected to be useful in developing anti-inflammatory agents for treatment of inflammatory diseases accompanied by the overproduction of NO. In this study, methanol/dichloromethane extracts (MDEs) of seven Sri Lankan medicinal plants *(Artocarpus nobilis, Curcuma albiflora, Dipterocarpus zeylanicus, Leucas zeylanica, Vernonia zeylanica, Walidda antidycentirica, Zingiber cylindricum)* were prepared and screened for inhibitory activity against NO production in carrageenan-induced rat peritoneal cells (RPC). Peritoneal cells were collected from rats by injecting carrageenan (5 mg/kg) intraperitoneally and then treated in vitro with different concentrations (15.6-1000 µg/mL) of MDEs in RPMI 1640 medium supplemented with 1% BSA for 30 min at 37°C. The viability of cells after 30 min incubation was assessed by Trypan blue exclusion test. The concentration range of 15.6-500 µg/mL was selected as non-toxic dilution range. In vitro cultured RPC were centrifuged and resuspended in complete culture medium and further cultured for 24h. RPC were treated with 1 mM NMMA in RMPI 1640 medium as positive control showed 70.1% inhibition of NO production. Among the seven plant extracts tested, highest NO inhibition was observed in *Vernonia zeylanica* (97.5 % at 500 µg/mL) and it was moderate in *Artocarpus nobilis* (76.6 % at 500 µg/mL) and *Dipterocarpus zeylanicus* (60.8% at 500 µg/mL). *Curcuma albiflora, Leucas zeylanica, Walidda antidycentirica and Zingiber cylindricum* showed lower inhibitory activity of NO production (35, 34, 23 and 13 % respectively at 500 µg/mL). Therefore, *Vernonia zeylanica, Artocarpus nobilis and Dipterocarpus zeylanicus* can be considered for further purification to the isolation of bioactive compounds.

This work was supported by Ministry of Higher Education Grant for Drug Leads from Medicinal Plants and constitutes part of PhD studies of BADR.

Medicinal Plants

OP 11:

Bioactive tetrahydro-protoberberine type alkaloids from plants of genus *Corydalis*

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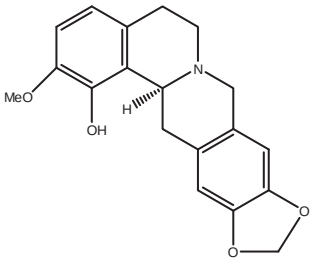
The genus *Corydalis* (family Fumariaceae) comprises 470 species. *Corydalis* is native to China, the Himalayas of Nepal, Pakistan and India, and also found in mountainous regions of Eastern Africa. Ethno medically, the roots have been used in the treatment of syphilis, scrofula, cutaneous infections, along with diarrhea and dysentery. Plant extracts, pure compounds, and alkaloids from different species of this genus showed an inhibitory effect against hepatitis virus, amoeba, tumors, liver cancer, as well as acesodyne and sedative, improved immunological function, hepatocirrhosis, ascites, etc. Tetrahydroprotoberberine-type alkaloids, isolated from genus *Corydalis* are identified as a new category of dopamine receptor ligands and anti-malarial agents.

Twelve Tetrahydroprotoberberine-type alkaloids have isolated from *Corydalis govaniana* Wall. and *Corydalis casimiriana* Duthie and Prain. We have find out different novel and potent activities of this class of alkaloids such as urease inhibition, *β*-glucuronidase inhibition, antioxidant, anticancer, and antileishmanial. Isolation, structural elucidation and bioactivity of



Govaniadine

compounds will be discussed in this presentation.

OP 12:

In vitro* anti-hepatocarcinogenic properties of a mangrove plant *Scyphiphora hydrophyllacea

Samarakoon SR, Chanthirika S, Tennekoon KH, Thabrew MI, Ediriweera PMK, de Silva ED.

Institute of Biochemistry, Molecular Biology and Biochemistry, University of Colombo. Sri Lanka.

Most of the mangroves are woody plants growing along seashores in tropical and subtropical areas and contain plenty of chemical constituents with potential medicinal values. *Scyphiphora hydrophyllacea* is a shrub mangrove plant belonging to the Rubiaceae family and its medicinal properties have not been evaluated. Thus, possible anti-hepatocarcinogenic activity of *S. hydrophyllacea* was evaluated in this study. Dried leaves of *S. hydrophyllacea* were sequentially extracted to hexane, chloroform, ethyl acetate and methanol respectively and tested for cytotoxicity on HepG2 cells by sulforhodamine B (SRB) assay. Most active hexane extract was further tested for pro apoptotic properties in HepG2 cells by DNA fragmentation assay, evaluating morphological changes, quantifying regulation of caspase 3 and caspase 9. Furthermore effects of hexane extract on *p53*, and *Bax*, gene expression were evaluated by real time RT-PCR. Results showed that hexane extract exerted a significant (IC50 = 82.52 ± 12.456 µg/mL) cytotoxicity. Hexane extract mediated apoptosis as shown by significant ($p<.001$) activation of caspase 3 and caspase 9 and significant ($p<0.05$) dose- dependent up regulation of *p53* and Bax. Overall results demonstrate that the hexane extract of *S. hydrophyllacea* is responsible for mediating the *in vitro* anti hepatocarcinogenic effects in HepG2 cells.

This work was supported by International Foundation for Science, Sweden

OP 13:

Hexane extract of *Mangifera zeylanica* bark exhibits cytotoxic activity through induction of apoptosis in triple negative breast cancer cells (MDA-MB-231) and ovarian cancer cells (SKOV3)

Ediriweera PMK[1], Tennekoon KH[1], Samarakoon SR[1], Thabrew MI[1], de Silva ED[2]

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[2]Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka.

Mangifera zeylanica (family: Anacardiaceae) an endemic Sri Lankan plant is used in the traditional system of medicine for cancer therapy. We previously reported that the hexane extract of *M. zeylanica* exerts cytotoxic effects on oestrogen receptor positive breast cancer cells (MCF 7) possibly via caspase independent apoptosis. Here we investigated possible cytotoxic and apoptic properties of the hexane extract of *M. zeylanica* bark on triple negative breast cancer cells (MDA-MB-231) and ovarian cancer cells (SKOV3). Hexane extract was cytotoxic (Sulforhodamine B assay) to ovarian cancer (IC50: 86.6 µg/mL) and triple negative breast cancer (IC50: 116.5 µg/mL) cells 24 h post incubation. Caspase 3 and 7 activities were significantly increased in a dose dependent manner at 4 h post incubation with the hexane extract (doses ranging from 25 to 200 µg/mL) with a fold change of 2.6 to 6.5 in triple negative breast cancer cells and 1.3 to 5.06 in ovarian cancer when compared to untreated controls (ApoTox-Glo™ triplex assay). Acridine orange / ethidium bromide staining and DNA fragmentation analysis further confirmed apoptosis induced by the hexane extract. Thus cytotoxic effects of the hexane extract of *M. zeylanica* on triple negative breast cancer cells and ovarian cancer cells are likely to be mediated via caspase dependent apoptosis. Based on the GC-MS profile the active fraction contained long chain fatty acids, sterols and unidentified compounds.

This work was supported by National Research Council of Sri Lanka (Grant No: 11-018) and constitutes part of PhD studies of PMKE.

OP 14:

Effects of *Flueggea leucopyrus* (Willd.) decoction on HSP 70 expression and apoptosis in triple negative breast cancer cells (MDA-MB-231)

Mendis AS, Thabrew MI, Samarakoon SR, Tennekoon KH

Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka.

Flueggea leucopyrus decoction is used by traditional and folk medical practitioners of Sri Lanka for treatment of breast cancer, despite lack of scientific evidence to validate its anticancer properties. Ongoing investigations carried out at the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB) have shown that *F. leucopyrus* decoction exerts significant cytotoxic effects in breast cancer cell line (MDA-MB-231). Cancer progression is highly dependent on heat shock proteins (HSP's) and they have emerged as promising new targets for anticancer drug discovery. HSP 70 is often over-expressed in tumour cells and reported to enhance cancer cell proliferation by inhibiting apoptosis. An investigation has therefore been carried out to determine whether enhanced apoptosis related to a reduction in HSP 70 expression is a mechanism by which the *F. leucopyrus* decoction mediates cytotoxic effects in MDA-MB-231 cells. HSP 70 expression was evaluated by real time PCR and immunofluorescence. Results showed statistically significant inhibition of HSP 70 expression mediated by the decoction. Effects of the decoction on apoptosis were evaluated by (a) fluorescent microscopic examination of apoptosis related morphological changes and (b) colorimetric estimation of Caspase 9. Results demonstrated that the decoction in a dose related manner, significantly increases apoptosis related morphological changes in MDA-MB-231 cells while enhancing Caspase 9 activity. Based on overall results, it may be concluded that inhibition of HSP 70 is one of the mechanisms utilized by the decoction to mediate its cytotoxic effects in MDA-MB-231 cells and this effect may correlate with enhanced apoptosis in these cells.

