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IBMBB

Institute of Biochemistry, Molecular Biology and Biotechnology

Third Annual Scientific Sessions

25th April 2008

Programme and Abstracts

Institute of Biochemistry, Molecular Biology and Biotechnology
University of Colombo

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Institute of Biochemistry, Molecular Biology and Biotechnology
University of Colombo

Our Vision

"To be
an
International
Centre of Excellence
in
Molecular Life Sciences"

PROGRAMME

09.00 h - Lighting of the Traditional Oil Lamp

09.10 h - Welcome address by Director, IBMBB

09.20 h - Address by Prof. Gamini Samaranayake
Chairman, University Grants Commission

09.30 h - Address by Prof. Kshanika Hirimburegama
Vice Chancellor, University of Colombo

09.40 h - **Professor Stanley Wijesundera Memorial Lecture**
What in my DNA makes me, me?
by Vidya Jyothi Prof. Eric H. Karunanayake
Chairman, National Research Council
and Emeritus Professor, University of Colombo

10.40 h - Vote of thanks

10.45 h - Reception

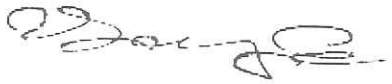
11.30-12.20 h: **Plenary Lecture**
Role of metabolic enzymes and insensitive target sites in mosquito resistance to insecticides
by Prof. S. H. P. P. Karunaratne, Professor of Zoology
and Dean, Faculty of Science, University of Peradeniya

12.20-12.25 h: Presentation of student research awards

13.30 h onwards: Research Presentations

Message from the Chairman/UGC

I am pleased to note that the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB) of the University of Colombo has organized its third Annual Scientific Sessions in April this year. It is commendable that despite being established in 2004, the IBMBB has conducted Annual Scientific Sessions during the last two years and has sustained it. It is mandatory that an academic institute specially with a research focus in an area like Molecular Biology and Biotechnology conduct research sessions in order to disseminate the new knowledge generated and also to share the experiences gained with other stakeholders. This occasion also would be an opportunity to portray the academic standards of this institute to attract more students as well as funds and recognition. In this respect I believe that the IBMBB would benefit immensely through this research sessions and open its resources for all those who need facilities to pursue specialized studies in Molecular Biology and Biotechnology in Sri Lanka.



Professor Gamini Samaranayake
Chairman
University Grants Commission

Message from the Vice-Chancellor/University of Colombo

It is with great pleasure that I issue this message on the occasion of the 3rd Annual Scientific Sessions of the Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB) of the University of Colombo, Sri Lanka.

It is heartening to note that the IBMBB within a short period of less than five years from its establishment has emerged to be a Centre of Excellence in terms of research and development in Life Sciences at Molecular level under the able leadership of Professor Kamani Tennakoon the Director. The Institute is also providing postgraduate training in the respective fields at Master of Science, Master of Philosophy and Doctor of Philosophy levels by attracting eminent academics & research fellows.

This Institute was established with funding from the Swedish International Agency for Research Co-operation with Developing Countries (Sida) mainly due to the vision and the academic insight of Vidya Jyothi Professor Eric H. Karunanayake who was also the founder Director of the Institute. Today, the IBMBB has excellent facilities and highly sophisticated equipment to provide postgraduate training and research at the cutting edge Molecular Life Sciences, meeting international standards. In addition to its international links, in particular with Swedish Universities, the IBMBB has developed collaborative programmes with other Sri Lankan Universities and Research Institutes to carry out need base research on national issues.

I congratulate Prof. Kamani Tennakoon, the Director and the academics and the other staff involved in organizing this Scientific Sessions for the third successive year. I also would like to thank the sponsors, donors and well-wishers for their generous contributions and support.

I wish the scientific session a success.

Professor Kshanika Hirimburegma
Vice-Chancellor

Message from the Director/ IBMBB

Institute of Biochemistry, Molecular Biology and Biotechnology is holding its 3rd Annual Scientific Sessions today. Highlights of the day will be the 3rd Professor Stanley Wijesundera Memorial Lecture which is to be delivered by Professor Eric H Karunanayake, founder Director of the IBMBB and a plenary lecture by Professor S.H.P.P. Karunaratne, Dean, Faculty of Science and Professor of Zoology, University of Peradeniya. Students of the IBMBB will be presenting their research findings at these sessions. In addition there will be two presentations from academics and students from other Higher Educational Institutes.

These research presentations would not have been a reality without our research collaborators, funding agencies and the hard work of students. Our collaborators are many and diverse coming from the Health Sector, Agricultural Sector and the Academia. Research projects were supported by Swedish Agency for Research Cooperation with Developing Countries, National Research Council, National Science Foundation and University Grants Commission. Our students are either direct IBMBB students registered for postgraduate degrees at the IBMBB or indirect, that is those who are registered at other Higher Educational Institutes for postgraduate degrees but carrying out research studies at the IBMBB. During the period from 2004 to 2006 an estimated 2.9 million rupees has been spent by staff of the IBMBB from competitive research funds that they had obtained in supporting these indirect students.

Professor Stanley Wijesundera Memorial Lecture is an annual event held during the annual scientific sessions to commemorate late Professor Wijesundera who served as Professor of Biochemistry and First Vice-Chancellor, University of Colombo from 1978 to 1987. He was a visionary leader par excellence. Many of us have reaped the fruits of his commitment to the development of the University of Colombo. He played a key role in supporting establishment of the discipline of Molecular Biology in the University of Colombo. First Professor Stanley Wijesundera Memorial Lecture was delivered by Professor Rune Liminga, former Director of the International Programme in Chemical Science, University of Uppsala, Sweden and the second lecture by Professor W.D. Lakshman, former Vice- Chancellor, University of Colombo. This year, Professor Eric H. Karunanayake will pay tribute to late Professor Wijesundera by delivering the 3rd Professor Stanley Wijesundera Memorial Lecture enlightening us on how DNA makes one, oneself.

On behalf of the Board of Management, staff and students of the IBMBB, I would like to thank Professor Gamini Samaranayake, Chairman, University Grants Commission and Professor Kshanika Hirimburegama, Vice-Chancellor, University of Colombo for messages given for this supplement and for agreeing to grace the occasion. Professor Eric Karunanayake is thanked for agreeing to deliver Professor Stanley Wijesundera memorial lecture and Professor S. H. P. P. Karunaratne for being the plenary lecturer for the sessions. A very special thank you is due to Mrs. Anoja Wijesundera, Mr. Shalitha Wijesundera and other family members of late Professor Wijesundera for sponsoring the Professor Stanley Wijesundera Memorial Lecture from its inception. Students, collaborators and funding agencies are thanked for their respective roles in making research programmes a reality and our advertisers for their generous sponsorship.

