University of Colombo

IBMBB

Institute of Biochemistry, Molecular Biology and Biotechnology

First Annual Scientific Sessions

28th April 2006

Programme and Abstracts

Institute of Biochemistry, Molecular Biology and Biotechnology
University of Colombo

Shriyanee Handunnetti
2006/04/28

Institute of Biochemistry, Molecular Biology and Biotechnology

University of Colombo

2nd B'day.

Our Vision

" Fostering a conducive culture for research in molecular life sciences and high quality human resources training to facilitate national development "

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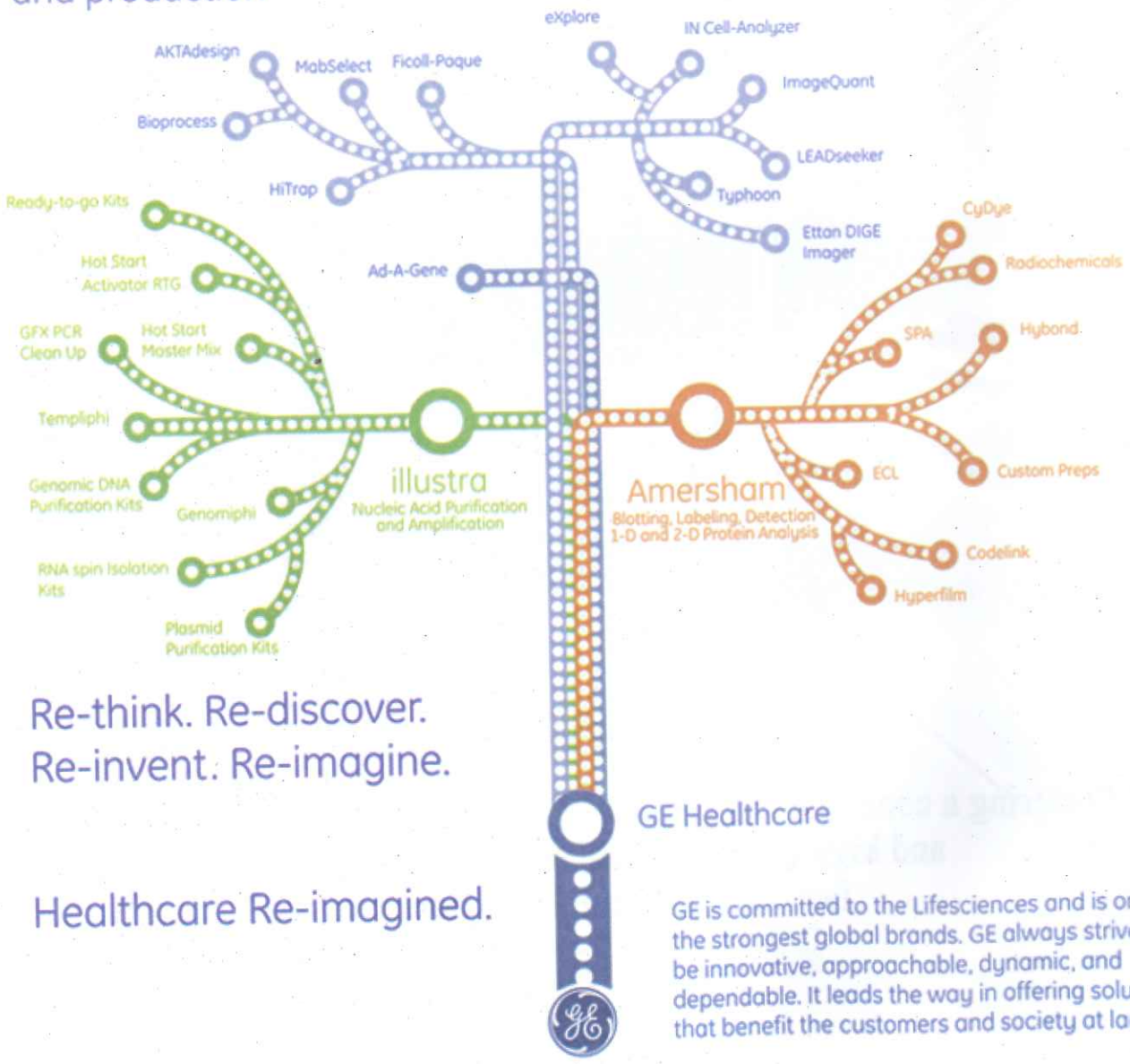

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PROGRAMME

08.00 h - Registration

09.00 h - Lighting of the Traditional Oil Lamp

09.10 h - Welcome address by Director, IBMBB

09.20 h - Address by the Chairman, University Grants Commission

09.30 h - Address by the Vice Chancellor, University of Colombo

09.40 h

Professor Stanley Wijesundera Memorial Lecture

Capacity Building in Science in Developing Countries

by Prof. Rune Liminga

Former Director

International Programme in Chemical Sciences

University of Uppsala, Sweden

10.40 h -Vote of thanks

10.45 h - Reception

11.30-12.30 h Plenary Lectures – Chairperson: Prof. E. R. Jansz

11.30 h - **Global gene expression profiling using microarray: an overview**

Prof. Kamani Tennekoon, Professor of Physiology

Faculty of Medicine, University of Colombo

12.00 h - **Molecular biological approaches in the development of new rice varieties**

