PROCEEDINGS
OF THE

NINETY EIGHTH SESSION OF THE

INDIAN SCIENCE CONGRESS

CHENNAI, 2011

PART II

SECTION OF NEW BIOLOGY

(Including Biochemistry, Biophysics & Molecular Biology and Biotechnology)

President : **Dr. Hasi Das**

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98th Indian Science Congress
January 3-7, 2011, Chennai

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President : Dr. Hasi Das*

SECTION OF NEW BIOLOGY
(Including Biochemistry, Biophysics & Molecular Biology and Biotechnology)

Honorable chairman, distinguished delegates and friends,

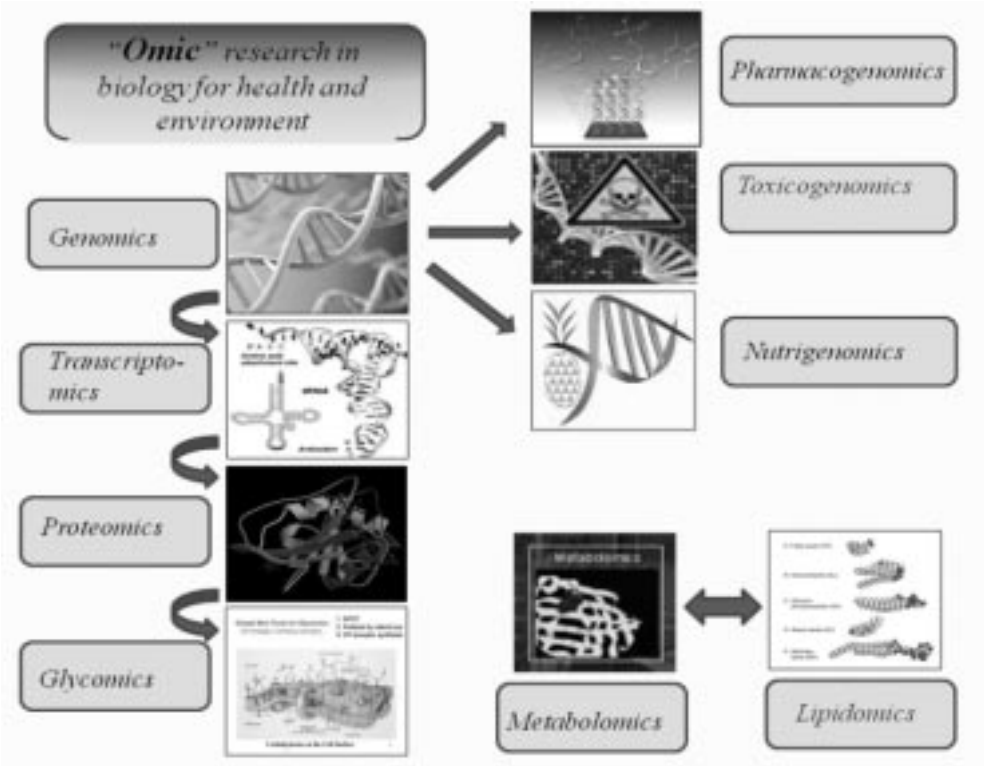
At the outset I would like to express my gratitude to the members of the New Biology Section of Indian Science Congress Association (ISCA) for electing me as their sectional president. I feel, it not only a great honor but a big responsibility. I believe, it is also a privilege to deliver the Presidential Address before this august audience. I take this opportunity to welcome you all to the scientific sessions organized under the New Biology section.

The focal theme of the 98th session of Indian Science Congress is "Quality education and excellence in scientific research in Indian Universities" and that of New Biology Section is "**Omic research in biology for health and environment**".

Following the currency of "Genomics", additional words have been coined to denote the compilation of databases as resources for future research and analysis; proteomics for proteins, glycomics/CHOnomics for carbohydrates, transcriptomics for transcriptome, metabolomics, lipidomics, pharmacogenomics, nutrigenomics and toxicogenomics. Together, these approaches are termed "Omic research". With 35,000 genes and thousands of proteins to identify, correlate and understand, it does not suffice to rely on studies of one gene, one gene product or one process at a time. We have entered the "**Omic**" era in biology. I hope the sessions planned in this section will provide the platform for all of us to interact with one another on various issues of omic research in biological sciences.

* Director Grade Scientist, Head, Proteomics & Structural Biology Division, Institute of Genomics and Integrative Biology, Mall Road, Delhi-110 007. E-mail : hdas@igib.res.in

The interrelation among different Omic disciplines



Genomics is the study of the genomes of organisms. The field includes intensive efforts to determine the entire DNA sequence of organisms and fine-scale genetic mapping efforts. Research on single gene does not fall into the definition of genomics unless the aim of this genetic pathway, and functional information analysis to elucidate its effect on, place in, and response to the entire genome's networks.

As of September 2007, the complete sequence was known of about 1879 viruses, 577 bacterial species and roughly 23 eukaryote organisms, of which about half are fungi. Most of the bacteria whose genomes have been completely sequenced are problematic disease-causing agents, such as *Haemophilus influenzae*. Of the other sequenced species, most were chosen because they were well-studied model organisms or promised to become good models. Yeast (*Saccharomyces cerevisiae*) has long been an important model organism for the eukaryotic cell, while the fruit fly *Drosophila melanogaster* has been a very important tool (notably in early pre-molecular genetics). The worm *Caenorhabditis elegans* is an often used simple model for multicellular organisms. The zebrafish *Brachydanio rerio* is used for many developmental studies at the molecular level and the flower

Arabidopsis thaliana is a model organism for flowering plants. The Japanese pufferfish (*Takifugu rubripes*) and the spotted green pufferfish (*Tetraodon nigroviridis*) are interesting because of their small and compact genomes, containing very little non-coding DNA compared to other most species. The mammals dog (*Canis familiaris*), brown rat (*Rattus norvegicus*), mouse (*Mus musculus*), and chimpanzee (*Pan troglodytes*) are all important model animals in medical research.

A rough draft of the human genome was completed by the Human Genome Project in early 2001, creating much fanfare. By 2007 the human sequence was declared "finished" (less than one error in 20,000 bases and all chromosomes assembled). Display of the results of the project required significant bioinformatics resources. The sequence of the human reference assembly can be explored using the UCSC Genome Browser.

Transcriptomics : The global study of gene expression at the RNA level.

The human genome contains the complete set of genes required to build a functional human being. However, the genome is only a source of information. In order to function, it must be expressed coordinately. The transcription of genes to produce RNA is the first stage of gene expression . The transcriptome is the complete set of RNA transcripts produced by the genome at any one time. In other words a transcriptome is a collection of all messenger RNA molecules in a population of cells. Along with DNA, these RNA molecules help create proteins. Transcriptomics provides tools that help researchers gain a better understanding of how genes and pathways are involved in biological processes.

The transcriptome is extremely dynamic. Most of our cells contain the same genome regardless of the type of cell, stage of development or environmental conditions. Conversely, the transcriptome varies considerably in these differing circumstances due to different patterns of gene expression. Transcriptomics, the study of the transcriptome, is therefore a global way of looking at gene expression patterns.

Why is transcriptomics of interest? There are many different ways in which the large-scale analysis of gene expression patterns can be useful. These include :

- To answer specific questions about gene expression. For example, which genes are activated by a particular transcription factor? This sort of questions can be addressed by comparing gene expression patterns in tissues in which the particular transcription factor is either active or inactive.
- General discovery experiments. These have no particular hypothesis but can be used to identify interesting genes. For example, which genes are highly expressed in brain tumours but not in healthy brain tissue? Can these be used as drug targets or diagnostic markers?

- Disease classification. Sometimes single markers are not sufficient to distinguish two similar diseases, as is often the case in cancer. Testing the expression profiles of a larger number of genes can provide accurate diagnoses.
- Functional annotation. Many DNA sequences that have been determined have no known function. However, if they show similar expression patterns to a characterised gene, it is likely that their functions are similar. Sometimes it is possible to identify conserved regulatory elements in such genes.
- To identify drug targets. If the gene expression profile caused by a mutation is similar to that caused by a drug, it is likely the drug interacts with and inactivates the protein affected by the mutation.

How is transcriptomics studied? There are a number of different methods but currently the most popular is the use of **DNA microarrays**.

However, mRNA is not the ultimate product of a gene, transcription is the first step in gene regulation and information about the transcript levels is needed for understanding gene regulatory networks. Thus, the new challenge is to identify all genes, their expression patterns and their function.

Transcriptomics or global analysis of gene expression, also called genome-wide expression profiling, is one of the tools that is used to get an understanding of genes and pathways involved in biological processes. The idea underlying this approach may be called “guilt by association”, which means that genes showing similarity in expression pattern may be functionally related and under the same genetic control mechanism. However, transcriptomics may be a part of genomics only.

Proteomics :

Proteomics can be considered the “next level” after genomics in the study of a biological system. With the advancement in structural and functional genome analysis, the sequence of genes and its function can be visualized. But the ultimate aim is to ascertain how the gene is expressed into a protein, which subsequently becomes functional or structural protein. Proteomics is the study of the structure and function of proteins, typically on a large scale. The word proteome is the entire set of proteins (including modification) produced by an organism. As most drug targets are proteins, a route to study the genome efficiently at the protein level is of immense value and that is what proteomics offers. The term “proteomics’ was first coined in 1997 to make an analogy with genomics, while “proteome” was coined by Marc Willkins in 1994.

Proteomics is a rapidly expanding field. The broad scope of proteomics might perhaps be broken down into six types of questions that are addressed in some

form : (1) identification of individual proteins, (2) recognition of protein interactions, (3) relative quantitation to distinguish differential expression of proteins, (4) characterization of post-translational modifications, (5) qualitative or quantitative measurements at high spatial and/or temporal resolution to address the dynamics of protein interactions, and (6) formulation of models based on results from components 1-5. It is important that in a field as complex and interdisciplinary as proteomics, technology development be pursued with a sound understanding of context. One area of particular interest is the development of technologies that will permit observations to be quantitative and made in real time, whether for clinical studies or experimental systems.

Proteomics can be considerably more complex than genomics due to the fluctutating nature of the proteome from cell-type to cell-type. At this point, the proteome is only known for the simplest organisms such as C elegans. We are far from knowing the entire human proteome.

Post-translational modifications

While an important part of proteomics is the quantitative measure of translated proteins, another important part of proteomics is the study of modifications to proteins after they are translated. Some well-known examples of post-translational modifications include, ubiquitination, sumoylation, methylation, acylation, acetylation, phosphorylation, sulphation, glycosylation, oxidation, nitrosylation. The main goal of any proteomic endeavour is to correlate the potential protein modifications to particular phenotypes.

Advantages of proteomics over genomics

The level of transcript (mRNA) bears only a rough estimate of the amount of translated protein. The transcript can be rapidly degraded or may be translated inefficiently. Proteomics directly measures protein levels, providing more accurate estimates of the importance of specific proteins. In addition, protein modifications can be measured and the impact of these modifications upon protein function and phenotype can provide much more information than genomics. Methods such as phosphoproteomics and glycoproteomics are used to study these post-translational modifications. Finally, genomics does not include protein-protein interactions which are important components of the proteome and play an important role in phenotype determination.

Proteomics methods

Specific protein-modifications can be studied by the development of an antibody specific for that particular modification. An example of this is the

development of antibodies specific for serine, threonine and tyrosine-phosphorylated proteins. Carbohydrate binding proteins, lectins are used to recognize specific sugars and can be used in the study of protein glycosylation. The most common way to study post-translational modification is through the use of 2D-gel electrophoresis. This allows for small differences in proteins to be visualized by separating the modified forms from the unmodified ones. Determining the existence of proteins in complex mixtures is more difficult and other techniques such as enzyme-linked immunosorbent assay (ELISA) or matrix-assisted laser desorption/ ionization (MALDI-TOF) can be used. In addition to the development of broadly applicable research tools that address the core technical challenges in proteomics, unique constraints in two subordinate areas merit special attention. Here, I wish to address the unique needs of glycomics and clinical proteomics.

Glycomics :

Glycomics research emerges from proteomics research. Current reliable proteomic methods reflect the convergence of the latest mass spectrometric technologies, protein chemistry and separation sciences, plus advances in genomics and bioinformatics. Yet, no proteomic platform available today is suitable for all areas of research. However, proteomics remains a viable option for improved biomarkers, diagnostics and treatment of various diseases. Meanwhile the advances in analytical tools for proteomics over the last decade, particularly in mass spectrometry (MS) methods, are applicable to sensitive and definitive glycan analysis.

The complexity and diversity of glycosylation significantly complicates the linkage between genetic sequence and mature, active proteins. Glycobiology-focused proteomics, or glycomics, requires the development of novel approaches and tools directed at the special challenges of glycobiology. Among post-translational modifications, glycosylation is the only one that requires structural characterization of the modifying moiety beyond noting its presence. Strategies for separation, profiling, quantitation, and detailed characterization of carbohydrate structures are central challenges. Informatics tools are needed for data handling and reduction, correlation of carbohydrate and protein information, and a variety of other purposes. Discovery-based analytical tools that can survey the complexities of glycosylation on a system-wide basis may have significant biological impact.