Professor Kamani H Tennekoon
Director / IBMBB

Oral Communications

Session 1: Reproduction and Molecular Biology

13.30 – 13.45 h (Abstract 01): Association of -2548 G/A polymorphism in the leptin gene with pre-eclampsia/ pregnancy induced hypertension
Sugathadasa BHKR, Tennekoon KH, Karunanayake EH, Kumarasiri JM,
Wijesundera APDS

13.45 – 14.00 h (Abstract 02): Study of *Apa 1* polymorphism of insulin like growth factor (IGF)-II gene and its association with birth indices
Jayanthiny P, Tennekoon KH, Karunanayake EH, Peiris TKG, Kumarasiri JM,
Wijesundara APDS

14.00 – 14.15 h (Abstract 03): Maternal serum levels of placental growth factor in pregnancy induced hypertension /pre-eclampsia.
Wijayasinghe YS, Sugathadasa BHKR, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundera APDS

14.15 – 14.30 h (Abstract 04): Promoter sequences targeting tissue specific gene expression of CD80 in murine
Vivehananthan K, Sahoo NC, Rao KVS

14.30 – 14.45 h (Abstract 05): Establishment of expression microarray analysis for rat uteri samples using oligo arrays
Premakumara KS, Tennekoon KH, Karunanayake EH

Session 2: Immunology, Cell Biology, Medicinal Plants, Plant Molecular Biology

14.45 – 15.00 h (Abstract 06): Determination of serum IgA and rheumatoid factor IgA-RF in association with clinical manifestations in a population of rheumatoid arthritis patients in Sri Lanka

Goonetilleke S, De Silva LWV, Rajapaksa GK, Wijayaratne LS, Udagama-Randeniya PV

15.00 – 15.15 h (Abstract 07): Establishment of primary cultures of the rat endometrial stromal cells

Eswaramohan T, Tennekoon KH, Karunanayake EH

15.15 – 15.30 h (Abstract 08): Anti inflammatory activity of leafs of *Ixora coccinea*: Use of *in vitro* and *in vivo* assays for activity-directed fractionation

Kumara RR, Nanayakkara AA, Deraniyagala SA, Ratnasooriya WD, Handunnetti SM

15.30 – 15.45 h (Abstract 09): Isolation and characterization of putative plant disease resistance gene analogs (RGA) from Sri Lankan traditional rice varieties.

Ariyawansa KGSU, Weerasena OVDSJ, Fernando KKS, Rajapkse RMT

Poster presentations

Cancer

PP 01 (Abstract 10): Charaterization of novel mutations and recurrent polymorphisms in exon 11 of *BRCA1* gene in Sri Lankan breast cancer patients and at risk individuals
De Silva JWN, Karunanayake EH, Tennekoon KH, Amarasinghe I, Angunawala P

PP 02 (Abstract 11): Preliminary screening for germ line mutations in exons 7 and 12 of *BRCA*-2 gene in a selected group of Sri Lankan breast cancer patients
Rodrigo HACIK, Wickramaratne HVGS, De Silva JWN, Tennekoon KH, Karunanayake EH, Amarasinghe I, Angunawala P

PP 03 (Abstract 12): Screening for germ-line mutations of exon-8 in the *TP53* gene in a family with a *BRCA-1* mutation
Rozani JG, De Silva JWN, Karunanayake EH, Tennekoon KH, Amarasinghe I

PP 04 (Abstract 13): A preliminary study on the detection of micrometastasis of breast cancer by immunohistochemical identification of K-19 antigen in axillary lymph nodes
Wittachchi RS, Weerasinghe A, Angunawela P, Handunnetti SM

Parasitic diseases

PP 05 (Abstract 14): Cloning, sequencing and characterization of filarial parasite's specific genes
Wickramanaryake M, Murugananthan A, Samarakoon SSR, Karunanayake EH

PP 06 (Abstract 15): Partial purification of a 50kDa protease secreted by the infective larvae of parasitic nematode *Toxocara canis*.
Jayananda YPL, Athauda SBP, Rajapakse RPVJ

Reproduction

PP 07 (Abstract 16): Gln223Arg polymorphism in the leptin receptor: prevalence among Sri Lankan women with pre-eclampsia/pregnancy induced hypertension
LiyanageWI, Sugathadasa BHKR, Tennekoon KH, Karunanayaka EH, Kumarasiri JM, Wijesundara APDS

PP 08 (Abstract 17): Insulin-like growth factor binding protein-1 in pre-eclampsia/pregnancy induced hypertension
Peiris TKJ, Sugathadasa BHKR, Tennekoon KH', Karunanayake EH, Kumarasiri JM, Wijesundera APDS

PP 09 (Abstract 18): Establishment of two dimensional gel electrophoresis technique for analysis of human placental proteome: A preliminary report
Wijayasinghe YS, Peiris TKG, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundera APDS.

Medicinal Plants

PP 10 (Abstract 19): Anti-inflammatory activity of rhizomes of *Alpinia calcarata*
Kalaiarashi K, Kumara RR, Ratnasooriya WD, Handunnetti SM

Plant Molecular Biology

PP 11 (Abstract 20): Preliminary studies on genetic differentiation of finger millet varieties using AFLP markers
Senanayake SMC, Dassanayake PN, Weerasena OVDSJ

PP 12 (Abstract 21): Development of simple sequence repeat (SSR) DNA polymorphism marker to detect salinity tolerant rice (Oryza sativa L.) varieties in Sri Lanka
Wijekoon RDWMASA, Niroshini E

Centre of Excellence in Molecular Life Sciences

Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), University of Colombo, established in 2004 with funding from the Swedish International Development Agency (SIDA) is now considered a Centre of Excellence in Molecular Life Sciences. The International Programme in Chemical Sciences, University of Uppsala, Sweden has identified IBMBB as a Resource Centre in Molecular Life Sciences in Asia. In the year 2007, the IBMBB was elected a member of the European Molecular Biology Network (EMBnet) as its national node for Sri Lanka.

Molecular Life Sciences encompass Biochemistry, Physiology and Genetics as the main disciplinary components. The unveiling of the Human Genome Sequence, the blue print of human life, in 2003, and prior and post sequencing of the genomes of the malarial parasite, *Plasmodium falciparum*, *Mycobacterium tuberculosis*, the rice genome, and hundreds of other genome sequences represent landmark achievements in molecular life sciences in the latter part of the 20th century. The advances in molecular technologies and the application of computer science in the study of molecular biology, defined as Bioinformatics have further contributed to revolutionary achievements in Biology. So much has been achieved, the scientists consider the 21st century as the century of Molecular Life Sciences. The application areas of rapid advances and developing technologies are numerous. The biomedical sciences, agriculture, environmental sciences, biodiversity, marine sciences, and food safety are few areas where molecular technologies are applicable to improve the quality of human life.

The world renowned scientists both in the advanced and developing countries are fully convinced that these technologies are of tremendous potential not only for advanced countries but for the developing countries to achieve rapid economic growth. These views have also been expressed by the Royal Society of UK, The National Academy of Sciences of the USA, Indian National Academy and hundreds of relevant reports have been published.