Dr. Jagath Weerasena, Senior Lecturer

Institute of Biochemistry, Molecular Biology and Biotechnology

Oral communications

Session 1: Cancer and Entomology

Chairperson: Prof. Ira Thabrew

14.00 -14.15 h (Abstract no: 01) Novel mutations and a novel polymorphism in breast cancer susceptibility gene, *BRCA1* in Sri Lankan breast cancer patients
De Silva JWN, Karunanayake EH, Tennekoon KH, Allen M, Amarasinghe I, Angunawala P

14.15-14.30 h (Abstract No: 02) The effect of C-KIT 816 mutation on differentiation and proliferation of myeloblasts
Kurukula Arachchi C W

14.30-14.45 h (Abstract No 03) An allele-specific polymerase chain reaction assay for the differentiation of species A and D from species B, C and E of the *Anopheles culicifacies* complex
Munasinghe MPCS, De Silva BGDN, Dassanayake RS, Karunanayake EH

14.45-15.00 h (Abstract No: 04) Use of RAPD-PCR markers for unveiling the breeding structure of *Aedes aegypti*
Dilruk DN, De Silva BGDN, Karunanayake EH, Jayasekera N, Weerasinghe I

Session 2: Reproduction, Plant Molecular Biology, Medicinal Plants

Chairperson: Prof. Eric H Karunanayake

15.00-15.15 h (Abstract No: 05) Expression of leptin and the long form of the leptin receptor isoform during the rat estrus cycle: a preliminary report
Eswaramohan T, Tennekoon KH, Karunanayake EH

15.15-15.30 h (Abstract No: 06) Genetic differentiation of Sri Lankan traditional rice varieties using AFLP markers
Indraguptha WAGD, Weerasena OVDSJ, Karunanayake EH, Rajapakse RMT, Fernando KKS, Bandara JHBH

15.30-15.45 h (Abstract No: 07) Development of early detection techniques for *Corynespora* leaf fall disease resistant clones using molecular markers
Jayakody A, Silva WPK, Karunanayake EH

15.45-16.00 h (Abstract No: 08) Investigations on anti-inflammatory potential and chemical constituents of *Ixora coccinea, Vitex negundo* and *Alpinia calcarata*
Kumara RR, Handunetti SM, Deraniyagala SA, Ratnasooriya WD

Poster presentations 16.00 – 18.00 h Abstracts 09 to 15

Cancer:

Abstract No: 09 - A study of mutations in exon 5 and 6 of TP53 gene in Sri Lankan breast cancer patients
Vivekanandarajah A, Karunanayake EH, Tennekoon KH, De Silva JWN, Amarasinghe I, Angunawala P

Reproduction:

Abstract No: 10 - Leptin dose not appear to stimulate insulin-like growth factor binding protein-1 synthesis by cultured rat endometrial stromal cells
Eswaramohan T, Tennekoon KH, Premakumara KS, Karunanayake EH

Abstract No: 11 - Cord blood leptin levels in normal pregnancies, pregnancy induced hypertension and gestational diabetes mellitus: a preliminary report
Silva NY, Tennekoon KH, Senanayake L, Karunanayake EH

Abstract No: 12 - Maternal and cord blood IGF-1, IGF-II and IGFBP- 1 levels in normal pregnancy, pregnancy induced hypertension and gestational diabetes mellitus
Jayanthiny P, Silva NY, Tennekoon KH, Senanayake L, Karunanayake EH

Bioinformatics:

Abstract No: 13 - Development of gene based PCR markers for comparative genomics: a bioinformatics approach
Pathmaja P, Weerasena OVDSJ, Dassanayake RS, Karunanayake EH

Abstract No: 14 - Characterisation of active site of Mevalonate Diphosphate Decarboxylase (MVD): a molecular docking and simulation based approach
Hulugalle DVK, Weerasinghe S, Dissanayake DP, Dassanayake RS

Parasitic Diseases

Abstract No: 15 - Partial characterization of excretory-secretory antigens and morphology of rumen pharamphistomes in neat cattle
Vamadevan A, Rajakaruna RS, Rajapakse RPVJ

Professor Stanley Wijesundera Memorial Lecture

Prof. Stanley Wijesundera served as the first Vice-Chancellor of the University of Colombo from 1978 to 1987. He also held the Chair of Biochemistry during the same period. Prof. Wijesundera was an academic with a great vision and made a significant contribution to the development of the University of Colombo. Some of his outstanding contributions include the infrastructure development of the Faculty of Law, University Library, Laboratory Complex for Chemistry, the Institute of Computer Technology and the Faculty of Graduate Studies. He strongly believed in human resource development at frontier disciplines and capacity building in science as essential requirements for national development. He made a significant contribution at the initial stage of the establishment of laboratory for Molecular Biology and Gene Technology at the Faculty of Medicine in 1986. The Institute of Biochemistry, Molecular Biology and Biotechnology is pleased to establish the Stanley Wijesundera Memorial Lecture as an annual event in his memory. His wife, Mrs. Anoja Wijesundera and children of late Prof. Wijesundera have agreed to sponsor this lecture. The inaugural Stanley Wijesundera Memorial Lecture will be delivered by Professor Rune Liminga, former Director, International Programme in Chemical Sciences, University of Uppsala, Sweden. Prof. Liminga has been associated with capacity building in science with developing countries in general, and with Sri Lankan universities in particular for more than 25 years.