The glycosylation of *N*-glycans represent a critically important post-translational modification reaction, and the comprehensive functional analysis of proteins is a target for the next stage of proteomic research. It is common knowledge that over 50% of all mammalian serum proteins and about 80% of cell membrane proteins are glycosylated and that glycans play crucial roles in various biological events

including cell recognition, adhesion, and cell – cell interaction. Glycans of glycoproteins, which are displayed on cell surface membranes, are structurally changed during carcinogenesis and development. Functional glycomics is an important strategy for the elucidation of the functions of sugar chains and this new field promises to provide fundamental answers in the area of functional proteomic research. Glycosylation can alter the charge, conformation and stability of proteins and induce heterogeneous profiles as a consequence of the production of variable glycoforms. A cell lysate of glycosyltransferase gene knock out or knock down cells in which glycoproteins are modified and a glycan structural analysis of the whole cell lysate is carried out, this is referred to as a glycomic analysis. Glycoproteomic analysis requires that a sample be purified prior to the glycan structural analysis. When we analyze a sugar containing glycoprotein of interest, the cell lysate can be purified or enriched by affinity chromatography using lectin(s) or sugar specific ligand(s); this is "glycoproteomic" analysis. Glycoproteomic analysis is useful for identification of target glycoprotein(s) of glycosyltransferase genes. On the other hand, using affinity columns which specifically recognize proteins such as antibody, specific binding proteins or specific ligands, are useful for purification or enrichment of the glycoproteins of interest from a cell lysate and the glycan structures of the binding fraction can be analyzed. This strategy is referred to as a "proteoglycomic" analysis and is useful for a certain types of clinical glycomics, because information can be obtained on glycan structural alterations in the particular protein. Finally the characterization of functional chains of glycoproteins due to the lack or addition of sugar chain(s) should be analyzed by various cellular and molecular approaches. This is referred to as functional glycomics. The advances in genomics have had a cascading effect as scientists can delve deeper into the structure and interactions among the biochemical bases of living matter and as a result, new areas of study have emerged. The progression of areas of study can be diagrammed as follows :

Genomics > ***Transcriptomics*** > ***Proteomics*** > ***Glycomics***

It is easy to recognize that the complexity of study in each field increases from genomics to glycomics. In fact, it is expected that the potential chemical information content rises exponentially from genomics to glycomics. Considering that there are approximately 25,000 human genes, the amount of information to be derived by glycomics is staggering. The study of functional glycomics is rapidly emerging as a mechanism to thrust carbohydrates into the mainstream of biology and biomedicine.

Why the interest in Glycomics?

Researchers have determined that minor differences in glycan structures can play a major role in biological functions. In fact, glycans are involved in all life

phases beginning with embryonic development. For example, consider oligosaccharides (also called simple sugars), a class of saccharides that contain a small number of component sugars. Oligosaccharides play a critical role in many biological processes including biorecognition, interactions between cells, immune response, infection and inflammation. The emerging field of glycomics may eventually result in new drugs; new uses for existing drugs; and/or existing drugs may be modified to make them more efficacious. Current methods of manufacturing protein-based drugs do not necessarily modify the artificially created proteins with the same sugars found in the human body. This discrepancy causes the liver to quickly flush the protein-based drugs out of the body. However, utilizing the appropriate sugars that are compatible with the human body could result in more efficient treatments and reduce the required dosage of protein-based medications.

Sugar chemistry is also involved in the progression of cancer, helping to transmit the signals that trigger unchecked cell growth. Glycomics may eventually play a critical role in the fight against cancer. Researchers are also investigating the role of sugars in the development of Parkinson's, Alzheimer's, inflammatory diseases and infectious diseases like AIDS and herpes. Sugars may also influence stem cell biology, organ transplantation and tissue engineering.

Metabolomics is the scientific study of chemical processes involving metabolites. In other words, it is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind" - specifically, the study of their small-molecule metabolite profiles. The metabolome represents the collection of all metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes. Thus, while mRNA gene expression data and proteomic analyses do not tell the whole story of what might be happening in a cell, metabolic profiling can give an instantaneous snapshot of the physiology of that cell. One of the challenges of systems biology and functional genomics is to integrate proteomic, transcriptomic, and metabolomic information to give a more complete picture of living organisms. Metabolomics in today's world carries on its shoulders, the responsibility of providing a detailed description of metabolic pathways and their workings, whether they are in humans, animals or plants. Metabolites are the intermediates and products of metabolism. Within the context of metabolomics, a metabolite is usually defined as any molecule less than 1 kDa in size. However, there are exceptions to this depending on the sample and detection method. For example, macromolecules such as lipoproteins and albumin are reliably detected in NMR-based metabolomic studies of blood plasma. In plant-based metabolomics, it is common to refer to "primary" and "secondary" metabolites. A primary metabolite is directly involved in the normal growth, development, and reproduction. A

secondary metabolite is not directly involved in those processes, but usually has important ecological function; examples include antibiotics and pigments. By contrast, in human-based metabolomics, it is more common to describe metabolites as being either endogenous (produced by the host organism) or exogenous. Metabolites of foreign substances such as drugs are termed xenometabolites.

Lipidomics may be defined as the large-scale study of pathways and networks of cellular lipids in biological systems. The word "lipidome" is used to describe the complete lipid profile within a cell, tissue or organism and is a subset of the "metabolome" which also includes the three other major classes of biological molecules: proteins/amino-acids, sugars and nucleic acids. Lipidomics is a relatively recent research field that has been driven by rapid advances in technologies such as mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, fluorescence spectroscopy, dual polarisation interferometry and computational methods, coupled with the recognition of the role of lipids in many metabolic diseases such as obesity, atherosclerosis, stroke, hypertension and diabetes. This rapidly expanding field complements the huge progress made in genomics and proteomics, all of which constitute the family of systems biology.

Lipidomics research involves the identification and quantitation of the thousands of cellular lipid molecular species and their interactions with other lipids, proteins, and other metabolites. Investigators in lipidomics examine the structures, functions, interactions, and dynamics of cellular lipids and the changes that occur during perturbation of the system. Han and Gross first defined the field of lipidomics through integrating the specific chemical properties inherent in lipid molecular species with a comprehensive mass spectrometric approach. Although lipidomics is under the umbrella of the more general field of "metabolomics", it is a distinct discipline due to the uniqueness and functional specificity of lipids relative to other metabolites.

Pharmacogenomics is the study of how an individual's genetic inheritance affects the body's response to drugs. The term comes from the words pharmacology and genomics and is thus the intersection of pharmaceuticals and genetics.

It is the branch of pharmacology which deals with the influence of variation on drug response in patients by correlating gene expression or single-nucleotide polymorphisms with a drug's efficacy or toxicity. By doing so, pharmacogenomics aims to develop rational means to optimize drug therapy, with respect to the patients' genotype, to ensure maximum efficacy with minimal adverse effects. Such approaches promise the advent of "personalized medicine"; in which drugs and drug combinations are optimized for each individual's unique genetic makeup.

Pharmacogenomics holds the promise that drugs might one day be tailor-made for individuals and adapted to each person's own genetic makeup. Environment, diet, age, lifestyle, and state of health, all can influence a person's response to medicines, but understanding an individual's genetic makeup is thought to be the key to creating personalized drugs with greater efficacy and safety. Pharmacogenomics combines traditional pharmaceutical sciences such as biochemistry with annotated knowledge of genes, proteins, and single nucleotide polymorphisms. Benefits of pharmacogenomics may be to have more powerful medicines, safer drugs, better vaccines, more accurate methods of determining appropriate drug dosages, advanced screening for disease and all decrease in the overall cost of health care. However, pharmacogenomics is a developing research field that is still in its infancy. Several barriers will have to be overcome before many pharmacogenomics benefits can be realized such as complexity of finding gene variations that affect drug response, limited drug alternatives.

Toxicogenomics is a rapidly developing discipline that promises to aid scientists in understanding the molecular and cellular effects of chemicals in biological systems. This field encompasses global assessment of biological effects using technologies such as DNA microarrays or high throughput NMR and protein expression analysis.

The rapid development and evolution of genomics, proteomics, and metabonomics, based technologies has accelerated the application of gene expression for understanding chemical and other environmental tressors' effects on biological systems. These technological advances have led to the development of the field of "toxicogenomics", which proposes to apply global mRNA, protein and metabolite analysis related technologies to study the effects of hazards on organisms

Impact of toxicogenomics on public health: Developments in toxicogenomics will have important impacts on toxicology, systems biology, and in improving public health. Toxicologists will look for unique or common signatures of toxicity. Gene expression profiling will enable researchers to determine genes and pathways that are activated and/or suppressed during cell injury and recovery, and whether organisms exhibit evolutionarily conserved responses to stress. Gene expression changes may also become a major tool in medicine for diagnosis/ prognosis, for determining drug sensitivities and effectiveness, and for personalized risk assessment based on genetic polymorphisms.

Toxicogenomics is a scientific field that studies how the genome is involved in responses to environmental stressors and toxicants. Toxicogenomics combines studies of genetics, mRNA expression, cell and tissue-wide protein expression and

metabonomics to understand the role of gene-environment interactions in disease. An important aspect of toxicogenomics research is the development and application of bioinformatics tools and databases in order to facilitate the analysis, mining, visualizing and sharing of the vast amount of biological information being generated in this field. This rapidly growing research area will have a large impact on many other scientific and medical disciplines, including systems biology, as researchers strive to generate complete descriptions of how components of biological systems work together and across organisms to respond to specific stresses, drugs, or toxicants.

Nutrigenomics, as a segment of the health, beauty and wellness industry, can potentially illuminate the path to avoid serious illnesses in the future. Could this provide a sense of direction, especially for people who are forced to take an alternative approach to pharmaceutical medications?

Nutrigenomics is a study of how different foods may interact with specific genes to increase the risk of common chronic diseases such as type 2 diabetes, obesity, heart disease, stroke and certain cancers. Nutrigenomics also seeks to provide a molecular understanding of how common chemicals in the diet affect health by altering the expression of genes and the structure of an individual's genome. The premise underlying nutrigenomics is that the influence of diet on health depends on an individual's genetic makeup. There is hope that nutrigenomics will ultimately enable such personalized dietary advice. It is still in its infancy and its contribution to public health over the next decade will dictate to adapt healthier food habits.

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II

ABSTRACTS OF PLATINUM JUBILEE LECTURE

PLATINUM JUBILEE LECTURE

Functional Genomics of Cotton Fiber Development

Reddy V. S[1]., Patil D[1]., Kumar S[1]., Pathhak R[1]., Reddy P.S[1], Padmalatha K.V[4]., Dhandapani G[4]., Prakash A H[3]., Reddy M K[1]., Katageri I[2]., Vamdevaiah. H M[2]., Leelavathi S[1]., Kumar P A[4]

[1]International Center for Genetic Engineering and Biotechnology (ICGEB), Aruna Asaf Ali Marg, New Delhi-110 067
Email : vsreddy@gmail.com
[2]University of Agricultural Sciences (UAS), Dharwad-580 005
[3] Central Institute for Cotton Research (CICR), Coimbatore-641 003
[4]National Research Centre on Plant Biotechnology (NRCPB), Pusa campus, New Delhi-110012

Key words : *Cotton fiber. Gossypium, Transcriptome, Proteome, Functional genomics, Microarray.*

Cotton is one of the most important commercial crops in India and provides livelihood to more than 60 million people. The genus *Gossypium* comprises 50 species and *G. hirsutum* or upland cotton (tetraploid with 2n = 52) is the most widely grown species, covering 95% worldwide acreage. Cotton fibers are seed coat trichomes that can elongate up to 5-6 cm in length, offer as a unique model for studying many basic biological processes in the plant cell. Subtraction based EST library showed the expression of over 7,500 genes during the fiber initiation and elongation stages, of which 500 ESTs have no significant homology with any known genes available in the database. A vast majority of ESTs belonged to cellular structure, carbohydrate and protein metabolism, correlating with massive cellulose biosynthesis during cell elongation stage. Microarray based studies involving lintless mutant have identified the genes that are differentially expressed in the initiation and elongation stages. In addition, proteomic analysis based on iTRAQ based labeling technology also identified differentially expressed proteins that are associated with the development of fiber cells. MYB transcription factors, sucrose synthase, structural proteins, cellulase synthase, tubulins, aquaporin, Superoxide dismutase, annexin, expansin are some of the proteins over expressed during the fiber elongation phase as compared to initiation phase. On the whole, genome wide transcriptomic and proteomic studies involving lintless mutant have identified about fifty genes that might be crucial for fiber development. However, a major challenge is to determine the role of several other genes with unknown function that are specifically up regulated during the fiber development in order to improve its quality through biotechnological approaches.

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III

ABSTRACTS OF AWARD LECTURE/ YOUNG SCIENTIST AWARDPROGRAMME

YOUNG SCIENTIST AWARD LECTURE

Preparation of cross-linked enzyme aggregates (CLEA) for enantioselective synthesis of unnatural amino acids.

Bhalchandra K. Vaidya, Sanjay N. Nene and Bhaskar D. Kulkarni

Chemical Engineering and Process Development Division,
National Chemical Laboratory,
Pune-411 008
E-mail : bk.vaidya@rediffmail.com

The present study deals with use of cross-linked enzyme aggregates (CLEA) of *Aspergillus melleus* aminoacylase for production of entiopure unnatural amino acids (namely: phenylglycine, homophenylalanine and 2-naphthylalanine). CLEA were preparaed by a novel method involving co-aggregation of the enzyme with polyethyleneimine (PEI) followed by cross-linking of enzyme-PEI co-aggregates using glutaraldehyde. Under optimum condition, CLEA expressed 74.9% activity recovery and 81.2% aggregation yield. CLEA gave higher pH and temperature stability and extended storage half life than free enzyme. Moreover, CLEA exhibited excellent enantioselectivity towards hydrolysis of unnatural amino acids amides (E> 120) and gave high operational stability over repetitive cycles of *rac*-homophenylalanine amide hydrolysis.