This work was supported by National Science Foundation of Sri Lanka, Ministry of Higher Education Grant for Drug Leads from Medicinal Plants and constitutes part of the PhD studies of ASM.

OP 15:

Antifilarial activity of *Curcuma zedoaria* against adult bovine *Setaria digitata*

Senathilake KS, Samarakoon SR, Karunanayake EH, Tennekoon KH

Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Sri Lanka.

Lymphatic filariasis is caused by infections with filarial nematode *Wuchereria bancrofti.* Lack of an effective adulticidal drug possesses a challenge to global filariasis elimination program. With the aim of identification of novel antifilarial compounds, *in vitro* antifilarial activity of sequential solvent extracts of medicinal plant *Curcuma zedoaria* were investigated using adult bovine filarial parasite *Setaria digitata* as a model organism. Extracts were screened in triplicates at 10 to 1000 µg/mL concentrations for 24 h and 48 h by worm motility assay. Motility of worms was recorded relative to solvent control, using an arbitrary score of 3 (highly active), 2 (moderately active), 1 (less active) and 0 (immotile). Cytotoxicity of active extracts to human normal lung epithelial cells (MRC-5 cell line) was investigated by SRB assay. Further fractionation and GC-MS analysis of non-cytotoxic antifilarial fraction was carried out. Hexane extracts of *C. zedoaria* exhibited dose dependent antifilarial activity with end points 250 µg/mL for 24 h, 100 µg/mL for 48 h. Fractionation of active extract yielded one fraction with antifilarial activity and another fraction with both antifilarial and cytotoxic activity (MRC-5 cell line). Further fractionation of active fraction having only antifilarial activity gave a single spot in TLC analysis. Two compounds were identified in this antifilarial fraction by GC-MS analysis. Out of two compounds, one was 99% identical to γ-sitosterol and the other did not show significant similarity (<48%) to any mass spectral pattern in NIST08 mass spectrum database.

This work was supported by the Ministry of Higher Education Grant for Drug Leads from Medicinal Plants and constitutes part of PhD studies of KSS.

OP 16:

Screening of selected medicinal plants for possible cytotoxic effects on breast cancer

Jayarathna DDP[1], Tennekoon KH[1], Samarakoon SR[1], Thabrew MI[1], Karunanayake EH[1], Shanmuganathan C[1], de Silva ED[2]

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[2] Department of Chemistry, University of Colombo, Sri Lanka.

We evaluated potential anti carcinogenic properties of ten medicinal plants using oestrogen receptor positive MCF-7 and triple negative MDAMB 231 breast cancer cells and normal breast epithelial cells (MCF-10A). Air dried leaves were subjected to sequential extraction. Cells were exposed to plant extracts (doses from 25 to 400 µg/mL) for 24 h, and cytotoxicity assessed by Sulforhodamine B assay. Total polyphenol (Folin–Ciocalteau method) and flavonoid (aluminum chloride method) contents in the extracts were quantified and free radical scavenging activity measured (DPPH assay). *Ochna jabotapita* ($IC_{50\text{- chloroform}}$ 80.1 µg/mL) and *Memecylon rostratum* ($IC5_{0\text{- ethyl acetate}}$ 90.33µg/mL) were cytotoxic to MCF-7 cells. *Cleistocalyx nervosum* ($IC5_{0\text{- ethyl acetate}}$ 65.78, $IC_{50\text{- methanol}}$ 71.33 µg/mL) and *Calophyllum moonii* (IC50- hexane 82.22, $IC_{50\text{- methanol}}$ 95.83 µg/mL) were cytotoxic to MDAMB-231 cells. These extracts were not significantly cytotoxic to MCF-10A cells. Highest polyphenol content (*Nepenthes distillatorya* methanol extract) and flavonoid content (*Actinodaphne stenophylla* ethyl acetate extract) were seen in non cytotoxic extracts. Hexane extract of *C. moonii* which was cytotoxic (IC50 1.659 µg/mL) and methanol extracts of *Calophyllum tomentosum* (IC50 1.53 µg/mL) and *N. distillatorya* (IC50 1.08 µg/mL) which were not cytotoxic showed free radical scavenging activity higher than the standard Ascorbic acid (IC50 3.55 µg/mL). Thus medicinal plants appear to exert phenotype specific anti carcinogenic effects which are not always related to their polyphenol or flavonoid content or to their free radical scavenging activity.

This work was supported by Ministry of Higher Education Grant for Drug Leads from Medicinal Plants and constitutes part of PhD studies of DDPJ.

OP 17:

New antileishmanials sesquiterpene coumarins from *Ferula narthex* Boiss

Bashir S[1], Alam M[1], Adhikari A[2], Yousuf S[2], Ahmad B[3], Parveen S[2], Aman A[1], Choudhary MI[2,4]

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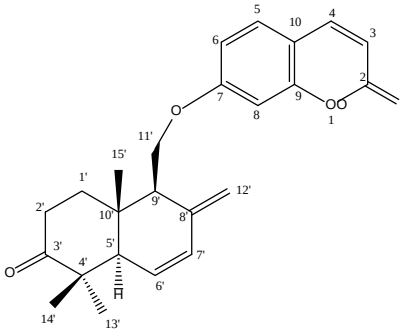
Two new sesquiterpene coumarins, fnarthexone (1) and fnarthexol (2), along with three known coumarin derivatives, conferol, conferone and umbelliferone, were isolated from the plant *Ferula narthex* Boiss. Structures of the compounds 1-5 were elucidated by using modern spectroscopic techniques. Structure of compound 3 was unambiguously deduced by single-crystal X-ray diffraction technique. Compounds 1-4 were tested for *in vitro* leishmanicidal activity against *Leishmania major* promastigotes. Conferol was found to be the most potent with IC50 value of 11.51 ± 0.09 µg/mL.



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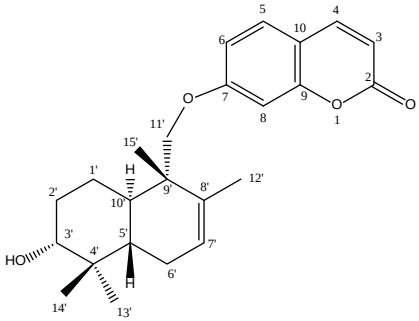

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Plant Molecular Biology

OP 18:

Diversity assessment in Sri Lankan aromatic rice using InDel and SSR markers.

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[1]Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University, Sri Lanka.
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Genetic diversity in traditional and modern rice varieties is important as it provides a basis for rice improvement. Sri Lanka has numerous varieties of traditional rice and some traditional aromatic varieties are preferred by consumers due to pleasant aroma although they have poor agronomic characters. Some of those aromatic rice varieties are perceived to have medicinal and nutritional properties. There are no reports on production of rice varieties using Sri Lankan aromatic rice germplasm. Molecular markers are useful to assess and exploit genetic variability for crop improvement. Therefore, we conducted a study to compare polymorphism among 18 accessions of Sri Lankan aromatic rice with 14 well known aromatic varieties from other countries. We tested the rice germplasm using 16 insertion/deletion (InDel) and 10 simple sequence repeat (SSR/microsatellite) markers that have previously been used for authentication of Basmati rice. The sizes of the amplified alleles were detected by fragment analysis conducted on a DNA sequencer. We found a high level of polymorphism in the Sri Lankan genotypes. Hondarawala, Kuruluthuda and Suwandal appeared to be highly diverse while Suwanda Samba (Ac: 3507) showed a close relationship with Basmati type aromatic rice. Few traditional rice varieties exhibited novel alleles. The results of the genetic diversity will be useful for the selection of the parents for developing rice breeding varieties and studies to authenticate rice varieties.

This work was supported by Commonwealth Academic Fellowship 2011, UK.

OP 19:

Construction of species specific suppression subtractive hybridization library from the endemic wild rice species *Oryza rhizomatis* and characterization of cDNA clones

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O. rhizomatis is a wild rice species endemic to Sri Lanka and is reported to have tolerance to biotic and abiotic stresses prevailing in the country. *O. rhizomatis* was selected as a "tester" plant and a popular cultivated variety Bg352 (*O.sativa*) was selected as a "reference" plant for construction of species specific cDNA library (Suppression Subtractive Hybridization (SSH) library) to isolate and characterize novel genes which are specifically present in this wild rice species and yet absent from the cultivar. Forty recombinant clones were randomly selected from the cDNA SSH library and the inserts were sequenced. Sequence analysis of all forty clones indicated that the suppression and hybridization procedures in the library construction were successful as most of the clones have significant alignment with other wild rice species including *O.minuta, O. punctata, O.officinalis, O.australiensis, O. glaberrimathan O.sativa*. Therefore the genes which were specifically expressed in the wild rice species O. rhizomatis enriched in the SSH library. Two conserved domains for two different proteins; nsLTP1 (non specific Lipid Transfer Protein 1) and Dirigent protein found in *O. rhizomatis* were identified from this cDNA clone analysis (pOr78 and pOr80). These two proteins are involved in control of plant defense responses. Since only 40 out of 250 clones of the SSH library were analyzed, further research should be carried out to isolate specific genes of interest that may found in this endemic wild rice species, *O. rhizomatis.*

Funded by National Research Council of Sri Lanka (Grant no 05-61) and constituted part of the PhD studies of GR.