Eric Karunanayake
Director
Institute of Biochemistry, Molecular Biology and Biotechnology
University of Colombo

Plenary Lecture 1

Global gene expression profiling using microarray: an overview

Kamani H Tennekoon[1]

Professor of Physiology, Faculty of Medicine, University of Colombo

Molecular descriptions of biological processes are required to map gene activity to normal or altered function of cells and organisms. Complexity of the genome(s) makes it essential that high throughput technologies are used to achieve this. Expression microarrays based on cDNA or oligonucleotide arrays allow study of expression of tens of thousands of genes at a given time. However, meticulous attention to experimental design, data pre processing and analysis is as important as meticulous laboratory experiments. The array consists of precisely positioned nucleic acid probes (cDNA or oligonucleotides) on a solid surface. Complementary DNA from the target is hybridized with the array. Data extracted need to be pre processed to remove systematic and other variations. Differentially expressed genes are identified using specially designed statistical methods. Differentially expressed genes are linked to appropriate data bases to map these to molecular functions and pathways. Gene expression patterns can also be identified to allow classification of samples, for example in molecular classification of tumours. In order to maintain standards in microarray experiments, Microarray Gene Expression Data Society has laid down guidelines for minimum information required in storing and reporting microarray data. Data stored in local databases need to be uploaded to public repositories for publication. At present expression microarrays can detect differences and direction of change in gene expression but not absolute gene expression level. The technique though powerful is still not robust enough for the average laboratory to yield consistent results. Expression microarrays are being used in a number of fields, Physiology, Pathophysiology, Molecular Oncology, Developmental Biology and Drug Development being a few examples.

[1]Presently at the Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo

Plenary Lecture 2

Molecular Biological approaches in the development of new rice varieties

Jagath Weerasena

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

Rice is used as the staple food by 40% of the world's population. 90% of the rice yield is produced and consumed in Asian countries. Rice belongs to the genus *Oryza*. The genus *Oryza* contains approximately 22 species and only two of them, *Oryza sativa* and *Oryza glaberima* are cultivated while others are considered as wild species. During last few decades, rice production has increased due to the introduction of high yielding varieties by world wide rice breeding programs. These varieties were developed by using conventional plant breeding methods based on classical Mendelian genetics. However it has been estimated that the rate of rice production has to be increased by 50% to meet the increasing demand for next few decades as population increases. Therefore it is essential to develop new varieties with high yield potential, pest and disease resistance and tolerance to abiotic stress to achieve this goal. This achievement is impossible with classical breeding methods as it takes a considerable time. Furthermore, there are some crossability barriers to introduce desired traits from some varieties. Therefore recent advances in molecular biology are used to enhance the breeding programs and overcome the barriers in conventional breeding. Some of these include PCR-based markers used in efficient selection of parents for breeding programs and transfer of desirable traits among different varieties, particularly to introgress novel genes from related wild species. The accumulation of DNA markers and construction of the linkage maps, made it possible to isolate important genes by positional cloning strategy to clone genes for important agronomic traits and subsequent production of transgenic rice plants.

Abstract No: 01

Novel mutations and a novel polymorphism in breast cancer susceptibility gene, *BRCA1* in Sri Lankan breast cancer patients

De Silva JWN[1], Karunanayake EH[1], Tennekoon KH[1,2], Allen M[3], Amarasinghe I[4]
Angunawala P[5]

[1]Institute of Biochemistry Molecular Biology and Biotechnology, Departments of [2]Physiology and [5]Pathology, Faculty of Medicine, University of Colombo

[3]Department of Genetics and Pathology, Rudbeck Laboratory, University of Uppsala, Sweden

[4]National Cancer Institute, Maharagama

Breast Cancer is the commonest noncutaneous malignancy in females, world wide and the most commonly diagnosed cancer among Sri Lankan women. Germline mutations in the susceptibility genes, *BRCA1* and *BRCA2* predispose development of hereditary breast/ovarian cancer. These mutations though low in prevalence, are highly penetrant and show geographical variations. Unlike from the west, there have been only a few reports from Asia on mutations in *BRCA1/2* genes, and none from Sri Lanka except our preliminary reports. A total of 128 patients (with and without a family history of breast cancer: N=63, 65 respectively), 70 unaffected individuals with a family history of breast cancer and 40 control subjects without a family history of any cancer were studied. Genomic DNA was isolated from peripheral blood lymphocytes and specific primers were used to amplify exon 21 of *BRCA-1*. The PCR products were screened by a combination of Single Strand Conformation Analysis (SSCP) and Heteroduplex Analysis (HA). DNA fragments were visualized by silver staining. PCR products which showed an abnormal pattern of SSCP and HA were reamplified from the original genomic DNA and both strands were directly sequenced using the MegaBACE 1000 automated DNA sequencer. Three novel mutations, *BRCA1* 5399insC, 5402insCA and 5404delG in exon 21 of *BRCA1* were found in two members of a single sib ship. An interesting novel polymorphism *BRCA1* 5401insT was also identified among some of the patients and normal controls. Mutations and the polymorphism found in this study are not previously reported in the breast cancer information core.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 02