The world food availability and stability, access to clean drinking water, prevention of tropical diseases and vaccination, utilization of natural resources and biodiversity, prevention of natural disasters, the use of traditional knowledge of agriculture and treatment of disease are major issues the world will face in the 21st century. The scientific knowledge that has emerged in the last century would provide the tools to solve many of the problems facing the developing countries. The developing countries are in fact at an advantageous position because the advance technologies are presently available. However, the application of these technologies to solve the problems of developing countries and achieve economic growth require highly trained scientists at cutting edge disciplines. The two most important requirements are, in my opinion, the human resource development and institutional capacity building for which a national commitment of funds is a *sine qua non.*

The IBMBB is fully equipped with all the advanced facilities available in any advanced country in the world. It can undertake the training of young scientists to PhD level in molecular life sciences. These programmes can be selected in areas relevant to national development such as biomedical sciences, agriculture, and biodiversity. However, initiations of such programmes are greatly hampered by severe shortage of funds for consumables, supplies and monthly stipends for the PhD students.

Two main objectives in the establishment of IBMBB were, firstly to undertake human resource development at MSc and PhD level in molecular life sciences and secondly to undertake research and development in areas relevant to the development of Sri Lanka using molecular tools. These two objectives are intimately linked to each other. Even today, in the advanced countries and at seats of scientific excellence, it is the PhD students who carry out the research in basic sciences required for industrial productivity. Though, the private sector is considered as the engine of growth, the science and technology capacity of a country is the power that drives the engine of growth

Soon after inauguration, in 2004, the IBMBB initiated two Master's programmes, namely an MSc in Molecular Life Sciences and an MSc in Cellular and Molecular Immunology, both first of its kind in Sri Lanka. The fourth batch of students was admitted in February, 2008. We are pleased to note that there is a growing demand for these two courses. These are fee-levying courses, charging Rs. 220,000 per student currently. It should, however, be emphasized that the research projects carried by these students are subsidized by research grants obtained by the academic staff from highly competitive funding agencies both foreign and local. Like the graduates, some of the successful MSc graduates also leave the country for further studies towards PhD. This is once again due to lack of funds and non availability of a national programme to support PhD training despite IBMBB having all the advanced facilities for such training. At current rates, the successful training of a PhD in Molecular Life Sciences in Sri Lanka is in the region of Rs. 6 - 7 million. Not only the country is loosing young bright scientists, but their departure from Sri Lanka also deprives the man power needed to initiate research projects relevant to the development of our country. In foreign institutions our talented young scientists will be carrying out research to develop national economies and industries of advanced countries. In contrast, if these young scientists are provided the opportunities to undertake PhD training at institutions like the IBMBB, they will inevitably conduct research on problems relevant to the development of Sri Lanka. Furthermore such training will also strengthen the capacity of local universities for research and their infrastructure.

Several recently established universities in Sri Lanka have academic departments which require expertise in Molecular Life Sciences. The University Grants Commission should provide funding for training probationary academic staff from such universities. The IBMBB could play a very important role in training such probationary staff in Molecular Life Sciences.

The current research activities at IBMBB include molecular genetics of cancer, human DNA variations, development and reproductive biology, Filariasis, Malaria, Molecular Entomology and Plant Molecular Biology (Tea, Rubber, Coconut and Rice) and

medicinal plants. The relevance of these programmes to national development needs no emphasis.

We hope the Ministry of Higher Education and the University Grants Commission make use of the excellent facilities available at IBMBB to promote postgraduate research in Molecular Life Sciences. IBMBB requires additional academic staff and proportionate increase in supporting staff, and funds for costly consumables. This is an essential and relevant investment to achieve excellence in postgraduate training and research in the university system in general and in particular maximize the use of cutting edge facilities available at IBMBB, the outcome of which will have a tremendous impact on the development of Sri Lanka.

VidyaJyothi Prof. Eric H Karunanayake
Founder Director/IBMBB
Emeritus Professor of Biochemistry

Plenary Lecture:

Role of metabolic enzymes and insensitive target sites in mosquito resistance to insecticides

Development of insecticide resistance creates severe threats to mosquito control programmes. Knowledge on the underlying mechanisms of resistance is essential to select appropriate insecticide/s and to design new insecticides against these acquired resistance mechanisms. Resistance to the commonly used insecticides, which attack the insect nervous system, is mainly caused by increased activity of insect metabolic enzymes and/or mutated target sites.

Esterases purified from Sri Lankan *Culex quinquefasciatus* and *Cx tritaeniorhynchus* could rapidly bind and inactivate organophosphates. Antiserum was used to isolate the respective genes and gene amplification was proved to be responsible for the increased enzyme production in resistant mosquitoes. Studies with the antiserum and electron microscopy showed that the heavy expression of esterases is localized to the mosquito sub-cuticular layer and the gut wall, which are the main entry points of insecticides. A strong negative correlation between esterase activity levels and parasite RNA levels could be observed in field collected as well as colonized Sri Lankan *Cx quinquefasciatus*. Predominance of the elevated esterase-based resistance mechanism in *Cx quinquefasciatus* may therefore be influenced by the dual roles of insecticide detoxication and reduction of microfilarial burdens. A normalised cDNA library was prepared and is being sequenced from *Cx quinquefasciatus* mosquitoes infected with various developmental stages of parasite, to identify the mosquito genes that are differentially expressed as a response to parasite development using microarrays.

Two mutant alleles of the voltage-gated sodium channel gene (gene of the target site of pyrethroids) were found from pyrethroid resistant *Cx quinquefasciatus*. Both mutations

resulted in the L1014F mutation either due to an A-to-T or an A-to-C substitution. Very high frequency of these mutations was found throughout the country with the A-to-C substitution predominant over the A-to-T, posing a serious threat to future pyrethroid based control programmes.

Pyrethroid resistance in *Anopheles culicifacies* and *An. subpictus,* was tested in two malarious districts of Sri Lanka. Partial sequencing of sodium channel genes revealed the presence of the classic leucine to phenylalanine substitution, TTA to TTT, in *An. subpictus.* It appears that both mutated target sites and monooxygenase-based resistance underlie pyrethroid resistance in malaria vectors of Sri Lanka.

Prof. S.H.P. Parakrama Karunaratne

B.Sc., M.Sc. (Perad.), Ph.D. (Lond), FRES (Lond.), FNASSL

Dean & Professor of Zoology, Faculty of Science, University of Peradeniya

Abstract No: 01

Association of -2548 G/A polymorphism in the leptin gene with pre-eclampsia/ pregnancy induced hypertension

Sugathadasa BHKR [1], Tennekoon KH [1], Karunanayake EH [1], Kumarasiri JM [2], Wijesundera AP DS [2]

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[2]Castle Street Hospital for Women, Colombo

OB gene which encodes for leptin has three exons, two introns and spans approximately 18kb of genomic DNA. The promoter region contains several putative binding sites for transcription factors. Cytosine enhancer binding protein (C/EBP) receptor and peroxisome proliferator-activated receptor-γ of the *OB* gene promoter are up regulated in pre-eclampsia (PE)/pregnancy induced hypertension (PIH). We investigated possible association of -2548 G/A polymorphism in the *OB* gene promoter and plasma leptin and soluble leptin receptor (SLR) concentrations in patients with PE/PIH.