OP 20:

Differential expression of defense-related genes in moderately-resistant *Sinapis alba* and susceptible *Brassica juncea* upon Alternaria brassicae challenge or defense inducer treatment

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Alternaria black spot of rapeseed-mustard caused by the necrotrophic fungal pathogen Alternaria brassicae is one of the most important diseases in India. A comparative study on defense gene induction was undertaken to explore the signaling pathways that underline the defense responses of susceptible Brassica juncea and moderately resistant *Sinapis alba* to alternaria leaf spot disease by using RT-PCR and Northern blot analysis. The expression of five selected defense-related genes viz., *PR1*, *PR2*, *PR3*, *NPR1* and *PDF1.2* was examined after inoculation of the seedlings of *B. juncea* and *S. alba* with A. brassicae. Transcripts of all five defense-related genes accumulated at a greater level and earlier in *S. alba* than in *B. juncea* upon challenge inoculation with A. brassicae. Methyl jasmonate (MJ) or salicylic acid (SA) also induced some or all of the above mentioned pathogen responsive defense genes in both species to varied levels. Marker genes of SA signaling pathway, such as *PR1*, and JA pathway, such as *PDF1.2*, were also induced by SA or JA or vice versa in both *B. juncea* and *S. alba*, deviating from the signaling pathways in Arabidopsis. Treatment with MJ 24 h before inoculation significantly reduced the size of the necrotic lesion in both *B. juncea* and *S. alba* up to 5 days after inoculation. These results suggest that the involvement of both JA and SA signaling pathways and their cross-talk in *S. alba* conferring its resistance to *A. brassicae*.

This work was supported by Sri Lanka Council for Agricultural Research Policy, Sri Lanka.

OP 21:

Cystathionine-β-synthase domains containing OsCBSCBSPB4 protein from rice confers abiotic stress tolerance to transgenic tobacco plants

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Salinity stress is a major cause which adversely affects the growth, development and productivity of crop plants worldwide. Therefore, it would be fruitful to isolate and functionally identify novel "salinity stress-induced candidate genes" for understanding the mechanism and developing abiotic stress tolerant crop plants. This study reports the cloning, characterization and functional validation of Cystathionine-β-Synthase (CBS) domain containing protein from rice, namely, OsCBSCBSPB4 that possesses two pairs of CBS domains and one Phox/Bemp1 (PB1) domain. Based on microarray and massively parallel signature sequencing (MPSS) data, we have identified and classified CBS domain containing protein (CDCP) family in Arabidopsis and rice and highlighted its possible role in salinity tolerance. CDCP comprises a large super family of evolutionary conserved proteins which are present in all kingdoms of life. OsCBSCBSPB4 protein is found to be localized in nucleus and interacts mainly with proteins that are involved in abiotic stress tolerance. Transgenic tobacco seedlings, over expressing OsCBSCBSPB4 under the control of CaMV35S promoter, when subjected to salinity, drought, oxidative and extreme temperature stress, exhibited delayed leaf senescence, were healthy with profuse roots and increased biomass in contrast to the wild-type seedlings. Overall, OsCBSCBSPB4, a novel CDC gene identified here can confer tolerance to various abiotic stresses, indicating that it might be a good candidate gene for genetic improvement to produce stress-tolerant plants.

OP 22:

The tea shot hole borer beetle (Euwallacea fornicatus) is a vector of the canker causing Fusarium spp of tea [Camellia sinensis (L.) O Kuntze] in Sri Lanka

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The causal agent of the recent outbreaks of stem canker and die back of tea in Sri Lanka was identified as *Fusarium solani* species complex based on colony and conidial morphology. The identification was confirmed by comparison of the TEF-1α sequences. The TEF-1α sequences of two isolates have been deposited at GenBank (AccessionNo.JX467676 and KC800803). A BLASTn search indicated that the sequences of the above isolates are most related to Fusarium spp associated with a die-back disease of Avocado in Israel, and Southern California vectored by Tea shot hole borer (TSHB). TSHB *(Euwallacea fornicatus)* is a known vector of various Fusarias having a symbiotic relationship with such fungi. Usually these fusaria are non-pathogenic on plants. The present study was undertaken to investigate whether TSHB contributes as a vector for canker causing fusaria in Sri Lankan tea gardens. TSHB were collected from symptomatic tea plants having canker and their symbiotic fungi were isolated from the macerated beetle heads. The isolated fungi were then screened for their pathogenicity on tea. The results indicated that some of the fungal strains recovered from the TSHB heads were strongly pathogenic on tea. Koch's postulates were confirmed by consistently reisolating F. solani from inoculated plants. The TEF sequences of the reisolated fungi were as same as the original isolates (JX467676 and KC800803). The above results confirm that the TSHB could act as a vector of canker causing *Fusarium* spp of tea in Sri Lanka.

This work was supported by the Tea Research Institute of Sri Lanka and constitutes part of PhD studies of NHLP.

OP 23:

Evolution of Jasmonic acid biosynthesis pathway from bryophytes to vascular plants

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Jasmonic acid and its metabolites (JAs) are ubiquitously occurring lipid-derived plant hormone molecules that regulate growth, development and defense processes in flowering plants. However, their functions in lower land plants have been overlooked. The model bryophyte, *Physcomitrella patens*, which represents a key evolutionary position between green algae and vascular plants, is an ideal model for studying JAs functions in lower land plants. According to the proposed land plant evolution, jasmonic acid signaling pathway has evolved in vascular plants after the evolutionary split of vascular plants and bryophytes. However, putative key homologous genes involved in jasmonic acid biosynthesis and its signaling pathway are available in *P. patens* genome. Allene oxide synthase (AOS) catalyzes the first committed step of jasmonic acid biosynthesis in flowering plants. Two *AOS* gene of *P. patens* were cloned and shown to have the same function as those of flowering plants. Moreover, based on substrate specificity, plant 12-oxophytodienoic acid reductases (OPRs) can be classified into two groups, group I and group II, of which only group II isozymes involve in jasmonic acid biosynthesis. Six OPR genes, among which only one represents group II, were identified in *P. patens* genome. Two *OPR* genes, which represent group I and group II, were cloned and characterized. Striking results were obtained as the group II OPR enzyme exhibited group I type activity. This unusual functional property clearly explains why *P. patens*, probably all the bryophytes, does not contain jasmonic acid and its signaling pathway though its genome contains the corresponding homologous genes.

This work was supported by Japan Society for the Promotion of Science (JSPS).

Research Papers – Poster presentations

Molecular Medicine

PP 01:

Establishment of DNA extraction from paraffin embedded tissues and preliminary analysis of somatic mutation of *TP53* in breast cancer

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Effectiveness of some of the cancer therapies depend on whether the cancer tissue carries wild type or mutated *TP53* gene. Often the tissue available for testing for such mutations is formalin fixed paraffin embedded tissue and application of molecular DNA-based techniques to such tissues is challenging. Therefore objective of this research was to establish a suitable DNA extraction from formalin fixed paraffin embedded (FFPE) tissue for mutation analysis. Tissue sections (10 micron thick) were taken from paraffin blocks of cancer tissues on to microscopic slides and to microcentrifuge tubes. Tumour tissue identified by comparing with hematoxylin and eosin stained slides was scraped out from sections collected on to microscopic slides. Both samples were processed using a modified organic extraction procedure and commercially available reagents. Quality and quantity of DNA extracted was assessed. Good quality DNA was amplified using target specific primers and polymerase chain reaction. Successful amplicons were sequenced on an ABI 3500 Dx Genetic Analyzer. Tumour tissue scraped out from sections collected on to microscopic slides when extracted with commercial reagents yielded the highest amount of less fragmented, good quality DNA. Amplification was successful for shorter amplicons (114 to 175 bp). Nucleotide sequences suitable for mutation analysis were obtained when *TP53* hot spot mutation regions were amplified using 8 sets of primers. The hot spot mutation regions contain approximately 90% of the *TP53* mutations known to date. This method is suitable for application in clinical settings thus facilitating institution of appropriate therapy for cancer patients.