PROFESSOR S. S. KATIYAR ENDOWMENT LECTURE

Adaptations at the Core of Life : Insights from Genomics

Mitali Mukerji

Institute of Genomics and Intcgrative Biology (CSIR),
Mall Road, Delhi-110007
mitali@igib.res.in

Humans have successfully colonized diverse geographical locations, adapted to various climalic conditions, dietary habits and resisted many pests and parasitic infections after their initial movement out of Africa. These adaptations have been possible due to continuous evolutions of human genomes under these selection conditions. In the genomics era, these events can be tracked through following genetic variations like single nucleotide polymorphisms, large insertion-deletions and segmental duplications in the human genome. It has been now realized that there is an enormous variability in the genome and there is no ”prototype human genome”. For instance from 3 billion bases that make up the human genome there are 11 million places where they could vary. A fraction of these variations have been linked to ancestry, diseases or different human phenotypes such as skin pigmentation, height etc. Studying genetic landscape across diverse world populations and integration of such genetic maps to epidemiology, demography, history and also anecdotal evidence could provide us interesting insights into patterns of human migrations and also allow us to trace mutational history as well as evolution of diseases. The Indian Genome Variation project which has provided the first comprchensive genetic map of Indian populations in conjunction with other global variation data has provided many such interesting insights. A few interesting examples related to high altitude adaptation, mutational history of neurodegenerative disorders, adaptation to malaria endemic regions as well as high salinity conditions would be illustrated.

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IV

ABSTRACTS OF SYMPOSIUM / INVITED LECTURES

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PART II : (Abstract of Symposium/Invited Lecture)

SECTION OF NEW BIOLOGY

(Including Biochemistry, Bipphysics & Molecular Biology and Biotechnology)

President : **Dr. Hasi Das**

OMIC RESEARCH IN BIOLOGY FOR HEALTH AND ENVIRONMENT

Invited Presentations

1. Rab7a Interacts with Cellular Prion Protein and Mediate its Intracellular Sorting and Trafficking

Saima Zafar[1], Nicolas V. Ahsen[1], Michael Oellerich[1], Walter J. Schulz-Schaeffer[2], Victor W. Armstrong[1], Abdul R. Asif[1]

[1]Department of Clinical Chemistry, University Medical Center Goettingen, Goettingen, Robert-Koch-Str. 40, 37075, Germany.
Email : asif@med.uni-goettengen.de
[2]Prion and Dementia Research Unit, Department of Neuropathology, University Medical Center Goettingen,
Robert-Koch-Str. 40, 37075, Goettingen, Germany.

Keywords : *Prion protein; STrEP-tag; Endosome; Microtubules Cellular prion.*

Protein (PrP^C) is highly conserved throughout the evolution of mammals and thought to play important but yet not fully understood cellular functions. Identification

of PrP^C interacting partners is crucial to appreciate its exact role in cellular physiology. The present study undertook a comprehensive strategy to purify the PrP^C along with its interacting proteins after expressing in prion protein-deficient murine hippocampus HpL3-4 neuronal cells. The interacting proteins were affinity purified, in-gel digested and identified by Q-TOF MS/MS analysis. The newly identified interacting proteins of PrP^C ranges from cytoskeleton proteins to proteins those are important for cell haemostasis maintence, cell communication, signal transduction, stress response and protein folding. We further characterized the novel interaction of GTPases (Rab7a and Arf1) along with alpha tubulin 1 with PrP^C using laser confocal microscopy and reverse co-immunoprecipitation. siRNA knockdown of Rab7a expression enhanced PrP^C accumulation in the endosomal compartments. Deactivation of Arf1 and disruption of microtubles resulted in the downregulation and accumulation of PrP^C respectively. These findings demonstrated that Rab7a and Arf1 are critical in the regulation and trafficking of PrP^C. Disturbance in the Rab7a and Arf1 interacting proteins may lead to the accumulation of PrP^C in the endosomal compartment, a potential scenario to study the diseases with protein aggregation.

2. Development of Multi-dimensional HPLC Mapping for N-glycans and its Application for Functional Glycomics

Hirokazu Yagi[1], Noriko Takahashi[1], and Koichi Kato[1,3,4,5]

[1]Graduate School of Pharmaceutical Sciences, Nagoya City University,
3-1 Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan
Email : hyagi@phar.nagoya-cu.ac.jp
[2] National Institute for Institute for Molecular Science and Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences,
5-1 Higashiyama Myodaiji, Okazaki 444-8787, Japan
[3]GLYENCE Co., Ltd., 2-22-8 Chikusa, Chikusa-ku, Nagoya 464-0858, Japan
[4]The Glycoscience Institute, Ochanomizu University, 2-1-1 Ohtsuka,
Bunkyo-ku, Tokyo 112-8610, Japan

Keywords : *Functional glycomics, HPLC, Mass spectrometry, Neural stem cells.*

Glycans mediate cell-cell communication and virus infection and govern protein folding, transport and degradation variety of linkage types connecting the monomeric units. Furthermore, the carbohydrate moieties of glycoproteins exhibit microheterogeneities resulting from the presence or absence of the terminal sugar units. Such structural complexity and diversity hamper our understanding of

biological functions of the individual sugar chains. The recently emerging glycomics projects aim at comprehensive identification and characterization of *N*- and *O*-glycans expressed by whole cells, tissues, organs, and bodies of a variety of organisms. In view of the situation, we have been developing HPLC mapping technique, which is a forceful method to identify the structures of *N*-glycans based on their elution positions on three different HPLC columns. The accumulating HPLC data combined with mass spectrometric data of approximately 600 different *N*-glycans are available in the web application GALAXY (http://www.glycoanalysis.info/galaxy2/). This technique is further applicable for profiling *N*-glycans possessing sulfate and/or glucuronyl groups.

Here we illustrate several examples of the GALAXY-based factional glycomics. For instance, we compared *N*-glycosylation profiles of neural stem cells before and after differentiation, demonstrating that the Lewis X-carrying glycans were specifically expressed on the undifferentiated cells. By knocking down fucosyltransferase 9 with short interfering RNA, we further revealed that the Lewis X carbohydrates are involved in the proliferation of neural stem cells. Our findings provide insights into the function of Lewis X carbohydrate epitopes in neural stem cells in the maintainance of stemness during neural development.

3. Glycomics of the Lysosomal Enzyme Sorting Machinery in the Invertebrates

Nadimpalli Siva Kumar

Protein Biochemistry Laboratory, Department of Biochemistry,
University of Hyderabad, Hyderabad-500 046
Email: drsk7@yahoo.co.in

Key words *: Mannose6-phosphate receptor, Lysosomal enzymes.*

The vertebrate Mannose 6-phosphate receptors MPR 300, (Mr 300 kDa) and MPR 46 (Mr 46 kDa) are transmembrane glycoproteins that function in the transport of newly synthesized lysosomal enzymes to lysosomes. Absence of the receptors or specific enzymes will lead to severe lysosomal storage diseases. Only the MPR 300 on the cell surface is designated as a multifunctional protein which involves not only in the internalization of mannose 6-phosphate containing ligands but also other ligands such as the IGF-II, thyroglobulin. Our laboratory has established that both MPR 300 and 46 proteins are structurally and functionally conserved from fish to mammals[1]. To further understand the evolution of lysosomal biogenesis pathway in

the animal kingdom, we focused our recent studies on invertebrates; echinodermates (star fish) and molluscs (*unio* and *Biomphalaria glabrata (Bge*) cells. In both species we have identified distinct lysosomal enzyme activities (alpha-fucosidase, ß-hexosaminidase and arylsulfatase) that were reactive with the respective mammalian antibodies. Furthermore, both receptors were affinity purified by phosphomannan chromatography. Cloning and sequencing of the starfish MPR 46 revealed that the receptor is structurally related to the vertebrate proteins. In a recent study we characterized the MPR 300 dependent pathway of lysosomal enzyme sorting in the *Biomphalaria glabrata* embryonic (*Bge*) cells. Exposure of *Bge* cells to *unio* MPR 300 antiserum resulted in a dramatic loss of MPR 300 protein with a shortened half life of approximately 20 min as compared to control cells exposed to pre-immune serum in which the half life of MPR 300 was of approximately 13 h. Loss of receptor resulted in the secretion of newly synthesized lysosomal enzymes into the cell culture medium as demonstrated by immunoprecipitation. Additionally, labeled lysosomal enzymes added externally were efficiently internalized by the MPR 300 protein in *Bge* cells suggesting the role of the receptor in targeting.

1. Siva Kumar Nadimpalli # and Praveen Kumar Amancha (2010) Evolution of the Mannose 6-phosphate receptors – the lysosomal enzyme sorting proteins– **Current Protein and Peptide Science** 11(1)68-90.

4. Matrix Metalloproteinases and Angiogenesis : a Cell –Cell Contact Dependent Regulatory Process

P. R. Sudhakaran, Athira. A. P. and Kiran. M. S*

School of Biological Sciences, Central University of Kerala, Kasaragod, Kerala,
Email : prslab@gmail.com
*Nanobioanalysis Team, Health Technology Research Center,
National Institute of Advanced Industrial Science and Technology (AIST),
Takamatsu, Kagawa, Japan.

Key words *: Angiogenesis, Matrix metalloproteinase.*

Angiogenesis - the formation of new capillary blood vessel from the pre existing vessel involves dissolution of basement membrane by proteases, cell proliferation and migration. Endothelial cells are the key cells involved in this process. Alterations in both cell–cell and cell–matrix interactions are associated with the activation of endothelial cells that initiate angiogenesis. During the initial stages, cell- cell and cell- basement membrane interactions are weakened whereas in the

later stages of angiogenesis, these interactions are strengthened. One of the molecular mechanisms involved in this is the action of neutral proteinases, particularly matrix metalloproteinases. To understand the involvement of MMPs in angiogenic processes, the *in vitro* model of human umbilical vein endothelial cells in culture was used. A temporal relationship between MMP production by endothelial cells and the onset of angiogenic event was observed. The results of investigations to examine the modulation of MMP expression in a cell contact dependent manner involving catenin will be discussed in the presentation.

5. Is there a Role for Transforming Growth Factor β1 (TGF-β1) Released from Platelets during Thrombus Formation in Delayed Sudden Cardiac Death?

Jasimuddin Ahamed, and Barry S. Coller

Blood and Vascular Biology Unit, Rockefeller University,
New York, NY 10065, U.S.A.
E-mail : jahamed@rockefeller.edu

Keywords *: Transforming growth factor β1 (TGF-β1), Platelets, Thrombosis, Fibrosis.*

Heart Attack, also known as myocardial infarction (MI) is the leading cause of death for both men and women worldwide. Platelet activation and aggregation contribute to heart attacks by occluding the blood vessels and causing immediate ischemic damage to the heart muscle. Platelet activation also occurs when coronary arteries are damaged by percutaneous coronary interventions (PCI) and during stent placement, procedures that are performed in more than 500,000 patients each year in the U.S alone. In fact, antiplatelet agents given at the time of PCI can not only reduce early mortality, but also reduce cardiac deaths months later, indicating that platelet-derived factors may contribute to disease progression. We have recently demonstrated that TGF-β1 released from platelets can be activated by shear force *in vitro*. Biologically significant amounts of active TGF-β1 were also detected *in vivo* after thrombus formation in the lumen of a mouse carotid artery. These data suggest that shear force could be a potential mechanism for activation of TGF-β1 *in vivo* and potentially contribute to normal physiology and disease states that are associated with high intravascular shear force. We have developed a mouse model of thrombosis to simulate MI, in which platelet microthrombi formed *in vitro* were injected into the left ventricle. We are currently assessing whether the platelet microthrombi enter the coronary arteries and blocking the blood circulation to the

heart muscle and ultimately resulting fibrosis in the heart. Data in humans suggest that alterations in TGF-β1 activity and/or signaling in a wide variety of disorders, including cardiovascular disease involving organ fibrosis. Thus, understanding the source(s) of TGF-β1 and its activation mechanism will provide new insights into its normal and pathologic roles. This study will also have the potential for the diagnosis and treatment of disease.

6. Development of Individual Barcode for Human by using Mitochondrial D-loop Hypervariable Regions : A Tool that may be Useful for the UID (Unique Identity) Project in India

Ghosh S K, Chakrabarty C, Laskar R S, Mondal, R Bhattacharjee MJ, Choudhury Y, Rajwanshi R, Ahmed A, Sherpa A, Bhaumik A, Haldar, A, Singha, B., Bhuiya, B., Tiwary, B., Choudhury, B., Sharma, I., Sharma, J., Sengupta, M., Pasha, M., Das, M., Bhattacharjee, M., Kumar, N.S, Ghosh, P.R, Ghosh, P., Majumdar, R., Devi, S., Bhattacharjee, S., Sen, S., Maitra, S S., Duttagupta, S. and Chakrabarti, S

Department of Biotechnology, Assam University, Silchar-788 011, Assam

E-mail : drsamkarghosh@gmail.com

Keywords : *UID card, Human DNA barcode, D-loop, mt-DNA, Hypervariable region, Indels.*

The UID (Unique Identity) project is a scheme undertaken by the Government of India with the agenda of implementing the envisioned Unique Identification card (UID Card) to most of the population of India. Here, we propose the use of the mitochondrial DNA (mtDNA) D-loop hypervariable regions as an individual identification marker as DNA barcode in human. Owing to its high mutation rate (2.8-5 fold higher than the mtDNA), the mitochondrial D-loop has been extensively used for population migration and forensic studies in human. Keeping these in view, we sequenced and analyzed 25 human D-loop hypervariable regions and observed significant variation (p=0.04 at 5% level of significance) in terms of total nucleotide differences (insertion-deletion and substitution) among the sequences. Moreover, our analysis revealed the presence of atleast one or more indels in each of the sequences, which makes them unique from each other. Based on the results obtained we recommend to exploit these characteristics of the mtD-loop as individual identification marker or barcode in human.