Non-fasting venous blood samples were obtained from women with PE/PIH (N=62) and with normal pregnancies (N=63). Plasma leptin and soluble leptin receptor (SLR) were determined using enzyme-immunoassays. Polymerase chain reaction followed by RFLP with C*fo*I was used to determine the -2548 G/A polymorphism. Banding patterns were visualized on agarose gel and the polymorphism confirmed by direct sequencing. In addition -2548 G/A polymorphism in 60 unrelated healthy controls was also studied.

Relative risk (RR) for PE/PIH in the presence of A allele (AA and AG genotypes) against carriers of GG genotype was significantly higher (RR=1.67, 95%CI 1.28-2.18, $p<0.0001$). PE/PIH ($p<0.0001$) but not the genotype had a significant effect on leptin, leptin/SLR, BMI or leptin/BMI with the presence of PE/PIH resulting in higher values (two way ANOVA). For SLR both PE/PIH ($p< 0.0001$) and genotype ($p< 0.05$) had a significant effect. Presence of PE/PIH resulted in lower SLR values and the AA and AG genotypes when compared to the AG genotype, resulted in higher SLR values.

Leptin system, in particular the leptin genotype could therefore be a contributory factor in the development of PE/PIH.

Supported by SAREC Grant for Molecular Biology and Biotechnology, and UGC Lead Initiative Grant

Abstract No: 2

Study of *Apa I* polymorphism of insulin-like growth factor (IGF)-II gene and its association with birth indices

Jayanthiny P[1], Tennekoon KH[1], Karunanayake EH[1], Peiris TKJ[1], Kumarasiri JM[2], Wijesundara APDS[2]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo

[2] Castle Street Hospital for Women, Colombo

Insulin-like growth factor (IGF) II gene plays an important role in fetal growth. The influence of IGFII genotype on birth indices was evaluated in a cohort of normal healthy newborns and their parents (N=61). Maternal, paternal and cord blood were genotyped for the IGFII gene *Apa I* polymorphism by PCR-based RFLP method and confirmed by direct sequencing. This polymorphism was also tested in an unrelated group of healthy individuals (N=62). The wild type sequence contains *Apa I* recognition site and is designated as the A allele. The presence of polymorphism results in the loss of the *Apa I* recognition site and is designated as the B allele. Distribution of genotype AA, AB and BB were 6%, 69%, 25% for mothers, 5%, 64%, 31% for fathers, 6% 62%, 31% for newborns and 10%, 60%, 30% for the unrelated group respectively. The frequencies of the A and B alleles were 0.41 and 0.59 for mothers, 0.37 and 0.63 for fathers, 0.38 and 0.62 for newborns, and 0.4 and 0.6 for the unrelated group. *Apa I* polymorphism of the newborns, mothers or fathers did not significantly affect birth indices (birth weight, length, chest circumference and head circumference) of the newborns. Chi-square test analysis showed no significant deviation of the observed genotype distribution from the expected distribution in accordance with Hardy Weinberg expectations in all groups. Polymorphic allele was more prevalent among Sri Lankans and the number of heterozygotes was more than the homozygotes compared to values reported for Caucasians by other investigators.

Supported by National Research Council Grant 05-28 and SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 3

Maternal serum levels of placental growth factor in pregnancy induced hypertension /pre-eclampsia.

Wijayasinghe YS[1], Sugathadasa BHKR[1], Tennekoon KH[1], Karunanayake EH[1], Kumarasiri JM[2], Wijesundera APDS[2].

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Preeclampsia (PE) is a serious complication of pregnancy leading to maternal and fetal morbidity and mortality. In pre-eclampsia hypertension (increments of systolic and diastolic blood pressures of 30 and 15 mmHg respectively, 6 or more hours apart, or an absolute blood pressure of 140/90 mmHg) develops with proteinuria (300 mg or more/24 h) after 20 weeks of gestation in previously normotensive women. Hypertension without proteinuria is considered as pregnancy induced hypertension (PIH). Placental growth factor (PlGF) is a member of the vascular endothelial growth factor family produced predominantly by cytotrophoblasts and syncytiotrophoblasts in the placenta. It is considered as a possible marker of endothelial dysfunction in PIH/PE. Ninety five subjects in the third trimester of pregnancy [PIH/PE (N=50); uncomplicated normal pregnancies (N=45)] were studied. Plasma levels of PlGF were measured using ELISA (DRG International, USA). Results were analyzed using Mann-Whitney U test after stratification according to gestational age (<36 and ≥37 weeks), birth weight (≤2500g vs >2500g) and presence or absence of urine albumin. PlGF levels were significantly higher in the control sample than in PIH/PE before 36 weeks of gestation [geometric mean, (95% CI): 544.7 (508.4, 799.3) vs 242.3 (234.0, 457.2) pg/ml respectively; P=0.0016]. PIH/PE women who had low birth weight babies had significantly lower PlGF levels than those with normal birth weight babies [geometric mean, (95% CI): 213.0 (193.4, 380.9) vs 369.3 (319.4, 587.7) pg/ml respectively; P=0.0146]. PIH/PE women with proteinuria had significantly lower PlGF levels than those without proteinuria [geometric mean, (95% CI): 180.2 (157.0, 332.1) vs 374.3 (355.5, 641.8) pg/ml respectively; P=0.0009]. This preliminary study suggests that PlGF can be used not only as a marker for PIH/PE, but also to indicate outcome of disease severity. Further studies are needed to correlate PlGF levels in early gestational ages with outcome in PIH/PE.

Supported by SAREC Grant for Molecular Biology and Biotechnology and National Research Council Grant 05-28

Abstract No: 4

Promoter sequences targeting tissue specific gene expression of CD80 in murine

Vivehananthan K*, Sahoo NC, Rao KVS

International Centre for Genetic Engineering and Biotechnology, New Delhi, India

It is an interesting task to analyze the functional regions of murine CD80 promoter as it does not contain conventional TATA element or any other obvious transcriptional control elements in its promoter region around the transcription initiation site. The CD80 (B7-1) co-stimulatory molecule is expressed on the surface of B cells and their expression is transcriptionally up-regulated upon stimuli. To identify the regions of murine CD80 promoter that direct tissue-specific gene expression, we generated four promoter constructs of CD80 with fragments –427 /+273 (700 bp), -953 /+273 (1.2 kb), -1623 /+273 (1.8 kb) and –3005/+273 (3.2 kb) fused to the GFP reporter gene. The pattern of promoter activity was assessed by measuring GFP activity in various murine cell lines by flow cytometry analysis. Curiously, significant levels of GFP activity were observed in CD80 positive cells, generated with all four promoter constructs. In contrast, CD80 negative cells lacked GFP activity with the same set of promoter constructs. We demonstrate that DNA sequences contained within –3005 to +273 bp of CD80 promoter possess promoter activity only for the cell lines which can express CD80, suggesting that the expression is tissue-specific. Our results also suggest that DNA sequences extending from –427 to +273 bp are sufficient to direct CD80 gene expression. In addition, confirming the endogenous CD80 expression in murine B cell line by RT-PCR using the primers specific to transcription start site, explains the transcriptional up-regulation of CD80 in B cells. In conclusion, our data clearly define the functional regions of the CD80 gene promoter that direct tissue-specific gene expression in murine B cells.