This work was supported by the IBMBB and constitutes part of MSc studies of MV *(previously presented at Sri Lanka Medical Association Annual Scientific Sessions)*

PP 02:

Association of endometriosis and *p53* gene codon 72 polymorphism in a group of Sri Lankan women

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Endometriosis is a polygenic disease present in 6 to 10% of women in the reproductive age group. Recent studies have explored the association between endometriosis and p53 gene codon 72 polymorphism. Published data on the genetic basis of endometriosis in Sri Lankan women is sparse. This study was designed to evaluate the association between endometriosis and the p53 gene polymorphism in a group of Sri Lankan women. A case control study was conducted in a tertiary care hospital where women with endometriosis (N=25) were compared with women without endometriosis (N=17), both confirmed by laparoscopy or laparotomy. Genotype distribution of the p53 codon 72 polymorphism was analyzed by allele specific polymerase chain reaction and direct sequencing. Allele frequency was compared using chi square test to determine the association. Allele frequencies of the three p53 genotypes, Arg/Arg, Arg/Pro and Pro/Pro in the study population (26%, 60% and 14% respectively) were in Hardy- Weinberg equilibrium. There was no statistically significant difference (p = 0.155) in the frequency of Proline allele between the cases and controls {odds ratio of 1.5 (95% CI 0.83- 2.73)}. However among the women with endometriosis the Proline allele frequency was 36.7% in stage IV and 50% in stage III compared to 25% and 16.7% respectively in stages II and I. In this group of Sri Lankan women, p53 codon 72 polymorphism was not associated with endometriosis although a higher frequency of Proline allele was observed in advanced stages of the disease.

This work was supported by the IBMBB and constitutes part of MSc studies of TN.

PP 03:

Extraction of DNA from old bones: optimization of methods and assessment of suitability for individual identification

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During archaeological and forensic investigations, successful individual identification based on DNA from old bones is hindered by numerous limitations of bone DNA extraction methods. In this study, six different DNA extraction methods were optimized and compared to find out a simple and efficient method of retrieving PCR-amplifiable DNA in terms of purity, quantity and cost using a human bone buried in soil for 75 years. Total demineralization method yielded more DNA than other methods. Mild PCR-amplification with minimal dilutions of bone DNA was attained for DNA isolated using a commercially available DNA extraction kit for formalin fixed paraffin embedded tissue. Amplifications of larger fragments (415 bp and 440 bp) of mitochondrial DNA (mtDNA) failed while successful amplifications and sequencing of mtDNA were accomplished for intermediate sized fragments (207 bp). However, amplification of nuclear DNA which generates shorter fragments (121 bp) failed. Sequence comparison of mtDNA of the 75 year old exhumed bone and whole blood mtDNA of a relative showed no maternal relationship between the deceased and the relative. Analysis of haplogroup informative mtDNA polymorphisms revealed that the examined individuals belonged either to M or N macro haplogroups. Successful amplifications and sequencing of hyper variable regions of mtDNA were achieved for DNA extracted from 10-20 year old bones with different levels of degradation which were neither buried nor burnt. Finally, to dispel the probability of contaminations and ensure the authenticity of results, typing of mtDNA of the analyst was performed.

This work was supported by the IBMBB and constitutes part of the MSc studies of NN.

PP 04:

Plasma free amino acid concentration in a group of apparently healthy adults

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Estimation of plasma free amino acids concentrations is imperative to the metabolic status of the body. Pattern of plasma free amino acid is associated with many metabolic disorders. Hence, the reference range for the plasma concentrations of the amino acid is obligatory for the interpretation of test result and the subsequent diagnosis. Thus, the study has been conceded with the general objective of estimating the plasma free amino acids concentrations in a group of apparently healthy adults. Forty eight healthy adults (aged 20 - 50 years) were chosen for the study. Blood samples were collected from fasting healthy males (N=17) and females (N=31). Acetonitrile was used to remove the protein and peptides from the plasma. Concentration of the amino acid was analyzed on online derivatization through Agilent-1260 High Performance Liquid Chromatography (HPLC).The optimal conditions were obtained with the use of standard. Ranges for the amino acids (N=21) of the healthy adult plasma were calculated parametrically by using SAS statistical analysis software. Glutamine (426 ± 90 µ mol/L) and alanine (380 ± 162 µ mol/ L) were highly detected and aspartic acid (13 ± 7 µ mol/ L), asparagine (15 ± 8 µ mol/ L), glutamic acid (41 ± 25 µ mol/ L), cysteine (36 ± 22 µ mol/ L), methionine (21 ± 11 µ mol/ L) , phenyl alanine (27 ± 10 µ mol/ L) and tryptophan (37 ± 5 µ mol/ L) were detected in low concentrations. Remaining amino acids (N=12) patterns were diverse within 60 – 250 µ mol/ L. Reference concentration profile of plasma free amino acid in healthy adults and method employed in this study could help to diagnose the several metabolic disorders in adult population.

This work was supported by the IBMBB and constitutes part of MSc studies of RK

PP 05:

Association between antibody-mediated platelet cytotoxicity and the severity of dengue infections in Sri Lanka

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The objectives of this study were to determine the association between antibody-mediated platelet cytotoxicity and the severity of dengue infections and also to determine whether platelet cytotoxicity and serum LDH levels could be used as prognostic indicators for disease severity. Two blood samples (on admission and on discharge) were collected from each clinically suspected dengue patient admitted to North Colombo Teaching Hospital (n=47) and the infection was confirmed by anti dengue IgM ELISA and RT -PCR tests. Twenty dengue fever patients (DF), twenty dengue hemorrhagic fever patients (DHF) (defined according to WHO guidelines, 2011) and twenty age-gender matched healthy controls (HC) were recruited and platelet cytotoxicity and serum LDH levels were measured using a colorimetric assay. Lactate dehydrogenate (LDH) released due to in vitro anti-dengue antibody-induced platelet destruction upon incubation of platelets with sera (with and without complement) was measured in order to assess platelet cytotoxicity levels. In the presence of complement, significantly higher mean platelet cytotoxicity levels were observed in DHF patients on admission (74.4%) compared to all other study groups; DHF on discharge (43.9%), DF on admission (33.6%), DF on discharge (19.8%) and HC (15.4%) ($p<0.001$). Platelet cytotoxicity levels of each group were significantly lower in the absence of complement (t-test, $p<0.001$). The serum LDH levels also exhibited the same pattern as platelet cytotoxicity. Further, platelet cytotoxicity levels were significantly correlated with the respective serum LDH values ($r = 0.65- 0.82$; $p<0.05$) suggesting that serum LDH levels may represent the destruction of platelets in vivo. According to the findings, patients who have platelet cytotoxicity and serum LDH levels above the cut-off values of 51.35% and 0.475 (OD 490nm) respectively upon admission, have a potential to develop DHF. The platelet cytotoxicity test had 100% sensitivity and specificity (Area Under the Curve/AUC =1.000, $p<0.001$) while serum LDH test had 73% sensitivity and 70% specificity (AUC=0.789, $p<0.001$) as obtained from Receiver operating characteristic (ROC) curve analysis. Therefore, platelet cytotoxicity levels can be used as a prognostic marker in determining the potential for developing severity in dengue infections while serum LDH levels can be a useful indicator for patient management.

This study was financially supported by Faculty of Science and IBMBB, University of Colombo and Genetech Research Institute, Sri Lanka

Immunology & Marine Molecular Biology

PP 06:

Bioinformatic analysis of four members from Teleostean complement pathway with their immune response against bacterial infections

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The complement system is the killing of bacteria by antibodies and phagocytic cells in an organism. It is a humoral system of innate immunity and a link to adaptive immune responses. This study we have characterized four complement genes, complement 1r (C1r), 1s (C1s), factor D (CfD) and carboxypeptidase N (CPN) from rock bream (Oplegnathus fasciatus).C1r and C1s, the enzymes responsible for the activation and proteolytic activity of the C1 complex and initiate the complement classical pathway. CFD is essential for the activation of the alternative complement pathway where it catalyzes the first enzymatic reaction. CPN inactivates the complement anaphylatoxins of C3a, C4a, and C5a by removal of their carboxy-terminal arginines. In silico analysis was done with molecular biological software and qPCR analysis was carried out to analyze the mRNA expression level of respective genes. C1r bear 704aa and C1s bear 691aa. Both C1r and C1s genes consist of conserved CUB, calcium binding EGF, CCP and trypsin domains. Cleavage site was identified at the 5' end of the trypsin domain. CFD bears 239aa and a conserved trypsin domain. CPN comprises 448aa, Zn binding signatures and PM14 Zn carboxypeptidase site. As complement genes, they exhibited an extremely strong transcription in liver spleen and blood tissues. As immune responses to the E. tarda and S. iniae C1r, C1s and CPN were significantly up-regulated at 6h post infection in liver tissue. Whereas, CFD was up-regulated at 12h post infection of the S. iniae and LPS challenge of spleen tissue. Collectively, our findings indicate that complement genes play a pivotal role in immune responses upon bacterial infection in rock bream as an immune modulator.