7. Mining the Human Genome for Novel Genes of Cellular Memory Modules

Vani Brahmachari

Dr. B. R. Ambedkar Centre for Biomedical Research
University of Delhi, Delhi
E-mail : vani.brahmachari@gmail.com

The coding capacity of the human genome is significantly limited relative to the complex functions and networks that exist. For instance, gene expression regulation through gene specific transcription factors itself demands a repertoire of genes of considerable size. The paradigm for generating unique regulatory complexes to maintain gene expression states through development with limited number of proteins is provided by the polycomb and trithorax complexes that function as global regulators of transcription through histone modification. It is in this context that our recent work has shown a novel function for a highly conserved chromatin remodeling protein, INO80. We mined the INO80 gene from the human genome by application of in silico analysis and experimentally validated the predicted functions. The contributions of the discipline of bioinformatics in deciphering and understanding the large body of data being generated from high-through-put genome sequencing, transcription profiling, proteomics, epigenomics and also in efforts to integrate these into meaningful biological networks is immense. The present talk will highlight the application of bioinformatics from a biologist's point of view and illustrates its application in the discovery and prediction of functions of a gene in the human genome, coding for a chromatin remodeling protein that helps in altering chromatin structure to facilitate regulated expression of genes during animal development. Further these predictions could be systematically tested biologically both in the context of cells in culture as well as at the organismal level. By in silico analysis, the protein was predicted to be a part of the module that is involved in maintaining developmental decisions at the cellular level, referred to as Cellular Memory Modules, predicting that it could be a dual function protein. The *in vivo* analysis in transgenic flies lent support to the in silico predictions. The presentation will trace the path of this exploration.

8. The Peopling of India : Origin and Health Perspectives

K. Thangaraj
Centre for Cellular and Molecular Biology,
Hyderabad
Email : thangs@ccmb.res.in

The peopling of the Indian subcontinent is the result of several historical migrations. This has had a significant bearing on the extant vast diversity of Indian populations, characterized by 4635 anthropologically well-defined populations. Since India represents one of the largest sources of human diversity, studies on Indian populations provide insight into their complex origin, history and relatedness. In order to answer long-held question on the origin of diverse Indian populations, we have analyzed ~16,000 individuals belonging to several tribal, caste and religious groups with ancestry informative genetic markers. We have shown that the enigmatic Andaman Islanders are the first anatomically modern humans migrated out-of-Africa about 65-70 thousand years ago. Recently, we have screened 560,123 SNPs across the genomes of 132 individuals belonging to 25 diverse groups from 14 Indian states, and six language groups, including two from the Andaman Islands. Our study revealed that a relatively small group of ancestors founded most Indian groups, which then remained largely isolated with limited gene flow for long periods of time. We also identified two main ancestral groups in India: an “Ancestral North Indian (ANI)”, which is distantly related to those in the Middle East, Central Asia, and Europe, and an “Ancestral South Indian (ASI)”, not related to groups outside India. Groups with only ASI ancestry may no longer exist in mainland India. However, the indigenous Andaman Islanders are unique in being ASI-related groups without ANI ancestry. Our results show that genetics patterns in Indian populations have been shaped by a long history of genetic isolation between different groups that predates the caste system in place in India during colonialism. Allele frequency differences between groups in India are larger than in Europe, reflecting strong founder effects whose signatures have been maintained for thousands of years owing to endogamy. We therefore predict that there will be an excess of recessive diseases in India.

9. Associative Study of GST Gene Polymorphism and Lung Function Decline in Coal Miners

B. G. Unni[1], Minakshi Bhattacharjee[1], Sangeeta Das[1], Anamika Das Chutia[1], P. K. Baruah[2], T. Borah[2], S. B. Wann[1], P. G. Rao

[1]Biotechnology Division, North-East Institute of Science and Technology (CSIR), Jorhat 785 006, Assam

[2]Clinical Centre, North-East Institute of Science and Technology (CSIR), Jorhat 785 006, Assam

E-mail: bgunni@rrljorhat.res.in

Keywords : *GST, Open cast coal mine, Lung function decline, COPD.*

Chronic obstructive pulmonary disease (COPD) ranks twelfth in the global burden of disease, but according to recent estimates it has been predicted to rise to the fifth highest burden by 2020. One of the risk factors for developing COPD is on account of the environmental triggering in genetically susceptible individuals. Atmospheric pollution from anthropogenic sources such as coal mining, industrial sources is a serious worldwide concern as it is associated with adverse health effects. This research work has been carried out to study the relative prevalence of the disease amongst the people residing in the vicinity of Open- cast coal mine areas in Assam and also to trace out the genetic susceptibility to the disease in the population. Extensive survey was carried out in the Open- cast coal mine areas in Assam and data were recorded in questionnaire formats by close interaction with the local people with their consent. Blood samples were collected (random sampling) from large number of villagers residing very near to the coal mine through health camps conducted in the area; and spirometry was carried out. There was significant air pollution in the study site and pulmonary function decline was observed amongst most of the villagers exposed to the study site. GSTM1 null type was significantly associated with lung function decline in smoker groups and the presence of at least one active allele (either GSTM1 /GSTT1) seemed to have a protective role in the development of COPD. GSTM1(null genotype) appears to be a risk factor for the rapid decline in lung function in smokers. The impact of potentially injurious environmental and other factors such as smoking status, respirable mixed coal dust will be presented and discussed.

10. New Insights into the Molecular Pathogenesis of Type 2 Diabetes with Special Reference to siRNA & miRNA Advancements

M. Balasubramanyam

Madras Diabetes Research Foundation, Chennai-600 086

E-mail : balusignal@gmail.com & drbalu@mdrf.in

Despite worldwide efforts to find susceptibility loci for type 2 diabetes there has been slow progress in the identification of any specific gene or genes predisposing to this condition. The genetic basis of type 2 diabetes is apparently heterogenous and may show ethnic variations across populations. Indians are one such ethnic group who are more insulin-resistant and considered to be vulnerable high-risk population for developing type 2 diabetes. The discovery of the regulatory function of miRNAs in several disease- states highlights the importance of continuing the investigation of the genome with miRNA profiling and RNAi technologies so as to dissect their role(s) in insulin secretion and insulin action. Recent studies also imply that miRNA, siRNA and epigenetic control of insulin secretion and insulin sensitization may prove to be a new frontier for future research. One of our recent studies demonstrated a role for O-glycosylation processes during the induction of insulin resistance in skeletal muscle. Cells treated with glucosamine or high-glucose exhibited increased UDP-GlcNAc levels and decreased insulin-stimulated glucose uptake. We used RNA interference (RNAi) for target-specific gene silencing as a powerful tool to dissect the underlying cause of insulin resistance. Cells silenced for O-glycosyl transferase (OGT) showed improved insulin-stimulated glucose uptake ($p<0.05$). While cells treated with either glucosamine or high-glucose exhibited increased JNK activity; silencing of OGT resulted in inhibition of JNK and normalization of glucose uptake. Of late, miRNAs are garnering attention on the biomedical front, startling researchers with ever expanding roles in various disease-states including Type 2 diabetes. Our preliminary studies imply a role of miRNA impairment linking inflammation and insulin resistance in Type 2 diabetes. miRNA microarray profiling done on human skeletal muscle obtained from clinically well-characterized individuals implied that altered miRNAs contribute to the disease mechanism(s) underlying not only Type 2 diabetes but also prediabetes. Recent studies have highlighted a role for specific miRNAs in pancreatic islet development and in the execution of specialized â-cell functions, including insulin synthesis and insulin release in response to secretagogues. Silencing of various kinases was also shown to be efficacious in the prevention of inhibitory signals that cause insulin resistance in skeletal muscle. The potential therapeutic application of siRNA will require effective delivery systems to target cells and tissues that may pose a challenge

for the treatment of metabolic diseases. Nevertheless, it is important to note that strategies to both mitigate off-target effects and advance siRNA delivery are currently being developed.

11. Unraveling the Genetic Complexity of Type 2 Diabetes : Past, Present and Future

Dwaipayan Bharadwaj
Institute of Genomics & Integrative Biology (CSIR),
Mall Road, Delhi 110 007
Email : db@igib.res.in

Solving the genetic puzzle for type 2 diabetes like other complex disorders has been very challenging owing to the complex genetic architecture and the small effect sizes of the genetic variants. India harbors the maximum number of type 2 diabetes patients in the world but little work has gone in to understand the factors predisposing Indians to this. One of the main focuses of our lab is to understand the genetic links of type 2 diabetes. Using the candidate gene approach we have two novel genes (*FOXA2* and *DOK5*) for type 2 diabetes. *FOXA2* and *DOK5* have important roles to play in the insulin signaling pathway and *DOK5* is also strong positional candidate for both type 2 diabete and obesity. We have also looked into the regulatory SNPs of 1q21 and 20q13, two chromosomal regions strongly linked to type 2 diabetes, and have come up with evidence for association of at least two more novel genes. Recently, we developed a new approach of gene prioritization for type 2 diabetes using the properties of a protein like its chromosomal location, interacting partners, biological function and position in interaction networks. We are now evaluating the association of the top signals from this approach. Genome wide association studies are the latest buzz word in the field of complex disorders and have led to the identification of a large number of genes. We evaluated the top eight signals of type 2 diabetes that have been shown to be associated with type 2 diabetes in the European population. Our analysis revealed significant association for all eight signals and four of them had effect sizes higher than that reported for Europeans. We are right now performing a large scale GWAS of type 2 diabetes where we are studying more than 12,000 subjects. This would be the largest genetic study on the Indian population. Though the above mention approaches have provided us with new insight, still the rare variants association, defining the causal variants and identifying additional variants at the same loci are matters to be looked into. With the cost of sequencing coming down we believe targeted region sequencing and whole genome sequencing will help us unravel the genetic complexity of complex disorders.

12. Metabolites, Metabolic Profiling and Proteomics in Relation to Metal Toxicity and Tolerance Mechanisms in Crop Plants

R. S. Dubey

Department of Biochemistry, Faculty of Science,
Banaras Hindu University,Varanasi-221 005
Email : rsdbhu@rediffmail.com

Keywords : *Metal Toxicity, Tolerance, Metabolites, Proteomics, Plants.*

Due to human activities, geochemical weathering of rocks, anthropogenic release of metals into the soil, pollution of soils by metals has become ever-growing global problem. Contamination of soil with Cd, Pb, As, Hg, Cr, Ni, Zn, Al, Mn and Cu has caused concern for plant and human health. Excessive levels of these metals in the soil can be highly phytotoxic. Plants have developed sophisticated mechanisms to sense metal-excess, and trigger signal transduction cascades, which in turn activate stress responsive genes and ultimately lead to changes at the physiological, biochemical and molecular levels. A myriad of metabolic alterations is induced within the plant tissues when metals are taken up by plant roots. Most common responses of metal toxicity in plants include overproduction of several compatible metabolites like amino acids, sugars, polyamines, phytochelatins and organic acids; alteration in activity of many enzymes and their increased synthesis and stress specific proteins. Most of these metals, when accumulate in plant tissues, cause increased production of reactive oxygen species and consequently elicit oxidative stress marked by increased lipid peroxidation, protein oxidation and fragmentation of nucleic acids**.** Plants expressing constitutively or targeted higher levels of many specific metabolites or components of the antioxidative defense system often show tolerance to metals. The specific responses of plants to a particular metal-excess and those associated with metal tolerance have served as basis to engineer crop plants suitable for cultivation in metal contaminated soils. Overall metabolite profiling analysis of plant tissues holds the promise to permit simultaneous monitoring of precursors, intermediates, and products of metabolic pathways. Proteomics offers a new platform for studying complex biological functions involving networks of proteins, comprehensive identification of proteins in the tissues and their functions. Metabolomics and proteomics are now defining critical functional and regulatory nodes important in our understanding of metal toxicity and tolerance mechanisms in plants in order to produce transgenic crop plants with enhanced metal tolerance for cultivation in excess-metal-prone areas.

13. Glutathione Metabolism in Yeasts

Anand K Bachhawat

Institute of Microbial Technology, Sector 39-A,
Chandigarh-160 036
Email : anand@imtech.res.in

Keywords : *Glutathione, Yeasts, Sulphur, Transporter, Degradation, Biosynthesis, Virulence.*

Glutathione is an essential metabolite in eukaryotes, playing an important role in redox homeostasis. Glutathione requirements need to be met either by endogenous biosynthesis or by uptake of exogenous glutathione. In addition to its other various functions, glutathione can also be used as a sulphur source in yeasts, and this requires the transport of glutathione by specific transporters (of the oligopeptide transporter family) followed by its degradation by a specific degradation complex. Genome sequence comparisons have revealed that the degradation complex proteins,Dug1p, Dug2p and Dug3p are fungal specific, present in almost all yeasts, with *Schizosaccharomyces pombe* being the only known exception. In contrast homologues of the glutathione transporter (Hgt1p) are present in all yeasts and plants and in some bacteria but are absent in metazoa. The only yeast known to lack members of the transporter family is the yeast pathogen *Candida glabrata.* We have evaluated glutathione metabolism in these and other different yeasts, and our studies reveal that yeasts have responded differently to meet their requirements. Studies with the pathogenic yeast*s C.glabrata and C.albicans* have also revealed that the ability to utilize and degrade glutathione is not essential for the virulence of these yeasts. In contrast, we observed an important role for glutathione biosynthesis. While glutathione biosynthesis is essential in *C. glabrata*, in *C.albicans*, although the enzyme is not essential, it is essential for virulence as seen in a murine model of disseminated Candidiasis. The essentiality of γ-glutamyl cysteine synthase in *C. glabrata*, and its essentiality for virulence in *C. albicans* underline the importance of expanding the repertoire of antifungals known to deplete glutathione in yeast and places the enzyme as a strong candidate for antifungal development.