*. Present address: Department of Biotechnology, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka

Abstract No: 5

Establishment of expression microarray analysis for rat uteri samples using oligo arrays

Premakumara KS, Tennekoon KH, Karunanayake EH

Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

DNA microarrays allow scientists to analyze expression of a set of genes or whole genome, in a single experiment quickly and efficiently. The gene sequences in a microarray are attached to a solid support in an orderly, fixed way. The spots themselves can be DNA, cDNA, or oligonucleotides. The location of each spot in the array is used to identify the expression of a particular gene sequence. In early attempts, amino allyl labeling and microarray hybridization was done using column purified rat uterine RNA samples. The cyscribe labeling of cDNA and hybridization was not successful with Amersham Cyscribe Post Labeling kit when used with rat oligo arrays. Thus the synthesis and labeling of cDNA was done using Ambion Amino Allyl cDNA labeling kit and the hybridization techniques were optimized for the rat oligo arrays. In order to have high quality hybridization signals the oligo arrays were washed according to the array manufacturer's recommendations as the experiments which were done following the recommendations given in the kit resulted in poor hybridization signals. Experiments were carried out to compare gene expression profiles between estrus and metestrus stages of *Rattus norvegicus* uterine cycle. Statistical analysis of the microarray images are currently in progress.

Supported by UGC/RPM Grant and SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 6

Determination of serum IgA and rheumatoid factor IgA-RF in association with clinical manifestations in a population of rheumatoid arthritis patients in Sri Lanka

Goonetilleke S[1], De Silva LWV[3], Rajapaksa GK[3], Wijayaratne LS[2], Udagama-Randeniya PV[3]

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Gaining insights into the immunological mechanisms that lead to the understanding of the aetiopathogenesis, together with socioeconomic aspects of rheumatoid arthritis (RA) would be of great importance in the proper management of the disease. This was a continuation of a case control study undertaken to assess the IgA rheumatoid factor (IgA-RF) and IgA profile and to sort associations with other investigated serum immunoglobulins (IgG, IgG1, IgG2, IgG3, IgG4, IgE and IgM), rheumatoid factors (IgG-RF and IgM-RF) and clinical manifestations, of RA patients in Sri Lanka. The study cohorts consisted of seropositive RA (N =100), and age-gender matched seronegative RA (N = 57) and osteoarthritis (OA) (N =30) patients from the National Hospital, Sri Lanka. Age-gender matched healthy normal individuals served as controls (N = 30). A modified indirect ELISA and a 'sandwich type' ELISA were established to assay serum IgA-RF and IgA, respectively. Socio-economic factors and clinical manifestations were accrued via an interviewer - administrated questionnaire. A significantly higher level of IgA was evident in the seropositive cohort than in the control cohorts (ANOVA; $P<0.05$). The magnitude of IgA-RF of seropositive patients was significantly higher (ANOVA; $P<0.05$) than in OA patients and healthy normal controls. In the seronegative cohort there were correlations between IgA and IgG-RF, and also IgA and IgG2. In the OA cohort there was a correlation between IgA-RF and IgM, which were evident for the first time in Sri Lanka. Gender was not significantly associated with the level of IgA or IgA-RF of the seropositive patients (Mann-Whitney U test; $P>0.05$). There were no significant correlations evident between IgA, and IgA-RF with either clinical manifestations (erosions, nodules or systemic involvements), socio-economic factors or with a positive family history (Pearson's correlation coefficient, $P < 0.01$). The results of this study would contribute to fill the existing knowledge gap of the preliminary study of the immunological parameters of the RA in Sri Lanka.

Supported by research funds of PVU-R and by the IBMBB.

Abstract No: 7

Establishment of primary cultures of the rat endometrial stromal cells

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Cell culture provides an alternative for use of whole animals to study the effect of chemical messengers on the endometrium. We report here the establishment of primary rat endometrial stromal cell cultures with modifications to the previously reported methods.

Virgin female rats (*Rattus norvegicus*, 3-4 months old, weight 140 ± 10 g) were sacrificed quickly by an intramuscular injection of sodium thiopental. Uteri were removed, trimmed of fat and chopped into small pieces (<1-2 mm^3). The minced tissues were incubated with different concentrations of collagenase (0.12% to 0.52% w/v) for varying time periods (30 to 120 min). Stromal cell separation was attempted using centrifugation at different speeds (100g to 1200g rcf) and filtration via filters with different pore sizes (30μm 100μm). After centrifugation, cell pellets were resuspended in the culture medium. The number of viable cells was estimated and the cells were seeded onto coverslips placed in 24-well cell culture cluster. Culture plates were incubated for 3 to 5 days until confluence and analysed by immunohistochemical staining for vimentin and cytokeratin.

Collagenase concentration at 0.42% w/v and a 30 min incubation period were found to be the best conditions for collagenase digestion. A single step of centrifugation at 800 g followed by filtration through 100 μm pore size gave the best separation of stromal cells. Best cell growth was achieved when rats were in the metestrus stage.

Supported by NRC Grant 00-07 and SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 8

Anti inflammatory activity of leafs of *Ixora coccinea*: Use of *in vitro* and *in vivo* assays for activity-directed fractionation

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Anti-inflammatory (AI) activity of both aqueous and methanol leaf extracts of *Ixora coccinea* has been scientifically validated previously using *in vivo* paw-edema assay. Activity directed fractionation is being carried out to identify AI active components using solvent fractionation and medium pressure liquid chromatography (MPLC). Larger quantities of the extract/fractions are required for the *in vivo* AI assay and this assay is not suitable for screening the MPLC fractions. The objective of the present work was to determine the *in vitro* assays that are suitable substitutes for screening the AI activity of MPLC fractions. Solvent fractions were first screened by the *in vivo* paw-edema assay. Secondly, *in vitro* assays on nitric oxide (NO) production and phagocytosis by rat peritoneal cells and tumor necrosis factor–alpha (TNF-α) secretion by human mononuclear cells were conducted to test these solvent fractions. Inhibition of NO production and secretion of pro-inflammatory cytokines such as TNF-α by immune cells and phagocytosis of microbes are important cellular mechanisms that contribute to the AI activity. Comparison of the *in vivo* AI activity of solvent fractions of *I. coccinea* with the *in vitro* AI activities demonstrated that the solvent fractions which had significant *in vivo* AI activity also had significant inhibitory activities on NO production, phagocytosis and TNF-α secretion. These findings indicate that the *in vitro* assays on NO production, phagocytosis and TNF-α secretion could thus be used for screening MPLC fractions for activity directed fractionation and identification of AI active constituents.

Supported by the National Science Foundation Grant RG/2005/HS/15 and National Research Council Grant 05-52.

Abstract No: 9

Isolation and characterization of putative plant disease resistance gene analogs (RGA) from Sri Lankan traditional rice varieties.