PP 07:

Molecular and biological characterization of two antimicrobial peptides, β defensin and piscidin, from rock bream *Oplegnathus fasciatus*

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Antimicrobial peptides (AMPs) are increasingly recognized as critical components of the host's defense against infection. Several types of AMPs have been recently identified from a number of teleosts. The current study reports the genomic structure, transcriptional profiling and functional activity of two AMPs, namely β defensin *(Of-β defensin)* and Piscidin *(Of-Pis)* from rock bream *Oplegnathus fasciatus.* The cDNA and genomic DNA sequences of these two AMPs were isolated from rock bream cDNA and BAC genomic libraries, respectively. The proteomics and genomics study of these peptides was conducted using standard bio-informatics software. The genomic analyses of *Of-β defensin* and *Of-Pis* demonstrated an arrangement of 3 exons (58 bp, 112 bp and 57 bp) and 4 exons (57bp, 91bp, 19bp and 302 bp), respectively. The full-length of cDNAs of *Of-β defensin* and Of-Piswas 256 bp and 485 bp, and had open reading frames of 192 bp and 213 bp encoding polypeptides of 63 aa (7.23 kDa) and 70 aa (7.78 kDa), respectively. The protein structural analysis revealed that the *Of-β defensin* contains PA2c domain (5-59), DEFSN domain (30-59) and three disulfide bridges between six cysteines; while the deduced peptide of Of-Pis possessed one major antimicrobial- 12 domain (1-42) and a signal peptide (1-19). Both AMPs have shown highest similarity with their corresponding fish counterparts. The quantitative real time PCR (qPCR) experiment was conducted to determine the tissue-specific expressional distribution of two AMPs and the mRNA expression in immune challenged tissues. The qPCR results revealed that the *Of-β defensin* and Of-P is transcripts were ubiquitously expressed in all tissues with the highest expression in heart and gills, followed by blood and skin tissues, respectively. The mRNA expression level of *Of-Pis* in gills was significantly up-regulated upon all the immune challenges including poly I:C-, LPS-, *Edwardsiella tarda-, Streptococcusiniae-* and Irido Virus-injections. Antibacterial activity of both AMPs was investigated by means of two short-synthetic peptides (SPs). The SPs of both AMPs displayed differential degree of antibacterial activities against various bacterial strains including *S. iniae, E. tarda, Listeria monocytogenes, Vibrio tapetis and Micrococcus luteus.* These findings imply that *Of-β defensin* and *Of-Pis* may play an important role in the innate immune response and elimination of invading microbial pathogens in rock bream.

PP 08:

Molecular characterization of two Galectins from *Oplegnathus fasciatus:* Transcriptional responses against immune stimuli and biological activities

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Galectins are carbohydrate binding lectins, response to pathogenic invasion as pattern recognition receptors in fishes. In the present study, we describe molecular and functional characteristics of two galectins from rock bream *Oplegnathus fasciatusupon immune* challenges. The full-length of two galectins named as Galectin-1 *(RBGal-1)* and Galectin like protein-B *(RBGal like-B)* were identified from a cDNA library, which comprised of 408bp (135 aa) and 438bp (145 aa) in the coding sequence, respectively. Both genes consisted of a characteristic conserved single galactoside binding domain and designated as proto type galectins. Homology and multiple alignment analyses revealed high conservation of these two proteins among vertebrates. Under normal physiological conditions, the mRNA expression of *RBGal-1* was significantly high in heart tissue followed by head kidney, intestine and brain; however, *RBGal like-B* was significantly high in gill tissue followed by kidney, intestine, heart and spleen. Transcriptional profiling of *RBGal-1* upon bacterial and viral inductions showed a significant up-regulation against bacterial pathogens: Streptococcus iniae, Edwardsiella tarda and immunostimulant LPS; meanwhile, *RbGal like-B* showed a significant up-regulation with both bacterial and viral pathogens: *S.iniae, E. tarda,* rock bream irido virus as well as with immuno stimulants (LPS and poly I:C). Sugar specificity of their recombinant proteins revealed that RBGal-1showed an affinity towards D- galactose, α-lactose and fructose. However, no sugar binding activity was observed forRbGal like-B. These two recombinant proteins showed agglutination activity with *S. iniae, E. tarda, Escherichia coli* and *Vibrio tapetis*. Results from the present study suggested significant role(s) for two galectins from rock bream against invading pathogens.

PP 09:

Two paralogs of NF-κB inhibitor-alpha (IκBα) genes from rock bream *(Oplegnathus fasciatus)*: Genomic organization and expressional analysis

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In this study, we identified two novel IκBα paralogs from rock bream *(Oplegnathus fasciatus)*, which are termed as RBIκBα-A and RBIκBα-B. The IκB family proteins play an important role in NF-κB activity. IκBα is a member of IκB family which sequesters NF-κB in an inactivate form in cytoplasm by blocking its translocation into nucleus. RBIκBα-A and RBIκBα-B proteins showed typical features of IκBα protein including, highly conserved IκB degradation motif, PEST sequence and six ankyrin repeats. However, identity and similarity among their amino acid sequences were only 55.6 and 69.7%, respectively suggesting that these two genes could be different isoforms of IκBα. The number (6) and size of the exons of RBIκBα-A and RBIκBα-B were conserved well with all compared vertebrate species, although they had significantly different genomic sizes. In phylogenetic examination, RBIκBα-A and RBIκBα-B proteins clustered with other IκBα family members; however, RBIκBα-A and RBIκBα-B were placed with two different sub-groups within IκBα family. Tissue specific expression of RBIκBα-A and RBIκBα-B mRNA was investigated by qPCR and found to be constitutively expressed in all the 11 tested juvenile tissues, and both of them showed the highest expression level in heart and liver. The RBIκBα-A and RBIκBα-B mRNA expression in gill and liver were significantly up-regulated by LPS, poly I:C and Edwardsiella tarda challenges. The RBIκBα-A and RBIκBα-B mRNA expression level was up-regulated in early injection time and rapidly restored. Meanwhile, in response to flagellin-injection, their transcript levels markedly increased in intestine, head kidney and spleen in a kinetically similar manner. These results suggest that RBIκBα-A and RBIκBα-B might be involved in rapid immune response reactions in rock bream against bacterial and viral pathogens, and they might also be associated with the flagellin-signalling pathway.

PP 10:

Ligand-receptor system of teleost IL8 signalling: Immune responsive expression and genomic organization of *IL8*, *CXCR1* and *CXCR2* in rock bream

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Interleukin (IL) 8 is a chemokine, which belongs to the cytokine superfamily and propagate the signals through CXC receptors (CXCR) 1 and/or 2. This ligand-receptor pair acts as an important immune-modulating system in mammals. The components of IL8-signaling, including two *IL8* paralogs and cognate receptors of IL8, *CXCR1* and *CXCR2*, were identified in rock bream (RB; *Oplegnathus fasciatus*) by a combined genomic and transcriptomic techniques. Genomic structure of *RBIL8* and *RBIL8-like (RBIL8-L)* genes contained 4 exons and 3 exons, respectively. Both *RBCXCR* genes demonstrated a bi-exonic structure. Coding sequence of *RBIL8, RBIL8-L, RBCXCR1* and *RBCXCR2* encoded proteins of 98, 105, 355 and 360 amino acids, respectively. Both RBIL8s contained a signal peptide, a chemokine module (SCY domain), which includes a CXC signature preceded by an ELR-like motif and 4 invariant Cys residues. RBCXCRs had a G-protein coupled receptors (GPCR) family 1 profile with a GPCR signature and a DRY motif. Seven conserved transmembrane regions were also predicted in both RBCXCRs. Amino acid sequences of these components revealed a strong relevance with other teleost counterparts in terms of homology and phylogeny. In inter-lineage genomic comparison, *RBIL8* complied with orthologous vertebrate gene structure; whereas, *RBIL8-L* revealed a novel genomic structure. Although *RBCXCR1* represented the typical vertebrate gene structure of *CXCR1*, teleost *CXCR2* gene structure was distinct from that of tetrapods. Furthermore, 5'-flanking regions of these genes harboured immune relevant transcription factor biding sites. The mRNA profiles were investigated by qPCR technique. *RBIL8* and *RBIL8-L* transcripts were non-identically expressed with the highest level in gills and spleen, respectively. Predominant levels of *RBCXCR1* and *RBCXCR2* were detected in head kidney, kidney and spleen. While their mRNA distribution highlighted their importance in immunity, it was further confirmed from their induced expression under pathologic conditions. Transcripts of *RBIL8* (gills), *RBIL8-L* (spleen) and *RBCXCR*s (head kidney and spleen) significantly increased post-injection of immunomodulators (LPS and poly I:C) and pathogens *(Edwardsiella tarda, Streptococcus iniae and rock bream irido virus)*. While the attempts to characterize the functional activities of RBIL8s are on-going, these initial evidences pinpoint the crucial role(s) of *RBIL8* and *RBCXCR* genes in the pathogenesis of rock bream fish. This study stands as the first report of a complete IL8-signaling system in a teleost.