14. Genetic Diversity of T-helper Cell Epitopic Regions of Circumsporozoite Protein of *Plasmodium Falciparum* Isolates from India

Jaiprakash N. Singh[1], Sanghmitra Verma[1], Sukla Biswas[1], Nalini Srivastava[2], Pramatha R. Bhattacharya[1]

[1]National Institute of Malaria Research, Delhi
[2]Department of Biochemistry, Jiwaji University, Gwalior, MP

Keywords *: T-helper cells, Epitope, Plasmodium falciparum.*

Genetic variations in the T-helper cell epitopic regions (Th2R and Th3R) of circumsporozoite protein of 642 *Plasmodium falciparum* isolates collected from different epidemic and endemic regions of India were studied. These isolates also collected at different phases of malaria transmission. Genomic DNA was isolated from the parasites and T-helper cell epitopic regions were PCR-amplified and the products were purified and then sequenceed. Sequence variations were found to exibit restricted polymorphism and can be biased, as different isolates collected from different regions were found to belong to the same group. The alleles of 2 groups in both Th2R and Th3R showed identical sequence variation with those observed in other geographical regions of the world. Since the variations are restricted, the prototype variant from the groups could be included in a subunit polyvalent vaccine against sporozoites.

15. From Rodents to Canines to Humans : A Comparative Evaluation of Xenobiotic-metabolizing Enzymes, Oxidative Stress, Cell Proliferation and Survival, and Apoptosis Evasion

Nagini S and Vinothini G

Department of Biochemistry and Biotechnology,
Faculty of Science, Annamalai University,
Annamalainagar- 608 002, Tamil Nadu
Email : s_nagini@yahoo.com

Keywords : *Apoptosis, Breast cancer, Canine mammary tumors, Caspases, Nuclear factor-kB, Oxidative stress, Xenobiotic-metabolizing enzymes.*

In human, canine, and rat mammary tumors, the expressions of estradiol and estrogen receptor, phase I (CYP total and as well as CYP1A1, 1A2 and 2B

isoforms), and phase II (GST and QR) XME and oxidative stress markers (4-HNE, HEL, and 8-OHdG) were significantly enhanced compared to the corresponding control tissues. This was associated with increased cell proliferation as evidenced by upregulation of PCNA and GST-P, and NFκB activation. Analysis of intrinsic apoptosis revealed altered balance of Bcl-2 family proteins with downregulation of cytochrome C, Apaf-1, and caspases indicating apoptosis evasion in mammary tumors. These changes reflect altered estrogen metabolism, imbalance in XME, oxidative stress, increased cell proliferation, aberrant NF-κB signaling and an apoptosis resistant phenotype in mammary tumors of all three species. The present study confirms the value of the dog as a suitable model for the development of prognostic molecular biomarkers in breast cancer, and the rat model for the evaluation of novel cancer therapeutics. In the era of comparative genomics, understanding the expression profiles of key molecular targets will provide opportunities to develop treatment regimens that can restore dysregulated pathways in breast cancer patients.

16. Molecular Characterization and Genetic Diversity of Begomovirus in Rajasthan

Kusum Dhakar, Rajneesh Prajapat and R. K. Gaur

Department of Science, FASC, Mody Institute of Technology and Science, Lakshmangarh-332 311, Rajasthan

Email : gaurrajarshi@hotmail.com

Keywords : *Begomovirus, Genetic diversity, Coat protein, Rajasthan.*

Begomovirus caused diseases are major problem for the production of economical important crops such as chilly, tomato, cotton, mainly in the tropical and sub-tropical region. The viruses are spread due to change in ecological condition, recombination of begomovirus and introduction of white fly. During the survey in 2009-10 samples showing the typical leaf curl, yellowing leaf curls and mosaic symptoms were collected from almost all the region of Rajasthan. The infected leaf samples were used for PCR based screening by using the coat protein (CP) universal primer. Out of seventy leaf samples 55 showed the positive result with the amplification product of ~ 530 kDa. The sequence analysis of the amplified product showed the homology of 70-90% with the other reported begomovirus, and that showed the maximum diversity of begomovirus in Rajasthan.

17. Urgent Need for New and Safe Drug in Place of Isoniazid : Current Scenario of Pharmacogenomics on Isoniazid

S.Rajarajan
Bioinformatics Infrastructure Facility Centre of DBT,
Govt. of India, Presidency College, Chennai
Email : drsrajarajan@gmail.com

Keywords : *Pharmacogenomics, Isoniazid, NAT, Mycobacterium Tuberculosis.*

Tuberculosis is a slow progressing long standing disease affecting the health and productivity of individual to the Nation. Pulmonary tuberculosis is very common among Indians. Inspite of the administration of single as well as multiple drugs twenty lakh Indians are infected afresh every year by tuberculosis bacillis (*Mycobacterium tuberculosis*). Among these, five lakh Indians die every year!! Isoniazid, used for primary treatment, has been a well proven efficacious drug for pulmonary tuberculosis. However the patients administrated with isoniazid for months exhibit severe adverse reaction inclusive of hepatotoxicity, cancer in urinary bladder or in colorectal region. These adverse reactions are due to inherent variations among individuals to absorb the drug. Isoniazid was the first drug in which pharmacogenomics was observed. Three levels of absorption have been reported for isoniazid in human namely fast, slow and intermediate acetylations. The absorption of isoniazid is 90% in fast acetylators, 67% in slow acetylators and 78% among intermediate acetylators. Thus 10-33% of isoniazid is unutilized and excreted out that result in adverse reaction. Neither clinical data nor genomic data on this varying absorption is available on tuberculosis patients of India. These three types of variations are by a polymorphic gene, NAT -2 (N-acetyl transferase). Except for an unpublished work by Maulana Azad Medical College, New Delhi, and on isolation of NAT-2 gene from few patients, comprehensive study to identify different acetylators among Indians and confirmation of them by isolation NAT- 2 will lead to personalized medicine and possible reduction of side effects. The differential acetylation of isoniazid is more complicated by findings on the presence of polymorphic NAT gene in *Mycobacterium tuberculosis* in addition to mutant Kat G that confers resistance of the bacterium to drug. Hence there is an urgent need to develop new and a safe antituberculosis drug in place of isoniazid.

18. Enantioselective Enzymatic Synthesis of Chiral Drugs and Drug Intermediates for the better Health Management

Uttam Chand Banerjee

Department of Pharmaceutical Technology,
National Institute of Pharmaceutical Education and Research,
Sec-67, SAS Nagar-160 062, Punjab
Email : ucbanerjee@niper.ac.in

Chirality is a key factor in the efficacy of many drugs and thus the production of single enantiomers of drug intermediates has become increasingly important. In this regard, biocatalysis is one of the key methodologies for the production of chiral compounds. Since the great advantages of chemical processes are simplicity, speed and low cost, why should biocatalysts be of interest? These advantages are however irrelevant when the product is labile and requires *regio-* or *chemo-* selective reactions. Biocatalysts are attractive because of their *regio-*, *chemo-* and *enantio-*selectivity and their activity under mild conditions. Although these characteristics can be useful, they can also limit their exploitation. Biocatalysis has therefore added an additional dimension to the production of high-value chiral compounds and coupled with synthetic chemistry, many more exciting developments should be forthcoming. Nitrilases are one such class of enzymes that have considerable potential and significant industrial interest but their applicability to the development of industrial-scale processes has been held back because of many such limitations. The presentation aims at elaborating the limitations of nitrilase-catalyzed biotransformation and how they can be alleviated to harness their catalytic potential. Nitrilases (EC 3.5.5.1) are an important class of hydrolases that convert nitriles to the corresponding carboxylic acids and ammonia. Nitrilases are useful biocatalysts for organic synthesis because this eco-friendly bioconversion allows clean and mild synthesis with high selectivity and yield. Enantiopure á-hydroxy acids especially (*R*)-(-)-mandelic acid is an important fine chemical which acts as a key intermediate for the synthesis of several pharmaceuticals. Nitrilase mediated production of optically pure mandelic acid provides significant advantages over other routes but also offers several challenges for the development of a competitive biotransformation process due to the lack of availability of readily usable biocatalyst, low stability of the existing enzymes and low solubility of the nitriles. The isolation of a potent organism which harbours enantio-selective nitrilases will be discussed here.

19. Integrating Omics with Traditional Medicine Provide Molecular Clues to Glaucomatous Neurodegeneration

Arijit Mukhopadhyay

Institute of Genomics and Integrative Biology (CSIR), Delhi

E-mail : arijit@igib.res.in

Keywords *: Glaucoma, Neurodegeneration, Genetic marker.*

In the post genomics era the ways and means of deciphering molecular causes for complex diseases has been revolutionized. The amount of publicly available datasets has enabled the researchers to ask broader questions where they can enrich their dataset and their conclusions by merging their data with the public domain information. Technology has now allowed querying more then a million loci in a genome if not sequencing the entire genome in matter of days. Yet it is puzzling that in spite of all these technological advancements, we actually achieved very little in order to understand the underlying biology of common complex diseases. Today, we are no longer limited by our ability to query the genome, but by our ability to select the correct study group to expedite disease gene identification. In our laboratory we try to use novel phenotyping approaches to 'homogenize' our cohorts to maximize the chance of genetic marker discovery. In this meeting our one such effort to decipher the molecular causes underlying glaucoma, a common complex neurodegenerative disease of the retina, will be discussed.

20. Proteomic and Genomic Tools to Validate Herbal Products : A Novel Approach

Yamini Bhusan Tripathi

Department of Medicinal Chemistry, Institute of Medical Sciences,
Banaras Hindu University, Varanasi-221 005

E-mail : yaminiok@yahoo.com

Keywords : *Peuraria tuberose, Nephroprotective, PKC inhibitor, Antiinflammatory.*

Use of herbal preparations are gradually gaining momentum, worldwide, specially for metabolic and age related chronic disorders. However, even after their long time clinical use, its global acceptability is still questionable. It is mainly because of lack of standardization parameters and also experimental studies to prove their

mechanism of action and associated side effects. Since these medicinal plants are mostly collected from wild, means they are naturally grown and not cultivated, so there is always a chance of variation in the quantity of secondary metabolites, because they are dependent on climatic changes. This can be overcome by HPTLC/ HPLC based method. The second issue is related to identification of actual active species. This can be addressed through RAPD and RFLP based techniques. It is similar to DNA finger printing to match 2 human individuals. Although this could be a strong tool, but still it is not common for angiospermic plants, because of their large DNA material. The third issue is to assess the effect of various plant extracts or their isolated secondary metabolites on various enzymes in animals to explore their mechanism of action. Here Western blot analysis and PCR based techniques would be of great importance. Based on this approach, we have identified the authentic sample of *Peuraria tuberose* Linn and explored its signaling cascade in macrophage culture. Based on the results, we could conclude that PTY-1, a patented product (*2751/DEL/2008)* from this plant, has nephro-protective property in cisplatin induced and streptozotocin-induced chronic diabetic nephrotoxicity and it is acting through its antioxidant and anti-inflammatory properties, which are finally inhibiting the PKC mediated signaling cascade.

21. From Venom to Drug : Therapeutic Application of Direct-acting Fibrinolytic Enzyme from Snake Venom for the Treatment of Thrombosis

A. K. Mukherjee

Department of Molecular Biology and Biotechnology,
Tezpur University, Tezpur-784 028
Email : akm@tezu.ernet.in

Thrombosis occurs due to accumulation of fibrin in the blood vessels and it leads to myocardial infraction and other cardiovascular diseases. Further, fibrinolytic enzymes may be used continuously for improving cardio-vascular health, lowering blood pressure and as natural cleanse of the blood vessels. Although t-PA, urokinase and other plasminogen activator capable of degradation of fibrin by converting inactive plasminogen to plasmin, have widely been used for the thrombolytic therapy; however, their expensive prices and undesirable side effects encourage researchers to search for cheaper and safer resources. During these decades, direct-acting fibrinolytic enzymes have attracted much more medical interest and it has been suggested that the direct fibrinolytic agents have impressive biochemical and pre-clinical foundations for ultimate clinical application for the treatment of thrombosis.

Snake venom contains many enzymes and toxins and it is a rich source of fibrinolytic enzymes. Many of the fibrinolytic enzymes derived from snake venom are commercially explored for the treatment of thrombosis. In this presentation an attempt will be made to present an overview of the direct-acting, plasmin like fibrinolytic enzymes derived from snake venom, their biochemical properties pertaining to therapeutic application, assessment of pre-clinical foundation and theraputic potential.