The effect of C-KIT 816 mutation on differentiation and proliferation of myeloblasts

Kurukula Arachchi CW

Department of Pathology, Faculty of Medicine, University of Ruhuna

Molecular Diagnostics Unit, Department of Haematology, Erasmus University Medical centre Rotterdam, The Netherlands

Acute myeloid leukemia (AML) is a heterogeneous group of malignancies carrying an ominous outcome in most patients. Associated genetic aberrations are used to classify and prognosticate AML. However, the patients of good prognostic groups showed resistance to therapy or early relapse when they had additional C-KIT mutations (especially C-KIT 816). In addition, increased resistance to therapy and treatment failure was observed in chronic myeloid leukemia patients carrying C-KIT mutations. Almost all primary systemic mastocytosis patients have activating C-KIT mutations specially C-KIT 816. C-KIT gene encodes a transmembrane tyrosine kinase type III receptor. Together with its natural ligand, stem cell factor (SCF), it regulates growth, function and survival of cells. Considering all these factors, it was postulated that, there could be an additional advantage to blasts carrying C-KIT mutations to survive and to resist therapy. To see the effect of C-KIT 816 mutation on proliferation and differentiation of myeloblasts, an in vitro study was performed using immortalized murine myeloblasts (32D cells). C-KIT 816 transfected 32D cells were propagated in cultures with wild type (WT) controls and proliferation and differentiation of blasts in each group compared. Although proliferation showed no difference, interestingly the blasts carrying C-KIT mutation showed significant impairment in differentiation compared to the controls. This confirms the inhibitory effect of C-KIT 816 mutation on cell differentiation which leads to accumulation of immature cells, probably the basis for adverse outcome in leukemia.

Abstract No: 03

An allele-specific polymerase chain reaction assay for the differentiation of species A and D from species B, C and E of the *Anopheles culicifacies* complex

Munasinghe MPCS[1], De Silva BGDN[2], Dassanayake RS[3], Karunanayake EH[1]

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

[2]Department of Zoology, Faculty of Applied Sciences, University of Sri Jayawardenepura

[3]Department of Chemistry, Faculty of Science, University of Colombo

Anopheles culicifacies, the principle vector of malaria in Indian subcontinent and Sri Lanka, is a complex of five cryptic species which are morphologically indistinguishable at any stage of life. In view of the practical difficulties associated with classical cytotaxonomic method for the identification of members of the complex, an allele-specific polymerase chain reaction (ASPCR) assay targeted to the ITS2 region of ribosomal DNA was developed. The assay discriminates *An. culicifacies* species A and D from species B, C and E. The assay was validated using chromosomally-identified species B & E specimens of *An. culicifacies* in Sri Lanka. Two of the four primers (FP1 B & FP2 B) produced strong, reliable and correctly sized products with chromosomally identified B and E and FP1 A did not produce a band as we expected.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 04

Use of RAPD-PCR markers for unveiling the breeding structure of *Aedes aegypti*

Dilruk DN[1], De Silva BGDN[2], Karunanayake EH[1], Jayasekera N[1], Weerasinghe I[3]

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[2] Department of Zoology, Faculty of Applied Sciences, University of Sri Jayawardenepura

[3]Medical Research Institute, Colombo

RAPD- PCR polymorphisms at 21 presumptive loci were used to examine the breeding structure of *Ae. aegypti*, the vector of dengue virus. Mosquitoes were sampled at one location in the Colombo city limits and one location in the suburb. A fair amount of genetic polymorphism was encountered among the mosquitoes that emerged in each container, signifying that the container possessed eggs from different females of the same species. Furthermore, females of this species laid very few eggs in discarded containers and dispersed their eggs around the vicinity.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 05

Expression of leptin and the long form of the leptin receptor isoform during the rat estrus cycle: a preliminary report

Eswaramohan T[1], Tennekoon KH[1,2], Karunanayake EH[1]