PP 11:

Molecular characterization of two STAT1 isoforms from rock bream, *Oplegnathus fasciatus* and their immune responses upon viral infection

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Signal transducer and activator of transcription factors (STATs) are important for the activation of several interferons (IFN) stimulated genes in JAK/STAT pathway. Most of the cytokines and growth factors including type I IFN, type II IFN and interleukins activate the signaling pathway by phosphorylating STATs and transduce the signal to the nucleus. Seven STATs (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6) have been identified and well characterized in mammals. In the present study, two different STAT1 isoforms (*RBSTAT1a and RBSTAT1b)* were identified and characterized from a commercially important fish, rock bream *(Oplegnathus fasciatus)*. The complete cDNA of *RBSTAT1a* and *RBSTAT1b* consisted of 250 and 266 amino acids, respectively. Both RBSTAT1s showed similar domain architecture as mammals. In comparison of RBSTATs with other orthologs, RBSTAT1a shared >90% identity with fish counterparts, whereas RBSTAT1b shared highest identity (65.1%) with *Haplochromis burtoni.* The residues which are important to form phosphotyrosine binding pockets and homodimer interfaces are conserved among all the sequences even though RBSTAT1a and RBSTAT1b shared 42.4% identity. According to the phylogenetic studies, RBSTAT1a clustered with mandarin fish within the fish clade. In contrast, RBSTAT1b clustered with puffer fish in a separated group. Genomic organization revealed that coding sequences of both *RBSTAT1* genes were dispersed into 23 exons disrupted by 22 introns. To understand the immune responses upon viral infection, we have challenged rock bream with poly I:C and iridovirus (RBIV). The mRNA expression levels were quantified by SYBR Green qPCR assay. Both *RBSTAT1*s transcripts were universally observed in all tissues analyzed and highest level was detected in blood. Significantly strong up-regulation of both *RBSTAT1*s was manifested upon poly I:C challenge in blood and liver tissues at middle phase. In contrast, mild up-regulations were detected in blood and liver tissues upon RBIV challenge. These results indicate a potential involvement of *RBSTAT1*sin immune responses against viral infection.

PP 12:

Genomic arrangement of vertebrate *MAPKs p38α* and *p38β* is highly conserved: Identification, comparison and expression of two rock bream *(Oplegnathus fasciatus)* MAPKs

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There are four types of p38 mitogen-activated protein kinases (MAPKs: *α*,*β*, γ, and δ) in vertebrates that play important roles in cellular response to extracellular stimuli. In the current study, we describe two p38 MAPK homologs, *p38α (RBp38α)* and *p38β (RBp38β)* which were identified in rock bream (RB) from a transcriptomic survey. Genomic sequences of *RBp38α* and *RBp38β* were determined from a BAC library and found to contain 12 exons. The coding sequences of *RBp38α* and *RBp38β* were 1080 bp and 1083 bp that encoded proteins of 360 and 361 amino acids. There was a characteristic serine/threonine protein kinase (S_TKc) domain in both MAPKs, which harboured an activation loop with a dual phosphorylation site of Thr-Gly-Tyr (TGY) motif and a substrate binding site Ala-Thr-Arg-Trp (ATRW). Homologically, RBp38α and RBp38β were similar to those of tilapia. Evolutionarily, they shared a closer relationship with other fish counterparts. The 3D molecular modelling of RBp38 proteins and comparison of their domains with human counterparts revealed their similar structure, except a few dissimilarities, suggesting that they may perform biologic functions through similar mechanisms. Comparison of vertebrate *p38α* and *p38β* homologs in terms of genomic arrangement clearly indicated that they are structured with 12 exons with exactly same lengths, except the exons in termini. The 5'-flanking regions of *RBp38α* and *RBp38β* contained transcription factor binding sites for Oct-1, C/EBP, HSF, Cdxa, AP-1, c-Myb and MZF1, suggesting that they are under tight transcriptional control. Differential roles of *RBp38α* and *RBp38β* were evidenced from its transcriptional distribution in normal fish tissues, where *RBp38α* was highly expressed in heart and brain, in contrast to RBp38*β* that was mainly expressed in brain and head kidney. To examine the involvement of these MAPKs in the flagellin (FLA)-sensing mediated by TLR5, we administered ultrapure FLA and examined the gene expression. *RBp38α* and *RBp38β* were differentially up-regulated at transcriptional level. These preliminary findings highlighted the conservation of *p38* MAPK across the vertebrates, their expressional feature in teleosts and their putative involvement in FLA-sensing pathway.

Medicinal Plants, Natural Products, Xenobiotics & Plant Molecular Biology

PP 13:

Investigation of antimicrobial properties of *Areca concinna* extracts

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Rapid increase in antibiotic resistance has prompted a worldwide search for new and more effective antibiotics. Medicinal plants are known to biosynthesize a wide variety of biologically active secondary metabolites and thus become an excellent potential source for the discovery of new antibiotics. *Areca concinna* (Leinthari) is used in traditional medicine of Sri Lanka to treat worm infestations in humans. In the present study antibacterial properties of this plant were evaluated. Different parts of the plant (root, leaves, bark and seeds) were air-dried and powdered samples were extracted with MeOH/dichloromethane mixtures to yield the total organic extracts. These extracts were tested for antibacterial properties against the pathogenic bacteria, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa* by agar disk diffusion method at 200 µg/disk concentration. Only the seed extract showed activity against S. aureus (11 mm zone of inhibition). Further purification of this extract by solvent/solvent partitioning gave four fractions. The water fraction was active against *S. aureus* (at 200 µg/disk concentration) while the other fractions (Hexane, CHCl3, and EtOAc) were not. Water fraction subjected to reverse phase column chromatography gave six fractions, A to F. Only fraction C showed activity against *S. aureus* (10 mm diameter of inhibition zone at 100 µg/disk concentration). Thus the polar fraction of the organic extract of *Areca concinna* seed possess good antibacterial activity against *S. aureus*. Further studies to isolate and characterize the active principle(s) is continuing.

This work was supported by Drug Leads from Medicinal Plants Grant from the Ministry of Higher Education, and constitutes part of the PhD studies of WSdeS.

PP 14:

***In vitro* genotoxicity evaluation of *Walidda antidysenterica* by comet assay**

Baragamaarachchi RY[1], Weerasena OVDSJ[1], Handunnetti SM[1], Samarasekara R[2]

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Walidda antidysenterica (Apocynaceae) is widely used in Asian traditional medicines a remedy for respiratory disorders, hematuria, spermatorrhoea, leprosy, dyspepsia, chest affections, helminthic disorders, dressing the oozing wounds, jaundice, piles, epilepsy, rheumatoid arthritis, osteoporosis, bacterial diseases and gut mobility disorders. Juice extracted from the bark is administrated to treat mouth sores. Seeds possess antibilious property and used to promote conception and making the muscles of vaginal tissue stronger and firmer after delivery. Flowers are used to treat snake bites, and leaves are used to treat several skin disorders such as psoriasis, nonspecific dermatitis etc. However, the presence of Pyrrolizidine alkaloids (a potent genotoxic compound) is also reported from this plant. Therefore this study was carried out to investigate the *in vitro* genotoxic effect of ethanol leaf, stem bark and flower extracts of *W.antidysenterica* on human lymphocytes, using alkaline comet assay. Human lymphocytes were exposed to different concentrations (10 – 50 µg/mL) of extracts (exhibited >70% viability after exposure) for 18 hours and Comet assay was performed according to the protocol described by N.P Singh, with some modifications. Results indicated that the leaf extract induced DNA damages at the concentrations of 30, 40 and 50 µg/mL ($p < 0.05$) and stem bark extract induced DNA damages at the concentrations of 40 and 50 µg/mL ($p < 0.05$). Flower extract did not induce DNA damages even at 50 µg/mL ($p > 0.05$). In conclusion, the results suggest that the leaf extract shows significant genotoxicity compared to stem bark extract, whereas the genotoxicity is insignificant in flower extract at all tested concentrations.

This work was supported by the IBMBB and constitutes part of MSc studies of RYB.

PP 15:

Antioxidant activity and total polyphenolic content of weedy herb *Commelina diffusa*

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Naturally occurring antioxidants in plants have gained an increasing attention worldwide due to their protection against human oxidative stress damage, which is associated with pathology of many diseases. Therefore, exploring medicinal plants for antioxidant activity bears a timely importance in advance. The objective of the present study is to determine the antioxidant activity and total polyphenolic content of Commelina diffusa (Commelinaceae) using five assays based on Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) reaction mechanisms. The whole plant of C. diffusa was extracted with ethanol, using cold extraction technique. Antioxidant activity of ethanol extract was investigated using Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, Ferric Reducing Antioxidant Power (FRAP), Ferrous Ion Chelating (FIC) and Oxygen Radical Absorbance Capacity (ORAC) assays. Total polyphenolic content (TPC) was determined by Folin-Ciocalteu (FC) method. The ethanol extract showed DPPH free radical scavenging activity, having a IC50 of 41.44±1.6 µg/ mL (Trolox: IC50= 5.29±0.09 µg/mL) and FIC activity, having a IC50 of 1916.97±35.98 µg/ mL (EDTA: IC50= 13.07±0.64 µg/ml). The FRAP and ORAC were found to be 581.67±9.31 mg Trolox Equivalents (TE)/ g and 1332.07±192 mg TE/ g respectively. Total polyphenolic content was found to be 59.04 ± 4.21 mg Gallic Acid Equivalents (GAE)/ g. The ethanol extract of *C. diffusa* has shown a good free radical scavenging activity, ferric reducing antioxidant power, peroxyl radical absorbance capacity, weak chelating activity and low polyphenolic content. The present study reveals the need of further investigations of the weedy herb *C. diffusa* for bio-active ingredients.