22. Recombinant Anthrax Vaccine : Clone to Clinical Trials

Rakesh Bhatnagar

School of Biotechnology, Jawaharlal Nehru University,
New Delhi-110 067
E-mail: rakbhat01@yahoo.com, rakeshbhatnagar@mail.jnu.ac.in

The nature of bio-terrorism resulting from anthrax attack is such that an aggressor is likely to strike at a time and place calculated to induce maximum terror through mass casualties. In the absence of specific intelligence in terms of medical surveillance and integrated real-time detection systems, the unpredictable nature of such events compels the development of medical countermeasures, which will enable the authorities to treat the exposed individuals. Early treatment is essential, when the disease reaches a point at which antibiotics are no longer effective owing to the accumulation of a lethal level of toxin, even though the organism is sensitive to the agent. The currently recommended post exposure treatment is a combination of an antibiotic (ciprofloxacin) and a licensed human vaccine AVA (highly toxic with side effects). We have PCR-cloned and over expressed the anthrax protective antigen gene. Bioprocess optimization was done to improve the yields of the genetically engineered protective antigen. The total yield of genetically engineered vaccine obtained was 25 g from a 5-liter bioreactor, which is equivalent to 1 million shots. The genetically engineered protein was found to be functionally and biologically identical to its *B. anthracis* antigen. Toxicity studies conducted on this protein indicated that the protein is devoid of any toxicity and can be safely used for the development of a safe and effective genetically engineered vaccine against anthrax. Technology for making genetically engineered vaccine against anthrax has already been transferred to Panacea Biotech Ltd., New Delhi, a pharmaceutical company already in the business of making polio and Hepatitis B vaccine. Phase II clinical trials have been completed.

23. Colorectal Carcinoma Cells Induce Dysfuntion of Dendritic Cells Through Glycosylation-depenent Interactions between Tumor-associated Glycans and DC-SIGN.

Motohiro Nonaka, Bruce Yong Ma, Nobuko Kawasaki, and Toshisuke Kawasaki

Research Center for Glycobiotechnology,
Ritsumeikan University, Japan
Email : mnonaka@fc.ritsumei.ac.jp

Keywords : *Dendritic cell, Tumor-associated glycans, Immune regulation.*

Dendritic cell (DC)-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) is one of the major C-type lectins expressed on DCs. Here, we demonstrated that interactions of DC-SIGN with colorectal tumor-associated Le glycans were important for cellular adhesions between DCs and colorectal carcinoma cells and that these adhesions strongly enhanced LPS-induced IL-6 and IL-10 secretion by monocyte-derived DCs (MoDCs). Additionally, LPS-induced maturation of MoDCs was strikingly inhibited by supernatants of cocultures with colorectal carcinoma cells, suggesting that DC dysfunction induced by the interactions between DC-SIGN and colorectal tumor-associated glycans is one of the critical mechanisms for tumor escaping from immune surveillance.

24. Affinity Purification of Biological Membranes : Application of Nanotagged Antibodies using Proteomics Approach

Dwijendra Gupta, Manish Dwivedi and Vijay Tripathi

1. Center of Bioinformatics (Institute of Interdisciplinary Studies)
2. Department of Biochemistry**,** University of Allahabad, Allahabad
Email : dwijenkumar@rediffmail.com

Nanoparticles *e.g.* Nanophosphors can be coated with monospecific antibodies, DNA, RNA or protein and coupled with advance technological tools (Scanned probe microscopies) for studying a number of cell biologic events, such as signal transduction, transporters function, receptor-mediated endocytosis, ionic flux through biological membranes, virus- host interaction, interaction between host and pathogens, DNA-protein interactions and regulation of transcription events. We are pursuing a proteomics based approach to study molecular basis of lysosomal storage diseases

resulting from defects of membrane transporter proteins. “Structural” lysosomal membrane proteins like LAMPS and tetraspanins are implicated in targeting of mannose 6-P receptors. Based on proteomics data on amino acid sequence targeting motifs in cytosolic domains in lysosomal membrane, one can design peptides and then raise yolk antibodies (IgYs) against these in layer hens and purify IgYs from egg yolk. These IgYs then can be adsorbed onto 40 nm nanogold particles to make nanogold –IgY conjugates, subsequently coated with nanophosphors or CNTs and employ these double-tagged immunoconjugates for affinity purification of lysosomal transport vesicles from human lymphocytic U937 cells by a combination of affinity or density perturbation and subcellular density-gradient ultracentrifugation protocol. To further this line of approach, we have prepared nanophosphor particles using ZnO, manganese doped ZnS and ***Eu:Y2O3*** nanophosphors and characterized these using XRD technique, phospholuminscence, UV spectra and the Wurtzite structures. We are also preparing other nanophosphors to see their feasibility for use in affinity purification of biological membranes. This proteomics-nanoparticle tagged approach can also be suitably adapted for dynamic studies on living cells as well as a potential route for delivery of medicine to appropriate cellular and subcellular targets.

25. Origin of Life on Earth and Astrobiology

V. C. Tewari

Sedimentology Group, Wadia Institute of Himalayan Geology,
Dehradun-248 001, Uttarakhand
E-mail : vtewari@wihg.res.in

Keywords : *Microfossils, Astrobiology, Meteorites, Laser Raman Spectroscopy.*

The origin of life on Earth is a fundamental question and still debate is going on whether life originated on Earth or brought to Earth by meteorites or comets (extraterrestrial source). The structure of the first cell and the elements of the building blocks of life (amino acids) , proteins, RNA, DNA molecules is still not well understood. The presence of amino acids in the meteorites supports the idea that life might have come from space and the main source of water on early Earth is from comets. The earliest record of microscopic life on Earth (bacteria) and megascopic (stromatolites) goes back to 3.5 billion years paleobiological history of

the planet. There could be nano sized life forms before that still to be discovered in the universe. Although the composition of early atmosphere of the Earth is not well known, however, there is enough ground to suggest that it contained all biogenic atoms and molecules in different forms. CH_4 and H_2O molecules were the most abundant in the early atmosphere. Water vapour contains electrically neutral water molecules. H, C, N, O, P atoms are necessary to form nitrogen bases (adenine, guanine, cytosine, and thymine). The main conditions for the origin of life include the water as a vapour and then liquid phase. Liquid form of water is the medium where nucleic acids can live and develop. The recent discovery of water molecule on Moon (also possibly frozen ice) and the evidence of paleochannels on Mars have confirmed the existence of extraterrestrial water in the past. The presence of water and bacteria below the ice sheets in Antarctica and the possibility of water below the ice covered Europa (Jupiter's satellite) indicate that life can survive in extreme conditions. Astrobiological studies and Laser Raman Spectroscopy in future can help in solving the carbon based origin of life in the universe.

26. Extremophiles in the Indian Desert : Source of Valuable Biomolecules

M. M. Saxena

Post Graduate Department of Zoology, Dungar College,
Maharaja Ganga Singh University,
Bikaner-334 001
E-mail : saxenamm@rediffmail.com

Keywords : *Extremophiles, Indian Desert, Biomolecules.*

Deserts are the extreme environments and organisms inhabiting deserts have evolved to develop adaptations to meet the challenges of extreme conditions, particularly those of temperature, aridity and salinity. Since adaptation is a genetic process, the secret of survival success of such organisms lies in their genetic makeup. Quite often, extremophiles is the term applied only to the microorganisms living in the extreme environments. Need there is to extend the term to higher forms including plants and cold-blooded animals that successfully inhabit such environments. The author, while surveying the Indian desert for over three decades and studying tolerance ranges of some groups of animals to given environmental parameters,

particularly temperature and salinity, has come across many ecotypes suited to match the extreme vagaries of nature. These in particular include some protozoans, sponges, leeches, shrimps and snails. Both field and laboratory studies vindicate their varied tolerance to an extremely wide range of conditions. Paper projects on such tolerance ranges that make these lower animal forms eurythermal, euryhydric or euryhaline. Certain biomolecules have been investigated, by different workers, as responsible for survival success of extremophiles, microbes in particular. Identification and isolation of such molecules could be extended to aforesaid animal groups, besides microbes, from the Indian desert. This research could be of significant experimental and industrial application in the fields of biotechnology, pharmaceutics, diagnostics, agro and food industry, oil and fuel, and chemicals.

27. Transportation of Membrane-Bound-Transcription Factor Myb : A Receptor Tyrosine Kinase-mediated Endocytosis

Mohamed Ali-Seyed[1, 2]

Department of Biotechnology, SRM University,
Kattankulathur, Chennai,Tamil Nadu[1],
Korea Research Institute of Biosciences and Biotechnology,
Republic of Korea (South) [2].

Emerging evidences suggest the existence of a new mode of receptor tyrosine kinases (RTKs) signaling pathway in which activated receptor including EGFR and ErbB2 undergoes nuclear translocalization and subsequently regulates gene expression and potentially mediates other cellular processes. This signaling route is distinct from the better-characterized, traditional RTKs signaling pathway that involves transduction of mitogenic signals through activation of multiple signaling cascades. On the other hand, using conventional biochemistry, the identification of transmembrane transcription factors (TMTFs) can be easily overlooked. Transcription factors are generally assumed to be soluble proteins and, consequently, membrane fractions are often discarded during purification so the sub cellular distributions of transcription factors are often not examined. Our laboratory

has not only identified the membrane bound transcription factor c-Myb and provided evidence for the interaction between c-Myb and one of the members in the ErbB receptor tyrosine kinase (RTK) family. We have used multiple novel strategies to demonstrate their interaction in many cellular compartments. Based on our findings we propose a model that depicts ErbB may act as a carrier cargo for membrane-bound c-Myb nuclear transport. If c-Myb or ErbB requires its partner to elicit its oncogenic crime to compensate for its own deficiencies fully or partially, then it might seem both molecules may be ideal candidates to target for cancer therapy. Thus the present study explains not only the interaction of c-Myb with ErbB and their nuclear transport but gives better insight for better understanding the receptor-mediated endocytosis.

28. Maternal Stress Factors Implicated in Down Syndrome Birth

S. K. Dey

School of Biotechnology & Biological Sciences : West Bengal
University of Technology BF-142,
Salt Lake, Sector-1 : Kolkata-700 064,
West Bengal
Email : subrata.dey@wbut.ac.in

Down syndrome due to trisomy 21 is the most common human chromosomal abnormality. In order to gain further insight into maternal stress factors responsible for nondisjunction, we have investigated the association between reduced recombination, maternal age and nondisjunction. We genotyped 12 microsatellite markers spanning along 21q from centromere to telomere in 138 individuals with free trisomy 21 and in their parents. Our DNA marker studies of parental origin were informative in 119 families with overwhelming majority 89.91% being maternal and 10.09% is paternal. Only cases of maternal origin were included in our analysis. The distribution of nondisjunction in maternal meiotic I and meiotic II stages were 81.19% and 19.81% respectively. The mean maternal age of nondisjunction in our Indian population sample is 27.58±6.4 which is significantly lower than that of Caucasians. We created a genetic map, using maternal meiotic

I nondisjunction data. The female genetic map was restricted to 21q. The distribution of chiasma shows a difference throughout the length of chromosome arm (21q) with more recombination towards telomeric end in comparison to control data. The telomeric exchange is found to be a significant risk factor for meiotic I nondisjunction, irrespective of the age of the mother. Analysis of crossover events indicates that in younger mother (<29) there was an increase in both zero- and one exchange events, suggesting reduction of recombination. The linkage map of 21q (39.58cM) was significantly shorter than the control female linkage map, indicating an overall reduction of recombination. Measurement of telomere length indicates that telomere length attrition may be associated in some way with meiosis I and meiosis II nondisjunctions of chromosome 21 and subsequent Down syndrome births at advanced maternal age.

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V

ABSTRACTS OF ORAL / POSTER PRESENTATIONS

PROCEEDINGS
OF THE

NINETY EIGHTH SESSION OF THE

INDIAN SCIENCE CONGRESS

CHENNAI, 2011

PART II : Abstracts of Oral/Poster Presentations

SECTION OF NEW BIOLOGY
(Including Biochemistry, Bipphysics & Molecular Biology and Biotechnology)

President : **Dr. Hasi Das**

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PART II : Abstracts of Oral/Poster Presentations

SECTION OF NEW BIOLOGY
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President : **Dr. Hasi Das**

I. *ORAL PRESENTATIONS*

1. *In Vitro* Antioxidant and Anticancer Activity in the Leaf Extracts of *Costus Igneus* N.E.Br.

Suresh Kumar P[1*] Jeyajothi C, Sudershana Deepa V, Rajaram K

Department of Biotechnology, Anna University,
Tiruchirappalli-20024, Tamil Nadu.

Keywords : *Anticancer, Antioxidant, Adenocarcinoma (A-549), Costus igneus, DPPH.*

The leaves of the *Costus igneus* were used as an ancient folklore medicine to cure many diseases. The present study was focused to screen the presence of potent phytochemicals, investigate the antioxidant and anticancer activity in the methanolic leaf extracts of *C.igneus*. The leaves were extracted with various solvents (ethyl acetate, methanol, ethanol and aqueous). The presence of high content of total phenol and flavonoid was observed only in the methanolic extracts. The methanolic leaf extracts exhibited the highest antioxidant activity (DPPH scavenging activity, superoxide scavenging activity and reducing power assay) compared to any other

extracts tried. Further the MTT assay reveals strong anticancer activity against human lung adenocarcinoma (A-549) cancer cell lines with the inhibitory activity of 47.90 % which was closely comparable to the standard drug (Cyclophosphamide). This is the first report on the antioxidant and anticancer activities of the methanolic leaf extract of *Costus igneus*. Further studies are in progress to identify the lead compound, which will unravel the reason for its potent antioxidant and anticancer activities.

2. *Zingiber Officinale* Roscoe Alone and in Combination with á-tocopherol Protect the Kidney against Diclofenac Sodium-induced Acute Renal Failure

Soumendra Darbar*, Nilendra Chatterjee, Tapas Kumar Chattaraj and Tapan Kumar Pal

Bioequivalence Study centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata.