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

[2]Department of Physiology, Faculty of Medicine, University of Colombo

Leptin, the product of the *ob* gene has been linked to reproductive function. It acts on its cognate receptor, ob-r, product of the *ob-r* gene. Leptin receptor exists in several isoforms of which the long form (ob-rb) has the full length cytoplasmic domain. Serum leptin levels and uterine and placental leptin receptor expression are modulated during pregnancy in the rat. Leptin and its receptor are implicated in embryo-maternal dialogue. In women, leptin receptor deficiency has been reported in infertility. However, the effect of the estrus cycle on the expression of leptin and its receptors in the rat uterus has not been studied. In the present study, we investigated expression of leptin and the long form of the receptor, in the rat uterus during different stages of the estrus cycle. Vaginal smear cytology was used to determine estrus stage. Semi quantitative reverse transcription polymerase chain reaction (RT-PCR) was used to study expression of leptin and its receptor, ob-rb. Leptin expression in all stages except during the estrus stage was almost equal. At estrus stage leptin expression was lowest. Expression of ob-rb was higher in metestrus and diestrus stages than in proestrus and estrus stages. These findings confirm that the rat uterus is a site for local production of leptin as well as a target tissue for circulating and locally produced leptin. Variation in the uterine expression of leptin and leptin receptor during the estrus cycle suggests that these are modulated by hormones and other chemical messengers in the reproductive system.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract no: 06

Genetic differentiation of Sri Lankan traditional rice varieties using AFLP markers

Indraguptha WAGD[1], Weerasena OVDSJ[1], Karunanayake EH[1], Rajapakse RMT[2], Fernando KKS[2], Bandara JHBH[2]

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

[2]Plant Genetic Resource Centre, Gannoruwa, Peradeniya

Rice varieties grown in Sri Lanka from ancient time are known as traditional rice varieties. In 1940's rice breeders in Sri Lanka started developing new varieties to increase the rice production to meet the increasing demand. Traditional varieties were crossed with foreign varieties to produce high yielding varieties known as old improved varieties (OIV). Later due to further developments new improved varieties (NIV) were developed. Although NIV's produce high yields, there is an increasing demand in the export market for Sri Lankan traditional rice varieties because of their grain qualities such as superior taste and high fiber content. Some of these varieties consist of important traits which are tolerant to drought and rice blast disease. They may also contain new genes which are resistant to newly evolving pathogens. Therefore the knowledge of genetic diversity of traditional varieties will be beneficial to plant breeders when developing new varieties. Here we used the Amplified Fragment Length Polymorphism (AFLP) markers to assess the genetic variation among 19 different traditional rice varieties. AFLP analysis was performed as described by Vos *et al* (1995) with minor modifications. Polymorphic bands were visually scored as presence (1) or absence (0). The genetic distances between each variety were calculated and UPGMA based dendrogram was constructed. According to the dendrogram rice varieties with resistance to rice thrips and those resistant to salinity were well differentiated. The varieties suitable for wet zone were also grouped into a different cluster.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 07

Development of early detection techniques for *Corynespora* leaf fall disease resistant clones using molecular markers

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

[2]Rubber Research Institute of Sri Lanka, Dartonfield, Agalawaththa

Corynespora leaf fall disease (CLF) caused by the fungus *Corynespora cassiicola* has now become a serious threat to natural rubber industry. In Sri Lanka the first epidemic was experienced in 1985 devastating the clone RRIC 103, the most prestigious clone produced by the Sri Lankan scientists. With regards to the management of the disease it has been agreed by the rubber pathologists that the use of genetic materials is the only hope as chemical control is not cost effective. From the past experience it seems that the field clearing should be at least 3-5 years old to determine whether a particular clone can tolerate the disease or not. Rubber being a perennial crop minimum of 20 years should be spent on experiments to evolve a 3-5 year old clearing since the hand pollination. In the light of this situation present studies were designed to identify possible disease resistance markers in rubber with the view of developing an early detection system for CLF resistance in potential clones. As a preliminary study Amplified Fragmented Length Polymorphism (AFLP) technique was developed to distinguish susceptible and resistance clones. However the specific bands for resistance were not seen in all the resistant samples. Hence it was decided that AFLP method is not very suitable to identify resistance to CLF. Investigations are underway using RGA (Resistance Gene Analog) marker system for the possibility of identifying a DNA marker for CLF disease resistance in rubber.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 08

Investigations on anti-inflammatory potential and chemical constituents of *Ixora coccinea, Vitex negundo* and *Alpinia calcarata*

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[2]Department of Chemistry, Faculty of Science, University of Colombo

[3]Department of Zoology, Faculty of Science, University of Colombo

The objective of this study was to re-examine the anti-inflammatory potential of aqueous extracts of leaves of *Ixora coccinea* and *Vitex negundo,* and rhizomes of *Alpinia calcarata* (recommended in Ayurvedha for inflammatory conditions), to determine their main classes of phytochemicals and, to quantify the metal ions. Anti-inflammatory activity was determined using carrageenan-induced rat paw edema model following oral administration of extracts. The results showed marked inhibition of edema both during the early and late phases; *Ixora coccinea* (79.2 & 26.6%), *Vitex negundo* (64.5 & 64.1%) and *Alpinia calcarata* (59.7 & 45.3%). This anti-inflammatory effect was higher than that has been reported previously. All the extracts were slightly acidic (pH 5.2-6.6). Qualitative and quantitative analysis for phytochemicals revealed the presence of alkaloids (1.6-4.7%), flavonoids (1.3-1.7%), saponins (0-4.8%), steroids, tannins, terpenoids, phlobatannins and cardiac glycosides. Leaves of *Ixora coccinea* and *Vitex negundo,* and rhizomes of *Alpinia calcarata* contained Ca, Fe, K, Na and Mg. In addition, *Vitex negundo,* and *Alpinia calcarata* contained Mn, *Ixora coccinea,* and *Alpinia calcarata* contained Zn. Interestingly, heavy toxic metals such as Cd, Cr, Cu, Ni and Hg were undetectable. In conclusion, this study confirmed the oral anti-inflammatory activity of aqueous extract of these three plants, identified their major classes of phytochemicals and demonstrated the lack of toxic heavy metals. A strong possibility thus exists for the development of potent anti inflammatory agent/s, especially from *Vitex negundo*.