The work was supported by National Research Council grant 12-100 and constitutes part of MPhil/PhD studies of HDSMP.

PP 16:

Acetyl cholinesterase inhibitory and antioxidant activities of leaves of *Toona ciliata*

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Toona ciliata (Meliaceae) has been used as an important medicinal plant. Pharmacological studies have reported antiulcer, antifungal, antifeedant and cytotoxic activity for the extracts of *T. ciliata*. This study was conducted to determine Acetyl cholinesterase inhibitory (AChEi) and antioxidant activities of ethanolic leaf extract of *T. ciliata*. Air-dried and powdered leaf was extracted with ethanol by cold extraction technique. AChEi activity was measured according to Ellman method. Antioxidant activities of the extracts were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging, ferrous iron chelating (FICA), ferric reducing antioxidant potential (FRAP) and oxygen radical absorbance capacity (ORAC) assays and total phenolic content (TPC). All assays were carried out using Spectra Max 96 well microplate-reader. Ethanolic leaf extract of *T. ciliata* exhibited AChEi activity with an IC50 of 77.49 ± 2.7 µg/mL while the IC50 of the Galanthamine is 0.46 µg/mL. Marked IC50 value of 4.7 ± 0.3 µg/mL was obtained for DPPH radical scavenging activity and that of Trolox is IC50 4.6 ± 0.0 µg/mL. Lower Fe2+ chelation (IC50 4763.27 ± 191.9 µg/mL) was detected compared to EDTA (IC50 12.74 ± 0.2 µg/mL). The FRAP value was 603.6 ± 2.1 mg TE/g of extract while the ORAC value (mg TE/g of extract) was 778.27 ± 0.0 (Green tea - 1362.82 ± 0.2). The extract exhibited phenolic content of 42.65 ± 0.1 mg GAE/g of extract. The results indicated that the ethanolic leaf extract of *T. ciliata* showed good AChEi, free radical scavenging and peroxide scavenging activities indicating further studies on this plant.

This work was supported by NRC grant 12 – 100 and constitutes part of MPhil/PhD studies of HDSMP.

PP 17:

Liquid chromatographic profiling of flavonols in Sri Lankan black tea and green tea

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Tea is the second most popular non-alcoholic beverage, derived from the plant *Camellia sinensis* L. Sri Lankan tea considered as one of the best tea products in the international market, is a major source of dietary polyphenols including flavanols and their derivatives. Flavonols have recently received much attention due to their health promoting effects, mainly high antioxidant activity. This is the first systematic study on the flavonol content of Sri Lankan black tea and green tea carried out using a high throughput technique. Representative samples of broken orange peckoe (BOP) from 42 different localities and 16 different types of green tea were selected for the study. The samples were hydrolysed with 6M HCl and 60% methanol mixture. The flavonol glycosides were hydrolysed into flavonol aglycones, namely kaempferol, quercetin and myricetin, prior to analysis. Analysis of individual flavonols was carried out in Agilent 1260 Infinity HPLC system with UV detection capability at 370 nm. Among the flavonols, quercetin was the major flavonol present in green tea (1.65±0.13 to 2.99±0.08 mg/g, dw- dry weight) followed by myricetin (1.02±0.02 to 2.02±0.31 mg/g dw) and kaempferol (0.64±0.03 to 1.12±0.13 mg/g, dw), whereas black tea was rich in quercetin (1.10±0.09 to 3.11±0.12 mg/g, dw) followed by kaempferol (0.54±0.05 to 1.33±0.13 mg/g, dw) and myricetin (0.18±<0.001 to 1.12±0.06 mg/g, dw).The current study highlights that quercetin is abundantly found in Sri Lankan tea. The flavonol content significantly ($P<0.05$) varies with the geographical location of black tea and type of green tea.

This work was supported by National research Council of Sri Lanka and constitutes part of MPhil studies of BJ.

PP 18:

Inhibition of iNOS expression and enhancement of CD40 and CD40L expression by combined hot water extract of *Coriandrum sativum and Coscinium fenestratum*

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The combined hot water extract (HWE) of *Coriandrum sativum* (*Kottamalli,* Coriander) and *Coscinium fenestratum (Veniwelgeta)* is a frequently used combination in both Sri Lankan traditional and Ayurvedic treatments as an anti-inflammatory agent. Our previous studies on HWE have shown its anti-inflammatory and immunostimulatory activities in rats. Aim of the present study was to determine the effects of HWE's specific immune mechanisms, i) expression of inducible nitric oxide synthase (iNOS), ii) expression of CD40 and CD40L in B- and T- cells respectively, leading B-lymphocyte activation and anti-oxidant capacity. Oral administration of rats with human equivalent dose (HED) of HWE resulted in significant inhibition of NO production (79.1±6.8%; $p=0.001$) and reduction of iNOS expression as shown by RT-PCR, however, it did not affect the expression of endothelial and neuronal NOS (eNOS and nNOS) which are the constitutive forms of NOS. HWE increased the phagocytic cell infiltration significantly (28.1±8.53%; $p=0.003$) compared to the control with a higher macrophage migration (16.1±3.5: $p<0.001$) to the site of inflammation. ABTS assays revealed that HWE has a dose-dependent antioxidant activity. HWE treatment had a significant effect in increasing the expression of both CD40 and CD40L on rat lymphocytes. The increased expression of CD40 and CD40L was detected by immunofluorescence and confirmed by the RT- PCR assays. In conclusion, this study shows that the reduction of iNOS expression and NO production may contribute to the anti- inflammatory activity of the HWE and the increased expression of CD40 and CD40L contribute to its immunostimulatory activity by enhancing lymphocyte activation reflecting an adjuvant effect of the HWE. These data supports the therapeutic claim for the use of HWE of *C. sativum* and *C. fenestratum* in traditional medicine.

This work was supported by IBMBB and constitutes part of the MSc studies of HDAJCH.

PP 19:

Comparative study of the cytotoxic effects of microcistin-LR and crude cyanotoxin extract from Beira lake cyanobacteria on human embryonic kidney (HEK 293) cell line

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Microcystins (MCs) are toxins produced by cyanobacteria from water environments and Microcystin-LR (MC-LR) has been described as a hepatotoxic polypeptide for the past three decades. Increasing evidence suggest that it might also be a nephrotoxin. The aim of this study was to evaluate the cytotoxicity and apoptotic effects of MC-LR and a purified cyanotoxin extract on human embryonic kidney cell line (HEK-293). The toxin extract was prepared from bloom sample collected from Beira Lake using absolute methanol. Filtered methanolic extract was subjected to HPLC-DAD analysis and the amount of MC-LR in the extract estimated. Cells were exposed for 24 h to different concentrations of MC-LR containing cyanobacterial crude extract or pure MC-LR (1.0–200 µg/mL). The cytotoxic effects were evaluated by SRB and MTT cell viability assays. Viability of HEK-293 cells was significantly decreased ($p<0.001$) after treatment with cyanobacterial crude extract (1 µg/mL) and pure MC-LR (10 µg/mL) for 24 h. Moreover, treated cells exhibited marked dose dependant loss of confluence as judged by phase contrast microscopy starting at 1 µg/mL of cyanobacterial crude extract and 10 µg/mL of pure MC-LR. Thus both the crude cyanobacterial extract containing MC-LR and pure MC-LR induce cytotoxic effects in HEK-293 cells. Higher toxicity observed with cyanobacterial crude extract may due to the presence of other toxins in the extract in addition to MC-LR. Cytotoxic and apoptotic effects exerted by MC-LR on kidney cells in-vitro confirm that MC-LR is a nephrotoxin.

This work was supported by National Research Council Grant 11-034 and constitutes part of PhD studies of MAPCP.

PP 20:

Development of markers to identify blister blight resistance and susceptibility in tea *(Camellia sinensis* L.) cultivars.

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[2] Tea Research Institute, Sri Lanka.