E-mail : darbarsoumendra@gmail.com

Keywords : *Antioxidant, Free radicals, Nephrotoxicity, Zingiber officinale, Diclofenac sodium.*

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most common prescription medicine in India, also freely available over-the-counter (OTC). Unfortunately, one of the main side effects of NSAID administration is renal function damage. NSAID are accountable for 7% of all cases of acute renal failure and for 37% incidents of drug-associated acute renal failure. Oxidative stress due to abnormal production of reactive oxygen molecules (ROM) is believed to be involved in the etiology of toxicities of many xenobiotics. Evidences suggested that ROM is involved in the nephrotoxicity of a widely used non steroidal anti-inflammatory drug, diclofenac sodium. The nephroprotective effects of ethanol extract of *Zingiber officinale* alone and in combination with vitamin E (á-tocopherol) were evaluated using diclofenac sodium (150 mg/kg body wt., i.p) induced acute renal damage in mice. The serum urea and creatinine levels in the diclofenac sodium alone treated group were significantly elevated ($P<0.001$) with respect to normal group of animals. The levels were reduced in the *Z. officinale* (250 and 500 mg/kg, orally) plus diclofnac sodium, vitamin E (250 mg/kg) plus diclofenac sodium, and *Z. officinale* (250 mg/kg) with vitamin E plus diclofenac sodium treated groups. The renal antioxidant enzymes such as superoxide dismutase (SOD, catalase (CAT), glutathione

peroxidase (GPx) activities and level of reduced glutathione (GSH) were declined; level of malondialdehyde (MDA) was elevated in the diclofenac sodium alone treated group. The activities of SOD, CAT, GPx and level of GSH were elevated and level of MDA declined significantly ($P<0.05$) in the *Z. officinale* (250 and 500 mg/kg) plus diclofenac sodium and *Z. officinale* (250 mg/kg) with vitamin E plus diclofenac sodium treated groups. The protective effect of *Z. officinale* (250 mg/kg) was found to be better than that of vitamin E (250 mg/kg body wt). The results also demonstrated that combination of *Z. officinale* (250 mg/kg) with vitamin E(250 mg/ kg body wt) showed a better protection compared to their 250 mg/kg alone treated groups. Moreover, *Z. officinale* along with Vitamin E prevented serum and tissue protein alteration as well as DNA fragmentation as compared with the groups treated with diclofenac alone. In the histopathological observation, kidney damage induced by diclofenac sodium was markedly improved in *Z. officinale* treated rats. The study concluded that ethanol extract of *Z. officinale* alone and in combination with vitamin E partially ameliorated diclofenac sodium-induced nephrotoxicity. This protection is mediated either by preventing the diclofenac sodium-induced decline of renal antioxidant defense system or by their direct free radical scavenging activity.

3. Studies on Cytotoxicity, DNA Binding Effect and Inhibition of Collagenase by *Withania Somnifera* and *Cardiospermum Halicacabum* Extracts

Ganesan Krishnamoorthy*, Praveen Kumar Sehgal*, #, Asit Baran Mandal and Sayeed Sadulla**
*Bioproducts Laboratory and **Chemical Laboratory,
Central Leather Research Institute (Council of Scientific & Industrial Research)
Adyar, Chennai - 600 020, Tamil Nadu.
***Indian Shoe Federation, Egmore, Chennai - 600 008, Tamil Nadu.
E-mail : krishnamoorthyganesh@yahoo.com; sehgal_pk@yahoo.co.in

Keywords : *Arthritis, Collagen, Collagenases, DNA, Antioxidant.*

The irreversible destruction of extracellular matrix (ECM) that comprises synovial joints are important in both rheumatoid arthritis (RA) and osteoarthritis (OA) by over expression of Matrix metalloproteinases (MMPs-collagenases). Recent evidence shows that plant extracts such as *Withania somnifera* (Ashwagandha) and *Cardiospermum halicacabum* (Balloon vine) have to lot of biological and

pharmacological applications. The aim of present study was to examine the evidence for anti-arthritic role of *W. somnifera* and *C. halicacabum* extract by following studies anti-oxidant, DNA binding effect, cell viability and inhibition of collagenases activity. We evaluated the anti-oxidant, cytotoxic, DNA binding activities by using different assay methods. This result demonstrates that both these *W. somnifera* and *C. halicacabum* extracts have an ability to serve as good agents to scavenge the free radicals. DNA electrophoresis, UV-Visible and CD spectra indicated the interaction of these extracts with DNA depended on the ratio of the concentration of these extracts versus DNA. These extract presented no cytotoxic effect by fibroblast viability assay. Inhibition to collagenolytic activity of ChC against ECM of collagen degradation and the inhibition were found to be concentration dependent.

4. A Novel Formulation for Modulation of Radiation Inflicted Tissue Injuries for Human Application

Manju Lata Gupta, Ajaswrata Dutta, Sandeep Kumar Shukla, Sanghmitra Sankhwar, Nitya Nand Srivastava, and Savita Verma

Radioprotective Drug Development Group, Division of Radiation Biosciences, Institute of Nuclear Medicine and Allied Sciences, Brig. SK Mazumdar Road, Delhi-110 054

Keywords : *Radioprotection, Ionization radiation, Podophyllum hexandrum.*

Current study is focused on the development of a biological radioprotector against accidental nuclear exposures for human applications. G-002M, a novel formulation from *Podophyllum hexandrum* rendered >90% protection in lethally exposed mice. ^{99m}Tc labelled G-002M was available to most of the vital organs of mice within 30min of administration and retained up to 4hrs. Peripheral blood counts and GI tract of irradiated mice were also significantly protected on G-002M pretreatment. Mechanistic studies revealed protection to DNA (ãH2AX), proliferation of hematopoietic stem cell marker (ScaI) and regulation of NF-κB and NRF2 as the possible mode of action of G-002M. Studies strongly suggest further exploration of this novel formulation for human application against undesired radiation exposures.

5. Effect of Composition and Peptide Bond Conformation in Modulating the Fragmentation Pathway of Cyclic Peptides : a Tandem Mass Spectrometric Approach

Raja Banerjee[1,2], S. Sudarslal[2] , R. S. Ranganayaki[2] & S. Raghothama[3]

[1]Deptartment of Bioinformatics, West Bengal University of Technology, BF-142, Salt Lake, Kolkata.

[2]Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012,

[3]NMR Research Centre, Indian Institute of Science, Bangalore 560 012

Keywords : *Tandem mass spectrometry, Metal ion interaction, Cyclodepsipeptide, Isariin and isaridin, NMR.*

Fragmentation behavior of two classes of cyclodepsipeptides, *isariins* and *isaridins*, in the presence of different metal ions using multistage tandem mass spectrometry with CID showed that the presence of β-hydroxy group in isariins led to follow the identical fragmentation pathway (generating product ions belonging to 'b-ion' series only) in its cyclic and its acyclic methyl ester derivatives in metal independent manner. However the presence of 'cis' peptide bonds in isaridins and its acyclic methyl ester derivatives, played a crucial role in sequence-specific charge remote fragmentation (generating b/y ions in a metal dependent manner for isridins while only y ions for its acyclic system in a metal independent manner). Complementary NMR data pointing towards metal-peptide interaction supports the fragmentation pattern. This study provides insights into peptide composition and peptide bond geometry in cyclic sequences for de novo sequencing in proteomics.

6. Role of Beta 3 Integrin-BMX Signaling for STAT3 Activation during Compensatory and Decompensatory Hypertrophy

Geetha Suryakumar

Defence Institute of Physiology & Allied Sciences,
Lucknow Road, Delhi-54..

Heart failure ensues when the compensatory hypertrophic process does not adequately meet the demand for increased cardiac load and/or when this growth phenomena differs qualitatively from that of physiologic growth. Our laboratory focuses on integrin-mediated signaling in pressure overload (PO) cardiac hypertrophy. Signal transducer and activator of transcription-3 (STAT3) mediates cardioprotection

in pathogenic settings of heart failure. Our studies on STAT3 in feline right ventricular (RV) PO model demonstrated distinction between compensated and decompensated hearts. During the initial phase of PO, there is a substantial increase in the level of STAT3 with a robust phosphorylation and translocation to the membrane skeletal (MSK) fraction.in addition, BMX, a non-receptor tyrosine kinase and an activator of STAT 3 also shows localization and activation similar to STAT3. However, in severely PO feline, STAT3 and BMX recruitment to the MSK and phosphorylation were reduced although their levels increased in the soluble fraction. Hence we hypothesized that the activation of a specific integrin causes cytoskeletal assembly and results the activation of STAT3 during compensatory cardiac hypertrophy. We chose beta3 integrin knockout (KO) mice and subjected them to transverse aortic constriction (TAC) pressure overload. Western blot analyses indicate that STAT3 level, association to the detergent insoluble MSK fraction and phosphorylation were substantially increased in the wild type mice following 72h TAC but considerably reduced in beta 3 integrin KO mice. In addition, BMX, a non-receptor tyrosine kinase activated downstream of integrin and an activator of STAT3 pathway, shows localization and activation pattern paralleling STAT3. Both these KO mice exhibited altered cellular localization as analyzed by confocal microscopy. Our data suggest that integrin-mediated BMX activation could be a mechanism for STAT3 activation during PO induced compensatory hypertrophy. These results form a molecular basis for defining adverse myocardial remodeling upon PO hypertrophy.

7. Anticancer Properties of Epicatechins Purified from Green Tea (*Camellia Sinensis*)

T. S. Saravanan

Jamal Mohamed College,
Trichirappalli-620 020, Tamilnadu.

Green tea is a perennial shrub belonging to the family *Camellia*, native to the mountainous southwest of China. Teas are usually categorized into two types: Chinese (Variety Sinensis) and Assam (Variety Assamica). All teas come from leaves that are picked and processed from the same type of tree. The specific method of processing differentiates the various types of teas into green, black, and oolong tea. In preparing green tea, the leaves are dried but not fermented. Commercially prepared green tea extracts contain 60 per cent polyphenols. There are four primary polyphenols in green tea and they are often collectively referred to as Catechins. This *in vitro* study examined the biological effects of catechin (EC), epigallocatechin

(EGC), EC 3-gallate (ECG) and EGC 3-gallate (EGCG) in cell lines from human gender-specific cancers. The cell lines developed from organ-confined (HH870) and metastatic (DU145) prostate cancer, and from moderately (HH450) and poorly differentiated (HH639) epithelial ovarian cancer were grown with or without EC, EGC, ECG or EGCG. When untreated cells reached confluency, viability and doubling time were measured for treated and untreated cells. Whereas EC treatment reduced proliferation of HH639 cells by 50%, EGCG suppressed proliferation of all cell lines by 50%. ECG was even more potent; it inhibited DU145, HH870, HH450 and HH639 cells at concentrations of 24, 27, 29 and 30 µM, whereas EGCG inhibited DU145, HH870, HH450 and HH639 cells at concentrations 89,45,62 and 42 µM. When compared with ECGC, ECG more effectively suppress the growth of prostate cancer and epithelial ovarian cancer cell lines derived from tumors of patients with different stages of disease. As powerful antioxidants, catechins have been shown in recent studies to fight viruses, slow aging, and antiproliferative effect on cancer cells and also have other beneficial effect on health.

8. The Influence of Osmolytes and Denaturants on the Structure and Enzyme Activity of á-chymotrypsin

Pannuru Venkatesu

Biophysical Chemistry Laboratory, Department of Chemistry,
University of Delhi, Delhi-110 007,
E-mail : pvenkatesu@chemistry.du.ac.in; venkatesup@hotmail.com

Keywords *: á-Chymotrypsin, Protein folding/unfolding, Osmolyte, Urea, Differential scanning calorimetry.*

Enzymes are very sensitive and highly complex systems, exhibiting a substantial degree of structural variability in their folded state. In the presence of co-solvents, the fluctuations among vast numbers of folded and unfolded conformations occur alternatively, enzymes can be stabilized or destabilized. To understand the osmolytes and denaturants contribution on the stabilization and enzyme activity of á-chymotrypsin (CT), we have monitored the differential scanning calorimetry (DSC), circular dichroism (CD), enzyme activity and gel electrophoresis as a function of osmolyte or denaturant concentrations. Evidently, we observed that naturally occurring osmolytes play dominant contribution on stabilization of CT while not enhances its enzyme activity.

II. *POSTER PRESENTATIONS*

i. GENOMICS

1. *rmpM* Gene as a Genetic Marker for Detection of Human Bacterial Meningitis

Sandip K. Dash[1], Simrita Singh[2], Shashi Khare[2], Minakshi Sharma[3] and Ashok Kumar[1]

[1]Institute of Genomics and Integrative Biology (CSIR),
Mall Road, Delhi-110 007
[2]National Center for Communicable Disease,
Sham Nath Marg, Delhi-110 054
[3]Department of Zoology, M. D. University,
Rohtak, Haryana-124 001

Keywords *: rmpM gene, Genetic markers, Meningitis.*

Bacterial meningitis is the most complicated and dreadful, as compared to viral and fungal meningitis. Among all divergent causes of bacterial meningitis, *Neisseria meningitidis*, is the chief most causative agent. Several investigators have attempted to establish a simple, sensitive and quantitative method for detection of bacterial meningitis from cerebrospinal fluid of patients. However, the conventional methods like LAL assay, LA test and Gram Stain are very less sensitive. Whereas, recently used methods like microarray and biosensor based detection methods are highly expensive. PCR is being used as a gold standard method for detection of bacterial meningitis since 1995. Reduction modifiable protein M (rmpM) or class 4 outer membrane protein is a very well studied protein found on the surface of *N. meningitidis*. This protein has two domains each for binding with peptidoglycan and outer membrane proteins. Hence, serves as an interlinking region between outer membrane protein and peptiodoglycan layer. This gene plays an essential role for stability and viability of bacteria. Targeting this gene, our lab has developed a quick PCR based diagnosis of bacterial meningitis in 35 min including agarose gel electrophoresis. An amplicon of 309 bp of surface protein coding gene (*rmpM*) can be used as genetic marker for detection of *N. meningitidis* in bacterial meningitis. Our method is highly economical, quick and equally sensitive as most of the advance methods.