Supported by National Science Foundation (Grant RG/2005/HS/15)

Abstract No: 09

A study of mutations in exons 5 and 6 of TP53 gene in Sri Lankan breast cancer patients

Vivekanandarajah A[1], Karunanayake EH[1], Tennekoon KH [1,2], De Silva JWN[1], Amarasinghe I[3], Angunawala P[4]

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Human TP53 is a tumour suppressor gene located on the short arm of chromosome 17. It encodes a protein of 53kDa. The protein plays an important role in arresting the cell cycle in G1 phase. It has been reported that TP53 is mutated in almost 50% of all human cancers. Furthermore, certain regions of the gene, exon 5, 6, 7 and 8 have been identified as hotspots for mutations. As a part of our on-going studies on mutations in breast cancer predisposing genes, we have investigated germline mutations in the exons 5 and 6 of TP53 gene in a limited number of breast cancer patients. Breast cancer patients with a family history of cancer (N=50), patients without family history of cancer (N=52) and unaffected individuals with a family history of breast cancer (N=43) were included in the study. Exons 5 and 6 were PCR amplified using exon specific primers. Exon 5 primer amplifies a product of 184bp and exon 6 amplification yields a fragment of 113bp. The amplified fragments were further analyzed by single strand conformation polymorphism (SSCP). In all cases studied SSCP did not reveal any mobility differences when compared with normal controls suggesting absence of mutations. Direct sequencing of selected samples is in progress to confirm the findings.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 10

Leptin dose not appear to stimulate insulin-like growth factor binding protien-1 synthesis by cultured rat endometrial stromal cells

Eswaramohan T[1,2], Tennekoon KH[1,2], Premakumara KS[1], Karunanayake EH[1]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo [2]Department of Physiology, Faculty of Medicine, University of Colombo

Our previous studies have shown that leptin increases prolactin but not IGF-I secretion from rat endometrial stromal cells (ESC) in a dose dependent manner. Insulin-like growth factors and Insulin-like growth factor binding proteins undergo unique changes during the menstrual cycle/estrus cycle and in response to different hormonal stimuli of endometrial cells *in vitro*. In the present study we examined the effect of leptin on insulin-like growth factor binding protein-1 (IGFBP-1) production by rat ESC. Confluent monolayer subcultures in the fourth passage established from primary rat ESC were stimulated with recombinant murine leptin (1 to1000 ng/mL) for 24 h. Cells were fixed and stained for IGFBP-1 by immunohistochemistry. Primary antibodies used were specific for rat/mouse and human IGFBP-1. Rat liver sections were used as the positive control. Universal negative control was used to account for non specific binding. Endometrial cell cultures showed a weak staining for IGFBP-1 and there was no difference between medium treated and leptin treated cells. Thus leptin at the doses used in the present experiment does not appear to have any effect on endometrial IGFBP-1 production within 24 h of stimulation.

Supported by National Research Council, World Health Organization-Rockefeller Foundation and SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 11

Cord blood leptin levels in normal pregnancies, pregnancy induced hypertension and gestational diabetes mellitus: a preliminary report

Silva NY[1], Tennekoon KH[1,2], Senanayake L[3], Karunanayake EH[1]

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

[2]Department of Physiology, Faculty of Medicine, University of Colombo

[3]Castle Street Hospital for Women, Colombo

As a part of our ongoing studies on genetic and endocrine determinants of birth weight, cord blood leptin levels were studied in normal uncomplicated pregnancies (N=18), pregnancy induced hypertension (PIH) (N=16) and gestational diabetes mellitus (GDM) (N=14). All women were in para 1 or 2 and delivered singleton infants at 36 to 40 weeks of gestation. Birth weights were not significantly different between the groups. Leptin levels were measured by enzyme immunoassay and compared between the three groups (Kruskal-Wallis ANOVA with Dunn's multiple comparison). Leptin levels significantly differed between the three groups (P=0.0064), the difference being due to higher levels in GDM compared to PIH [geometric mean (95% CI) for GDM: 10.889 (6.301, 18.836) vs PIH: 3.485 (2.136, 5.686) ng/mL]. The difference persisted when leptin levels were normalized to the Ponderal index (P=0.0035). In normal uncomplicated pregnancies leptin levels were lower than in GDM but higher than in PIH. Cord blood leptin levels showed a significant positive correlation with birth weight in PIH (Spearman r=0.5463, P=0.029) and with Ponderal index in normal pregnancy (Spearman r=0.507, P=0.032) but did not correlate with any birth size indices in GDM. Higher placental synthesis of leptin in diabetic mothers may partly account for higher cord blood leptin seen in the GDM group in the present study. Absence of a significant difference in birth weight between the three groups despite exposure to higher leptin levels in-utero in GDM, suggests a leptin resistance in the GDM fetus. This may contribute for the development of obesity and diabetes mellitus later in life in the GDM offspring. Further studies are needed to confirm this.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 12