Blister blight (BB) leaf disease of tea, caused by the fungus *Exobasidium vexans* Masse, is one of the major diseases which controls mainly by chemical means. Due to high labour/ cost involved in controlling and most importantly pesticide residue related issues, use of resistant cultivars for cultivation is identified essential and hence high priority is given to develop BB resistance cultivars. To enhance the efficiency of such programs, integration of marker assisted selection (MAS) for early selection of BB resistant individuals is fundamental and therefore the objective of this study was to characterize segregating population of BB by morphological, biochemical and EST-SSRs to identify potential marker/s for BB resistance. Field assessments for BB were carried out for 300 F1 individual (derived from a cross between cultivars BB susceptible TRI2023 and resistant TRI2043). Based on BB disease index (BBdiv), 3 extremely susceptible (BBdiv >0.5) and 3 extremely resistant (BBdiv < 0.1) F1 individuals were selected for further studies. Out of 14 morphological characters assessed (based on IPGRI descriptors), leaf color, leaf base habit, leaf pubescence, upper leaf surface, leaf length to width ratio, leaf width, leaf length are found to be highly correlated to BB resistant trait. To identify potential biochemical marker/s, individual catechins (i.e. EC, EGC, ECGC, ECG, EGCG) contents of the selected individuals were quantified by High performance liquid chromatography [ISO 14502-2:2005(E) standard procedure]. Results revealed that epigallocatechin (EGC) level is significantly higher in BB resistant F1 individuals (38.4+03.08 mg/g) than in susceptible individuals (23.62+1.12 mg/g). For identification of DNA marker, characterization of above six F1 individuals was performed using 12 EST-SSR primers. The primer designated as EST-SSR073, generated a distinct amplicon specific to BB resistant individuals. EGC level and the specific amplicon generated by EST-SSR073 for BB resistant individuals show greater potential in early selection of the trait than morphological characters.

This work was supported by National Research Council (Grant No: NRC-09-066) and constitutes part of MPhil/PhD studies of KHTK

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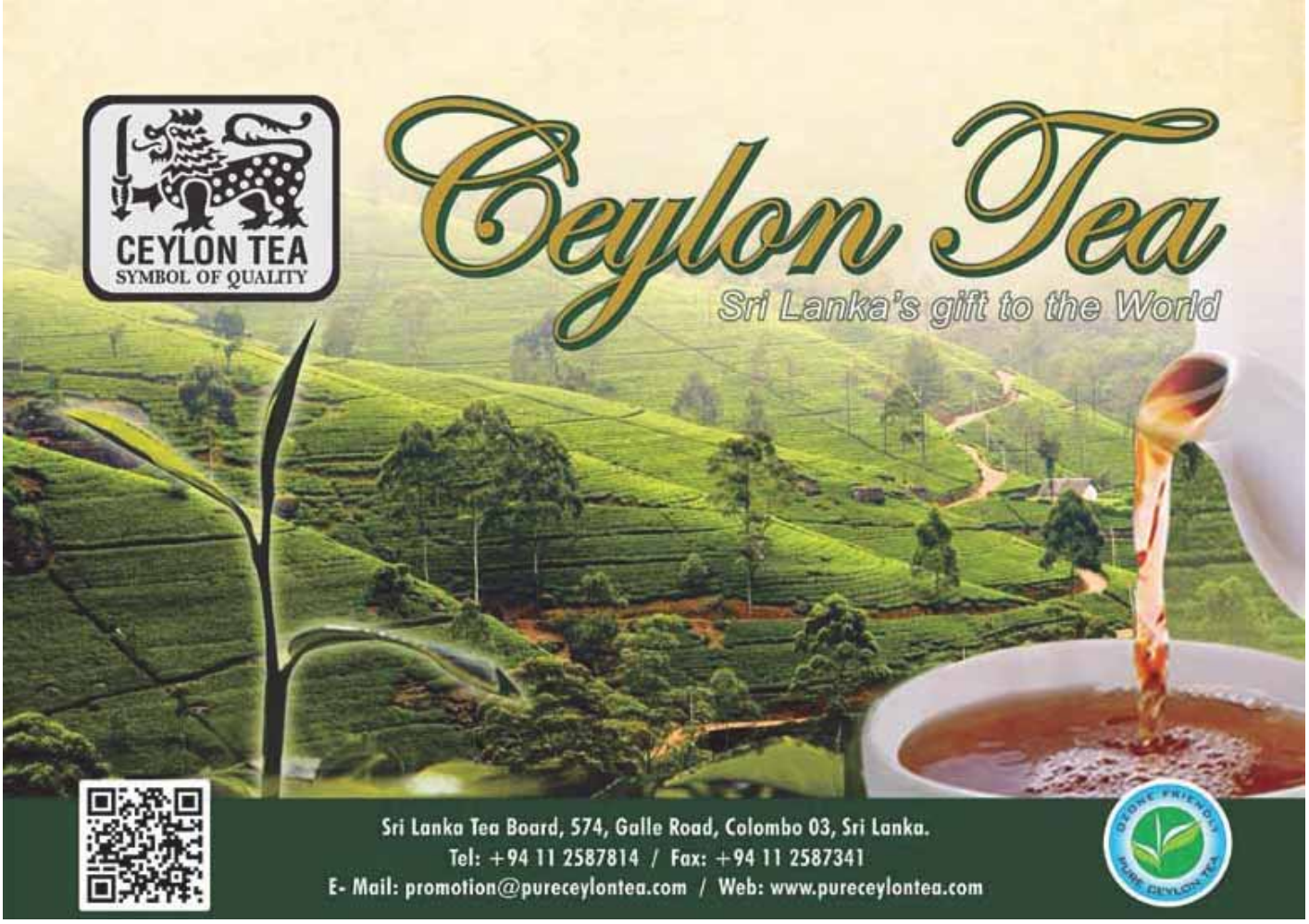
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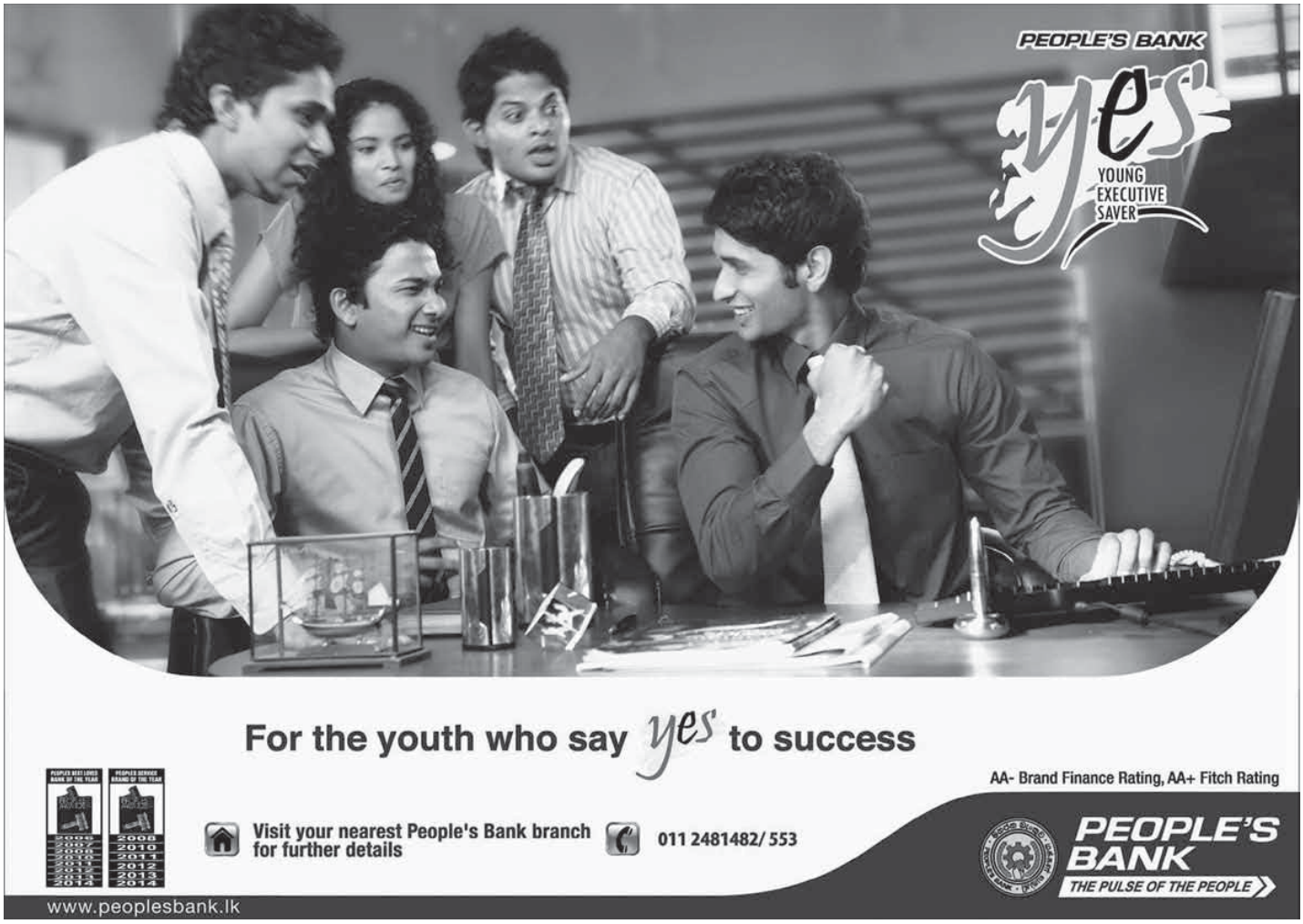

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