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Many disease resistance genes in plants are found to have conserved sequences that are presumably involved in pathogen recognition and signal transduction. A polymerase chain reaction approach using degenerate primers that target these conserved domains of known plant disease resistance genes (*R* genes) can be used to identify resistance gene analogs (RGAs) from different rice varieties. A pair of primers (LM637, and LM 638) designed to recognize such conserved regions were used to amplify genomic DNA from 20 different traditional rice varieties found in Sri Lanka. The fragments were separated by polyacrylamide gel electrophoresis and the rare and variety specific bands excised from the gels were reamplified and sequenced. Sequences were analyzed using genomic and protein data bases. A fragment isolated from the variety Rathuheenati (4922) showed an alignment with part of a putative plant disease resistance gene which corresponds to a retrotransposon type protein found in chromosome 3 of the rice genome. The putative plant disease resistance gene consisted of 1875 bp and encoded a relatively small polypeptide of 624 residues which contains three conserved domains (putative active site, putative NTP binding site and a putative nucleic acid binding site). Therefore it is suggested that the fragment isolated from the variety Rathuheenati (4922) represents a plant disease resistance gene analog.

Supported by National Research Council of Sri Lanka, Grant 05-61

Abstract No: 10

Charaterization of novel mutations and recurrent polymorphisms in exon 11 of *BRCA1* gene in Sri Lankan breast cancer patients and at risk individuals

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Breast Cancer is the most frequently diagnosed cancer among Sri Lankans. Germline mutations in the breast cancer susceptibility genes *BRCA1* and *BRCA2* though low in prevalence are highly penetrant and show geographical variations. There have been only a few reports from Asia on mutations in *BRCA1/2* genes and none from Sri Lanka. We have previously reported a novel mutation in exon 21 and three recurrent polymorphisms in exon 16 of *BRCA1* gene from Sri Lanka. This study reports the characterization of two novel sequence variants and six recurrent polymorphisms in exon 11 of *BRCA1* gene in Sri Lankan breast cancer patients and their relatives. A total of 51 patients with family history of breast cancer, 22 unaffected relatives of breast cancer patients with deleterious mutations in exons 11 and 21 / *BRCA1* and 22 control subjects were analysed for exon 11 of *BRCA1* mutations. Genomic DNA was isolated from peripheral blood lymphocytes. Since the exon 11 is the largest exon in *BRCA1* gene (3426 bp) five sets of specific primers were used for each sample. Two ends of the exon 11 were screened by SSCP/HD analysis and the other three sets were directly sequenced using the MegaBACE 1000 automated DNA sequencer. A novel deleterious frame-shift mutation; c.3086delT (stop at aa. 999) and a novel missense mutation; c.856T>G (aa. Leu > Try at codon 246) were identified. Six previously reported polymorphisms; c.2196G>A, c.2201C>T, c.2430T>C, c.2731C>T, c.3232A>G, c.3667A>G were identified. Four polymorphisms were higly prevalent in Sri Lankans (c.2201C>T, c.2430T>C & c.3667A>G:65%, c.3232A>G: 82%).

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 11

Preliminary screening for germ line mutations in exons 7 and 12 of *BRCA*-2 gene in a selected group of Sri Lankan breast cancer patients

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Breast cancer is the most common malignant growth diagnosed in females. Most cases of breast cancer are not inherited. But the fact that the disease has a high prevalence in certain families led to the notion that this subset of breast cancer cases may be attributed to inheritance of predisposing germ line mutations in cancer susceptibility genes *BRCA* – 1/2. *BRCA*-1 and *BRCA*-2 genes are located in chromosome 17 and chromosome 13 respectively and both are implicated in transcriptional regulation.

This was a preliminary study carried out on Sri Lankan breast cancer patients to screen for possible germ line mutations in exon 7 and exon 12 of *BRCA*-2 gene. Twenty breast cancer patients with a family history of breast cancer or breast and ovarian cancer were screened. DNA was extracted from peripheral venous blood. Exon 7 and exon 12 of *BRCA*-2 gene were amplified by polymerase chain reaction technique using exon specific primers under optimized conditions. PCR products were screened for mutations using single stranded conformation polymorphism and DNA fragments were visualized by silver staining. The results obtained for the 20 patients studied were negative for mutations. Absence of mutations was confirmed by directly sequencing representative samples. A larger number of patients need to be studied to identify possible germ line mutations in *BRCA*-2 in Sri Lankan breast cancer patients.

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Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 12

Screening for germ-line mutations of exon-8 in the *TP53* gene in a family with a *BRCA-1* mutation

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The tumor suppressor gene *TP53* has a well established role in protecting against cancer development in human and in other mammals. It is located on the short arm of human chromosome 17 at position 13.1. The presence of *TP53* mutation has been associated with 50% human cancer including various brain tumors, invasive bladder cancer and aggressive breast cancer. Deleterious germ line mutations in *T*P53 gene have been reported in less than 1% of all breast cancer patients. Thus contribution of exonic mutations of *P53* to familial breast cancer risk is small. However, a higher frequency of *P53* mutations has been observed in breast tumors from carriers of *BRCA-1* mutation than from non-carriers .This study examined the possible mutations of exon-8 in *TP53* gene by PCR, single strand conformation polymorphism (SSCP) and DNA sequencing in a cancer patient and her first and second degree relatives (N=20) having a family history of breast cancer as well as a *BRCA-1* mutation. DNA was extracted and exon-8 was amplified using polymerase chain reaction method. PCR products were subjected to SSCP analysis to screen for mutations. SSCP results did not show any evidence of mutations. Sequencing of representative samples confirmed SSCP results. Further studies are in progress to screen for possible mutations in exons 4, 5, 6 and 7 of the *TP53* gene in the samples screened for exon 8 mutations.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 13

A preliminary study on the detection of micrometastasis of breast cancer by immunohistochemical identification of K-19 antigen in axillary lymph nodes

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Metastasis of breast cancer is currently detected by standard histology using Hemotoxylene and Eosin (H & E) staining. Early detection of micrometastasis could improve diagnosis and treatment of metastatic disease in secondary sites and requires more sensitive methods. Focus of this study was on detection of micrometastasis based on cytokeratin-19 (K-19) antigen on breast cancer cells that migrate to the axillary lymph nodes (ALNs). Previous studies have shown that H & E negative ALNs become positive for micrometastasis by K19-based immunohistochemistry (IHC). The objectives of the present study were to establish and validate K19-based IHC assay and to find the feasibility of conducting a retrospective study to detect micrometastasis in ALN tissues obtained from Sri Lankan breast cancer patients. ALN tissue sections of breast cancer patients which were positive H & E staining (n=3) were used to establish the IHC assay and 28 histology negative ALN samples were tested by IHC. IHC assay was optimized successfully, using an IgG1 mouse monoclonal antibody specific for K19, IgG1 isotype specific control and DAKO cytomation kit. The results demonstrated a high (100%) sensitivity, specificity, accuracy, negative predicted value and positive predicted value compared to the same parameters observed with standard histology. This preliminary study has shown that 44% (8/18) of histology negative ALNs were positive by the K19-based IHC, reflecting a higher chance for detection of micrometastasis. These findings indicate that the K19-based IHC assay is a highly sensitive method for detecting metastatic cancer cells and would be a useful diagnostic tool for detection of micrometastasis compared to the currently used histological methods in Sri Lanka.