Maternal and cord blood IGF-1, IGF-II and IGFBP- 1 levels in normal pregnancy, pregnancy induced hypertension and gestational diabetes mellitus

Jayanthiny P[1], Silva NY[1], Tennekoon KH[1,2], Senanayake L[3], Karunanayake EH[1]

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[3]Castle Street Hospital for Women, Colombo

Insulin-like growth factors (IGFs) and IGF binding proteins (IGFBPs) are implicated in fetal growth. We studied maternal and cord blood IGF-I, -II and IGFBP-1 in women with normal uncomplicated pregnancies (N=15), pregnancy induced hypertension (PIH, N=15) or gestational diabetes mellitus (GDM, N=14). Birth weight did not significantly differ between the groups. Cord blood IGF-I concentration was significantly lower in PIH than in other groups [Kruskal-Wallis ANOVA P= 0.004, geometric mean (95%CI) for normal: 22.080 (19.143, 25.468), PIH: 14.093 (12.331, 16.144) and GDM: 22.646 (16.558, 30.974) ng/mL] and the difference persisted even when normalized to Ponderal index (P=0.0016). Cord blood IGF-II and IGFBP-1, and maternal IGF-I, IGF-II and IGFBP-1 were not significantly different between the three groups. In normal pregnancies cord blood IGF-I correlated (Spearman rank correlation) positively with head circumference (r=0.5680, P=0.0272) and IGF-II with birth weight (r=0.5676, P=0.0273). Maternal IGF-II positively correlated (Spearman rank correlation) with birth weight (r=0.6703, P=0.0063), and head circumference (r=0.8071, P=0.0003) in PIH, and with birth weight (r=0.5680, P=0.0272) and length (r=0.5976, P=0.0186) in GDM. Low cord blood IGF-I levels seen in PIH even when accounted for birth size may reflect reduced placental synthesis. Correlations between IGF-I and IGF-II (maternal or cord) with infant anthropometric indices differed between the groups and larger numbers need to be studied to clarify these associations.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 13

Development of gene based PCR markers for comparative genomics: a bioinformatics approach

Pathmaja P[2], Weerasena, OVDSJ[1], Dassanayake RS [2], Karunanayake EH[1]

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Similar genes are conserved among widely divergent species, performing identical or similar functions. Large numbers of such sequencing data are available in protein and nucleotide data bases. These sequences can be retrieved and used to design PCR primers to develop gene based markers useful for many applications such as studying the homologous genes and phylogenetic relationships of related organisms when their genomic sequences are unavailable. However, direct designing of PCR primers is not possible because the conservation can be found only in amino acid sequences for most of the conserved domains due to the degeneracy of codons. Here we used a bioinformatics approach to design primers for amplification of genomic sequences responsible for the targeted conserved amino acid sequences. Consensus-Degenerate Hybrid Oligonucleotide Primer (CODEHOP) design strategy was used to design degenerate oligonucleotide primers for PCR amplification of multiply-aligned protein sequences of various conserved domains (Myosin motor domain, Cyclooxygenase-1 domain, Tyrosine Kinase, Arv1 domain and Nucleotide binding domain). This strategy develops primers that consist of short 3' degenerate core region (about 11-12bp) and a longer 5' consensus clamp region (about 18-25bp) for specific amplification of the target. Initially phylogenetic analysis was carried out for each conserved domain sequences to group them according to their phylogenetic relationship and then forward and reverse degenerate primer pools were designed for each group to flank the target sequence. However, experimental studies should be carried out to ascertain the reliability of these primers in different applications.

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Abstract No: 14

Characterization of active site of Mevalonate Diphosphate Decarboxylase (MVD): A Molecular docking and simulation – based approach

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Mevalonate diphosphate decarboxylase (MVD) is a key enzyme in sterol biosynthetic pathway that catalyzes the decarboxylation of mevalonate diphosphate (MDP) to isopentynyl diphosphate (IPP) while hydrolyzing ATP to ADP. IPP is a key building block of a large family of fundamental biomolecules, showing essentiality for cell viability. To characterize active site of MVD hitherto unknown, rigid Cartesian coordinates of Saccharomyces cerevisiae MVD were retrieved from the PDB database and 3D atomic coordinates of MDP and ATP were constructed using Gaussian – 98 program. Putative active sites of MVD were identified using ClastalW aligned MVD sequences in databases and inter domain clefts in MVD 3D structure. Both MVD and ATP were docked into the putative active sites and minimum binding energies (MBE) and orientations were studied using Hex-4.2. Molecular dynamics (MD) simulations were carried out for MPD and ATP docked MVD complexes with MBE to ascertain global and local conformational changes using Gromacs software with ffG43a2 force field. In MD simulation, enzyme-substrate complexes were immersed in a rectangle box with the dimensions of 15x15x13 nm^3 and surrounded by 8950 water molecules while incorporating 13 Na^+ ions to maintain electro neutrality of the system. Analysis of bond distances of simulated enzyme-substrate complex and computer based mutagenesis suggest the possible involvement of strictly conserved Ser 121 and Lys 203 in MDP binding; Ala 15 and Lys 18 in stabilizing the carboxylate group of MDP; conserved Tyr 19 and Ser 208 in á-PO4 group of ATP in binding and Asp 302 in catalytic activity. Finally, analysis of binding energy of docked Trifluoromevalonate diphosphate (TFMD) to MVD revealed TFMD is a potential inhibitor of MVD.