Supported by the IBMBB

Abstract No: 14

Cloning, sequencing and characterization of filarial parasite's specific genes

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Filariasis is a parasitic disease and a major public health problem in tropical and subtropical countries, affecting more than 170 million people all over the world. Filariasis is classified into three distinct types such as lymphatic filariasis, subcutaneous filariasis and serous cavity filariasis. Lymphatic filariasis is caused by *Wuchereria bancrofti, Brugia malayi* and *Brugia timor.* In Sri Lanka, lymphatic filariasis is caused by *W. bancrofti. W. bancrofti* cannot be cultured. *Setaria digitata* is a related filarial parasite found in the peritoneal cavity of cattle. In view of the inability to culture *W. bancrofti,* and paucity of parasite material we have used *S. digitata* as a model organism to study the molecular biology of filarial parasites. Genomic library and cDNA library of *S. digitata* were constructed in the laboratory of IBMBB using ZAP Express Genomic and cDNA synthesis kit. The entire cDNA library of *S. digitata* was in vivo excised and the recombinant clones were selected by using the blue and white colony selection method. DNA from selected clones was extracted by using the plasmid mini preparation method. Then extracted plasmid DNA was analyzed by restriction digestion to estimate the size of the insert. Finally the clones will be sequenced by using automated Mega BACE (1000) sequencer. The sequences will be analyzed using bioinformatics methods.

Supported by the SAREC grant for Molecular Biology and Biotechnology

Abstract No: 15

Partial purification of a 50kDa protease secreted by the infective larvae of parasitic nematode *Toxocara canis*.

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Toxocariasis is a parasitic disease caused by the accidental ingestion of eggs of parasitic nematode *Toxocara canis*. Proteolytic enzymes secreted by the larval stages of *T.canis* are suggested as important biological molecules in their tissue migration process. Therefore, an attempt has been taken to identify a larval protease which might be important in tissue migration, thereby, targeting the particular enzymes against toxocara infection.

Excretory secretory products (ESP), collected from in vitro cultures of second stage larvae of *T.canis* were examined for the presence of proteolytic enzymes by gelatin-zymography and were characterized according to pH optima, substrate specificity and inhibitor susceptibility. There were seven proteolytic bands in *T.canis* ESP with their molecular weights ranging between 175 kDa to 20 kDa. The optimal pH value for these protease activities was observed between 5.5 to 6.5. Activity was optimum against albumin over gelatin and casein. Serine, cystein and metalloprotease activities were identified where metalloprotease found to be predominated. A 50kDa protease was partially purified by using DEAE-anion exchange chromatography and its activity was inhibited by metalloprotease inhibitor EDTA (10mM). Its optimum activity was detected in pH 8.5 at 70^0C.

T.canis ESP proteases demonstrate heterogeneity based on their differential migration in polyacrylamide gels containing gelatin, so these proteases might have functional variations during larval migration. The 50 kDa enzyme might play a specific function in the course of migration. Inhibition of its activity might result in the collapsing of larval migration thus, can be instrumental in arresting toxocariasis.

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Previously presented at the SLAAS Annual Scientific Sessions.

Abstract No: 16

Gln223Arg polymorphism in the leptin receptor: prevalence among Sri Lankan women with pre-eclampsia/pregnancy induced hypertension

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Human leptin receptor gene (LEPR) located at chromosome 1; 1p31 region transcribes a trance-membrane protein with several isoforms. Extra-cellular domain, trans-membrane domain & first 29 amino acids of intracellular domain are identical between these isoforms. Several single nucleotide polymorphisms (SNP) have been reported for LEPR. Previous studies have shown that the Gln223Arg polymorphism in exon 6 which lies in the extra-cellular domain is significantly associated with obesity, familial combine hyperlipidemia and increased circulatory leptin levels in postmenopausal women. In this study, prevalence of Gln223Arg polymorphism was compared between women with pre-eclampsia/pregnancy induced hypertension (PE/PIH) (N=30) and women with uncomplicated pregnancy (N=30). DNA extracted from venous blood was amplified with LEPR exon 6 specific primers. PCR products were subjected to restriction fragment length polymorphism with Msp1 restriction enzyme. Prevalence of genotypes Gln/Gln, Arg/Arg, and Gln/Arg were 20%, 16.67%, 63.33% respectively in the PE/PIH group and 26.67%, 23.33%, 50% respectively in women with normal pregnancy. These did not significantly differ between the two groups (χ^2 test). Thus LEPR Gln223Arg polymorphism does not appear to be associated with pre-eclampsia/pregnancy induced hypertension in our study population.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 17

Insulin-like growth factor binding protein-1 in pre-eclampsia/pregnancy induced hypertension

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Insulin-like growth factor binding protein-1 (IGFBP-1) is synthesized by the decidual stoma, and is thought to act locally to inhibit IGF activity and so limit trophoblast invasion. Cross sectional studies have reported conflicting data on maternal circulating concentrations of IGFBP-1 in early pregnancy before the development of pre-eclampsia/pregnancy induced hypertension. Maternal IGFBP-1 levels were compared between women who developed pre-eclampsia/pregnancy induced hypertension (n=30) and those with normal pregnancies (n=30). Pre-eclampsia was diagnosed if there were increments in systolic and diastolic blood pressure levels of 30 and 15 mmHg, respectively, or greater, 6 or more hours apart, or an absolute blood pressure of 140/90 mmHg or greater after the 20th gestational week in normotensive women. Those with proteinuria of 300 mg or more per day in additionally were considered to have pre-eclampsia.

Study participants were recruited from Castle Street hospital for Women at Colombo. Circulating IGFBP-1 levels were measured in non-fasting venous blood samples obtained during the third trimester by enzyme linked immunosorbent assay. Maternal IGFBP-1 levels were not significantly different between pre-eclampsia/pregnancy induced hypertension {Geometric mean (95% Confidence Intervals) 131.7 (109.4, 153.9) µg/L} and normal pregnancy {Geometric mean (95% Confidence Intervals) 115.345 (108.3, 157.7) µg/L}. In conclusion maternal IGFBP-1 levels were not altered by pre-eclampsia/pregnancy induced hypertension in our study population. Further studies involving large numbers stratified according to the severity of the disease are warranted.

Supported by SAREC Grant for Molecular Biology and Biotechnology and UGC Lead Initiative Grant

Abstract No: 18

Establishment of two dimensional gel electrophoresis technique for analysis of human placental proteome: A preliminary report

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Two dimensional gel electrophoresis (2D GE) is a valuable tool in proteomic research and still it is the best single technique for resolving proteins in a complex mixture. 2D GE is a combination of two different types of separating methods. It resolves the proteins in the first dimension by iso-electric point (Iso-Electric Focusing-IEF) and in the second dimension by molecular weight. Pregnancy induced hypertension/pre-eclampsia and intrauterine growth restriction (IUGR) are complications of abnormal placentation. It is shown that IUGR placenta is not simply the smaller versions of a term placenta. Therefore, we carried out this study to analyze the differences of protein profiles of placentae in different pathological conditions. Human term placentae from women with normal uncomplicated pregnancy (n=2), women with pregnancy induced hypertension (n=1) and women with IUGR pregnancy (n=2) were obtained during the Caesarean section. The placental proteome was extracted using the extraction buffer and quantified by the Bradford test. 2D GE was carried out using ATTO (Japan) Electrophoresis system using two different IEF gels (pH 3-10 & pH 5-8) and the polyacrylamide gel (5-20%). Experiments are in progress to compare the protein profiles under different conditions.