Abstract No: 15

Partial characterization of excretory-secretory antigens and morphology of rumen pharamphistomes in neat cattle

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In cattle immature stages of rumen paramphistomes cause intestinal pharamphistomiasis, mainly in the dry zone in Sri Lanka. The adult flukes show apparently sub clinical infection and usually occur as mixed infections with different genera. Scanty information is available on taxonomic diversity of rumen paramphistomes in Sri Lanka as genus identification is difficult and laborious. This study has attempted to isolate and characterise excretory-secretory (ES) antigens of rumen flukes which could be used in taxonomic identification. Adult paramphistome samples were collected from rumen of infected cattle at the Municipal Abattoir, Kandy. ES antigens were prepared by *in vitro* culture of adults in MEM medium at 37^{0}C, 5% CO_2 up to four days. Analysis of ES antigens was done using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The antigenic specificity of collected ES antigens were analysed by immunoblotting using known positive cattle serum. Morphological analysis by taxonomic keys distinguished four different genera of paramphistomes belonging to two families: Genera *Carmyerius* and *Fischoederius* representing family Gastrothylacidae and genera *Cotylophoron* and *Calicophoron* representing family Paramphistomidae. The ES products showed significant difference in the protein profile where *Calicophoron sp.* showed minimum of 11 bands with 4 characteristic to the group while *Cotylophoron sp.* showed 9 bands with 3 characteristic. *Fischoederius sp.* showed 7 bands with one characteristic and *Carmyerius sp.* showed 14 with 5 characteristic. All groups shared at least 2 bands of molecular weight <20kDa. The immunoblotting data show considerable variation in the antibody binding pattern indicating specificity in antigenicity

Morphological analysis by taxonomic keys distinguished five different genera of paramphistomes belonging to two families: Genera *Carmyerius* and *Fischoederius* representing family Gastrothylacidae and genera *Cotylophoron*, *Calicophoron* and *Paramphistomum* representing family Paramphistomidae. The ES products showed significant difference in the protein profile where *Calicophoron sp.* showed minimum of 20 bands with 3 characteristic to the group while *Cotylophoron sp.* showed 17 bands with 3 characteristic and *Paramphistomum* showed 14 bands with 2 characteristic. *Fischoederius sp.* showed 14 bands with 3 characteristic and *Carmyerius sp.* showed 20 with 5 characteristic. All groups shared at least 2 bands of molecular weight <20kDa.

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Programme and abstract book edited and compiled by Prof. Kamani Tennekoon and designed by Mr. Kanchana Senanayake on behalf of the organizing committee.

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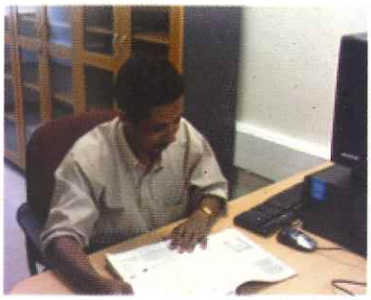

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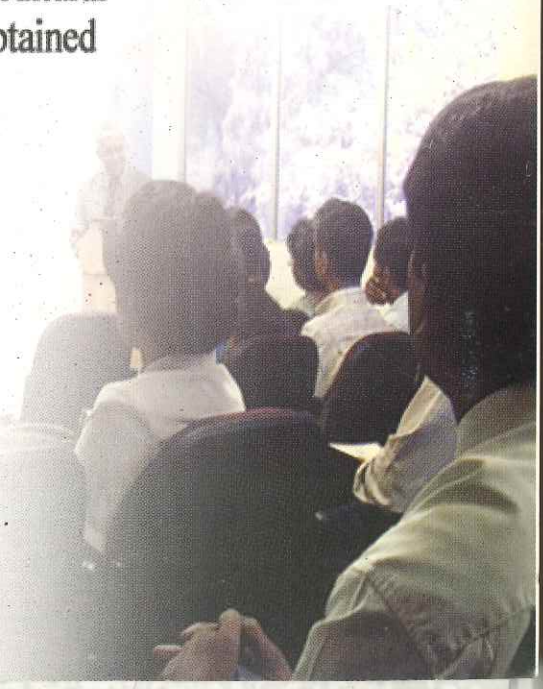

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