Supported by SAREC grant for Molecular Biology and Biotechnology, and National Research Council Grant 05-28

Abstract No: 19

Anti-inflammatory activity of rhizomes of *Alpinia calcarata*

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Alpinia calcarata is used as an anti-inflammatory agent in indigenous medicinal practices in Sri Lanka. The *in vivo* anti-inflammatory activity of both aqueous and methanolic rhizome extracts (ARE and MRE) have been scientifically validated previously. The objectives of this study were to examine the *in vivo* anti-inflammatory activity of different solvent fractions and the underlying mechanisms using *in vitro* assays on nitric oxide (NO) production, membrane stabilization and scavenging of free radicals. Of the four solvent fractions tested, the ethyl acetate fraction showed the highest *in vivo* anti-inflammatory activity, which persisted up to the 4th hour (32%-36%). This was comparable to the activity of ARE, MRE and the reference drug, prednisolone. Hexane fraction showed high activity only at the 1st hour (56%; $P<0.001$) which thereafter decreased markedly to low levels. The other fractions did not show any significant *in vivo* anti-inflammatory activity. ARE demonstrated a strong inhibition of NO production by rat peritoneal cells, 79% reduction at 1000µg/ml of ARE ($P=0.015$). Dose-dependent membrane stabilization activity was observed with ARE, MRE and the four solvent fractions, ranging from 78% - 91% at 10 mg/ml ($P<0.001$) and were comparable to that of aspirin. The anti-oxidant activity of ARE, MRE and two solvent fractions (methylene chloride and ethyl acetate) were significantly higher compared to the control (68%-82% at 500 µg/ml; $P<0.001$). These anti-oxidant activities were comparable to that of Vitamin C. In conclusion, these results re-confirm the anti-inflammatory activity of *A. calcarata* and of the four solvent fractions tested. Ethyl acetate fraction contains the highest anti-inflammatory activity.

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Abstract No: 20

Preliminary studies on Genetic Differentiation of Finger Millet accessions using AFLP markers

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Finger millet is the primary food source of millions of people in many regions of the world. Great attention has been paid on the improvement of this crop through breeding programmes and the germplasm maintenance due to its high nutritional and therapeutic values. Substantial and smaller germplasm collections are available and hence the understanding of genetic diversity of those germplasms is very important to improve the crop.

This study was carried out to understand the genetic diversity of finger millet accessions available in Sri Lanka. Amplified Fragment Length Polymorphism markers were used for the analysis of 22 finger millet accessions and 2 varieties, using three different AFLP primer combinations.

It was possible to optimize AFLP protocol to amplify finger millet DNA. Only Fifteen accessions out of 24 which gave clear amplification profiles. These were selected for further studies. Genetic distance matrix was created using the modified Nei and Li algorithm and it exhibits genetic differences of finger millet accessions. Three phylogenetic trees were constructed using three different methods. Grouping of some accessions clearly exhibits the power of AFLP markers to explain the genetic diversity of finger millet. It is difficult to make conclusion at this stage as only 3 primer combinations were used and due to the large genome size of finger millet. Therefore it is necessary to use more AFLP primer combinations to unveil the true genetic structure of finger millet accessions available in Sri Lanka.

Abstract No: 21

Development of simple sequence repeat (SSR) DNA polymorphism marker to detect salinity tolerant rice (Oryza sativa L.) varieties in Sri Lanka

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The present advanced knowledge and techniques will be helpful to improve the rice production in the future by improving the rice varieties by identifying the varieties which can withstand different abiotic and biotic stress conditions. Salinity becomes one of the major problems due to improper irrigation management. Identification of salinity tolerant rice varieties through conventional breeding methods demands extensive labour and longer time period to obtain results. The use of molecular biological methods to identify salt tolerant varieties at the earliest of breeding process may give an edge over conventional breeding methods. One such application is development of a microsatellite /simple sequence repeat (SSR) marker in rice varieties associated with salt tolerance. DNA was extracted from five salinity tolerant rice varieties, Pokkali, At 401, At 353, BW 400, BW 451 and a susceptible variety, Nona bokkra. Six microsatellite primers were designed targeting the quantitative trait loci (QTL) located close to a gene known as *OSA3* linked with salinity tolerance of rice. DNA was subjected to polymerase chain reaction under optimum conditions. The primers, SALTSSR2 and SALTSSR4 amplified two DNA fragments 143 bp and 194 bp from Pokkali DNA visualized after electrophoresis on 10% polyacrylamide gel. These fragments may be associated with salt tolerance of Sri Lankan rice varieties.

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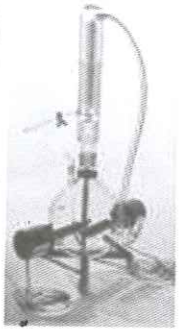

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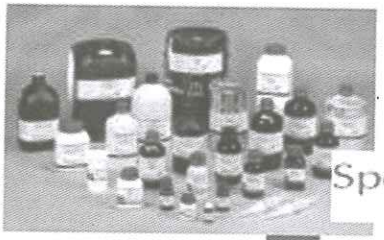

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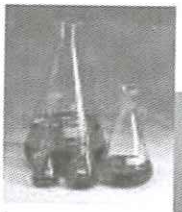

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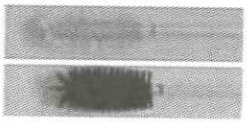

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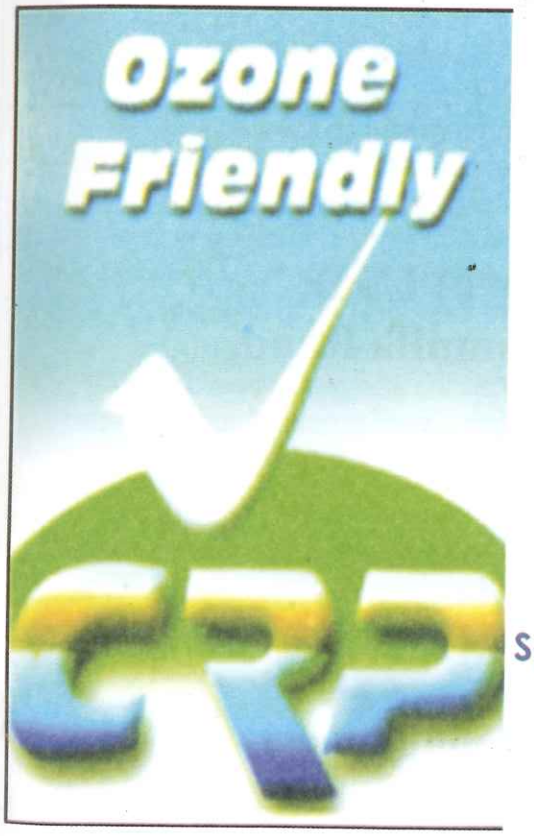

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