2. Studies on Genetic Diversity Among *Saccharum Spontaneum* Clones and Commercial Hybrids Through ssr Markers

Neha Mishra, M. L. Sharma and R. K. Singh

Center for Sugarcane Biotechnology, Sugarcane Research Institute, Shahjahanpur-242 001

Keywords : *Saccharum spontaneum, Commercial hybrids, Similarity coefficient, Genetic diversity, ssr markers.*

Genetic diversity within the *Saccharum spontaneum* germplasm and commercial cultivars from sub tropical part of India has not been extensively examined with molecular markers as it was quite difficult to recognize by morphological and physiological characters. The similarity coefficient ranging from 0.347 (CoS96268 and Calcutta) to 0.865 (CoS95255 and CoS767) revealed that there was sympatric relationship between variety x variety and allopatric (0.347) relationship between variety x species. This has also confirmed by the higher variation observed for quality traits in *S. spontaneum* (71.4%) than in commercial cultivars (23.11%). These diverse genotypes had not been extensively utilized in the development of other sugarcane cultivars; they can be used as progenitors for the creation of cultivars with a wider genetic base.

3. Genotyping and Phenotyping for Aroma of F_{10} rils Mapping Population Developed from a Cross Between CSR 10 x HBC 19

Pummy Kumari[1], Uma Ahuja[2], R. K. Jain[3] and A. K. Sarial[2]

[1]Department of Genetics, [3]Department of Biotechnology and Molecular Biology, CCS Haryana Agricultural University, Hisar-125 004

[2]College of Agriculture, CCS Haryana Agricultural University, Kaul, District Kaithal-136 021

Keywords *: Rice, Recombinant inbred lines, Aroma.*

Recombinant inbred lines (RILs) of rice among various mapping populations provide a noble material for linkage of marker and trait. A F_{10} mapping population comprising 208 RILs was developed from a diverse cross between CSR 10 (non-aromatic) and HBC 19 (aromatic) rice lins. Genotyping of this population was carried out with 14 microsatellite markers including three specifically developed for aroma.

Mentel test of significance detected by phenotyping with 1.7 % KOH revealed 97 %, 95% and 90.1% association of aroma with the markers BAD 2, BADEX7-5 and SCUSSRI, respectively. Remaining microsatellite markers did not exhibit association with aroma. The results revealed that the markers could be used for marker assisted selection and population for mapping yield and aroma QTLs/genes.

4. *mga* Gene as a Genetic Marker for Streptococcal Pharyngitis and Rheumatic Heart Disease Caused by *Streptococcus Pyogenes*

Ashok Kumar[1], Richa Chitransh [1], S. Karthik[1], Simrita Singh[2], Shashi Khare[2] and Suman[3]

[1]Institute of Genomics and Integrative Biology (CSIR),
Mall Road, Delhi-110 007
[2]National Centre for Disease Control (NCDC),
Sham Nath Marg, Delhi-110 054
[3]Institute of Advance Research and Studies,
Amity University, Noida-201 303

Keywords *: Rheumatic heart disease, Streptococcal pharyngitis, Streptococcus pyogene.*

Rheumatic heart disease (RHD) continues to be a common health problem in the developing world, causing morbidity and mortality among both children and adults being more common in the age group of 5-15 years and rare in below the age of 5 years. RHD occurs frequently in the winter season when the streptococcal pharyngitis is also on the rise. *Streptococcus pyogenes* also known as group A streptococcus, is a beta-hemolytic bacterium causing streptococcal pharyngitis and rheumatic fever. Rheumatic heart disease is a sequel of rheumatic fever that results from an untreated strep throat causing damage of the heart valves. The usual detection methods are Gram staining, culture, virulence test, catalase test, hippurate test, antibiotic sensitivity, CRP, ESR and PCR. All these methods are expensive, time consuming and have some limitations. Here, we have developed a quick and direct PCR based diagnosis of *Streptococcus pyogenes* using specific primers of virulent *mga* gene (multiple gene activator) of *S. pyogenes.* The overall analysis including electrophoresis completes in less than 1 h which is the least time reported so far for the confirmation of the disease. Amplicon of 394 bp of *mga* gene does not show homology with other organisms and can be used as specific genetic marker for detection of *S. pyogenes.*

5. Increased Chromosomal Aberration Frequency in Peripheral Blood Lymphocytes of Road Construction Workers and Influence ofGSTM1 Polymorphism

Anil Kumar[1], Anita Yadav[1*], Shiv Kumar Giri[1], Kapil Dev[1], Sanjeev Kumar Gautam[1], Ranjan Gupta[2] and Neeraj Aggarwal[3]

[1]Department of Biotechnology, Kurukshetra University Kurukshetra, Haryana
[2]Department of Biochemistry, Kurukshetra University Kurukshetra, Haryana
[3]Department of Microbiology, Kurukshetra University Kurukshetra, Haryana
***Email** : ntydv@yahoo.com

Keywords : *Glutathione-S transferase, Chromosome aberrations, Polymorphism, Road construction workers.*

The frequency of chromosome aberration in peripheral blood lymphocytes have been widely used in assessing exposure to various environmental mutagens and carcinogens. The present work was conducted among 70 unexposed individuals and 75 occupationally exposed road construction workers. The main objective of our study was to explore if there is any influence of polymorphism of GSTM1 genotype on chromosomal aberration (CAs) frequency. We found higher mean value of CAs among exposed workers and controls having GSTM1 null genotype but results were not found significant at $p < 0.05$. Our findings suggest that individuals having null genotype of GSTM1 gene are more susceptible to cytogenetic damage regardless of confounding factors but to arrive at any conclusion more studies are needed.

6. Evaluation of Genetic Damage in Different Age Group of Human Population Due to Radiations from Mobile Towers

Sachin Gulati[1], Anita Yadav[1]*, Shiv Kumar Giri[1], Kapil Dev[1], Anil Kumar[1], Ranjan Gupta[2], Neeraj Aggarwal[3] and Sanjeev Kumar Gautam[1]

[1]Deptt. of Biotechnology, Kurukshetra University, Kurukshetra (Haryana)-136119
[2]Deptt. of Biochemistry, Kurukshetra University, Kurukshetra (Haryana)-136119
[3]Deptt. of Microbiology, Kurukshetra University, Kurukshetra (Haryana)-136119
***E-mail** : anitayadavkuk@yahoo.com

Keywords : *Mobile phone towers, Electromagnetic radiation, Radiofrequency, Health hazards of non ionizing radiation.*

The effect of radiations on human health especially from mobile phones is most interesting and innovative area of research now a days because of enormous

increase in mobile phone usage throughout the world. Present study was focused on effects of electromagnetic radiations emerging from mobile phone base stations or towers on human population using comet assay. This work was carried out in 25 radiation exposed subjects living nearby mobile towers and 25 unexposed controls. Our results showed a significant increase in comet frequency among exposed subjects having age group of 25-40 years old at $P < 0.05$. Our findings suggest that mobile towers are affecting human health therefore more studies are needed to be carried out to make an effective conclusion.

7. RAPD-PCR Based Marker Approach for the Genetic Differentiation of Two Species of Cockroach (Order-Dictyoptera)

Divya Pandey*, Bharat Neekhra, Ankita Pathak and Subodh Kumar Jain

Molecular Biology Laboratory, Department of Biotechnology,
Dr. Hari Singh Gour Vishwavidyalaya, Sagar-470 003, M.P.
***E-mail** : divyabiotek@gmail.com

Keywords *: RAPD, PCR, Cockroach, Random primers.*

Random amplified polymorphic DNA (RAPD) analysis was conducted for the differentiation of two most commonly occuring insect species *Periplaneta americana* and *Biatella germanicana*. The RAPD-PCR proved to be a quick and effective technique to establish genetic markers to differentiate two populations of nearly morphologically similar cockroach species *Peripleneta americana* and *Biatella germanicana*. During the present study ten random primers were used for PCR. Many such bands were obtained, which differentiated between the two species. Based on criteria of interpretability, simplicity and reproducibility, six primers PL (GATGACCGCC), P3 (GGCACGTAAC), P6 (GGTGCGCCTT), P7 (GTCAGAGTCG), P8 (GTCGCCGTCT) and P10 (GTGCCCGATG) were considered for further screening using genomic DNA from each insect species. A series of bands ranging from -300 bp to -1000 bp were observed by using these primers indicated that those two species are related however they also exhibit variation, indicating the presence of conserved regions sharing ancestral relationship. Some of the fragments were unique in both the species which could be used for diagnosis. The study concludes that the RAPD-PCR technique is useful for the study of molecular taxonomy in insects.

8. A highly Conservative Amino Acid Substitution Pattern in the Barcode Region of Cytochrome Oxidase 1 (*cox1*) Gene : Signifying its Efficiency in Species Identification

Fazlur Rahman, Ruhina Shirin Laskar and Sankar Kumar Ghosh

Department of Biotechnology,
Assam University Silchar, Assam-788 811

Keywords : *Species specific marker, DNA barcoding, Mitochondrial genome, COX1, Base substitution.*

The barcode region of the cytochrome c oxidase I (*cox1*) gene consists of around 650 base pairs which is widely used as species-specific identification marker in animals. In this study, nucleotide and amino acid substitution pattern in the barcode region of the *cox1* gene was compared with the other frequently used gene sequences for species identification in animals' *viz.* Cytochrome oxidase b (*cyt b*) and the exon 1 of the nuclear interstitial retinol-binding protein 3 (*rbp3*) gene. From the analysis, it can be inferred that the property of species identification of all these sequences under study, comes from the variable nature of the third codon position. Interestingly, among the three compared sequences, the barcode sequence of *cox1* gene exhibits a very low percentage of amino acid variability (15.38%) inspite of high percentage of variation in its nucleotide sequence (40.76%) as well as a significantly ($p<0.0001$) low level of amino acid sequence divergence among the species. Hence, a significantly conservative amino acid substitution pattern seems to be a unique feature of barcode region of the *cox1* gene facilitating its better efficiency in species identification.

9. Cytogenetic Analysis of *Eudrilus Euginea* in Western Ghats, Tamilnadu, India

K. Poornima[1], A. Divya[2] and C. Gunasekaran[2]

[1]Department of Zoology, Government Arts College, Coimbatore-641 018
[2]Department of Zoology, Conseravation Biology Lab, Bharathiar University, Coimbatore-641 046

Keywords **: *Eudrilus euginea*, Vermicompost, Cytogenetic analysis.**

Currently agriculture is facing severe alterations of soil qualities such as erosion, depletion of organic components and persistence of residues of heavy metals

and pesticides. To restore soil health the practice of organic farming and encouraging the activities of soil invertebrates in agriculture has been an essential step. Among various components of organic farming vermicomposting has been a key component for making compost through earthworms. Chromosome analysis is an essential part of systematic studies of a group of organisms and it plays a vital role in taxonomically complicated genera. Description of the relative size of chromosomes and position of centromeres provide an information suitable to compare species involved in such processes. In the present study, the cytogenetical analysis of **Eudrilus euginea** (Kinberg 1867), the most commonly exploited species for vermicomposting in the Western Ghats region of Tamil Nadu is being investigated and analysed.

10. Association Between *XRCC1* Gene Polymorphisms and DNA Damage in Peripheral Blood Lymphocytes of Asbestos Industry Workers Occupationally Exposed to Asbestos Fibres

K. Kalaivani and Sheela Priyadarshinee

Post-Graduate and Research Department of Zoology,
Government Arts College (Autonomus), Coimbatore-641 018, Tamilnadu

Keywords *: Chromosomal aberrations, Micronuclei, Arginine, Phenylalanine.*

The asbestos industry workers are occupationally exposed to asbestos dust. Asbestos dust is associated with systemic disorders including mesothelioma, lung cancer, and asbestosis. The present study identified the XRCC1 genotype of the subjects and further attempted to associate the polymorphisms with the DNA damage (chromosomal aberrations and micronuclei frequency) observed. The study population consisted of 35 experimental subjects exposed to asbestos and 37 control subjects unexposed to asbestos. The blood samples from subjects were used for karyotype analysis, micronucleus study and genotype analysis. The present investigation has revealed that the DNA damage as measured by chromosomal aberrations and micronuclei were more pronounced in the Group II asbestos exposed subjects (> than 35 years of age) as compared to the Group I asbestos exposed subjects (<35 years of age). DNA repair pathways play a vital role in maintaining genetic integrity and it is becoming clear that defects in repair pathways are connected to many different types of diseases. DNA repair system maintains genomic integrity, in the face of environmental insults, cumulative effects of age and general DNA replication errors. The present study examined the association between Arg 399 Gln XRCC1 polymorphism and risk of DNA damage. The results suggest a high risk for DNA

damage in asbestos exposed individuals with the XRCC1 Arg 399 Gln aminoacid change. These findings support the hypothesis that XRCC1 Arg 399 Gln aminoacid change may alter the phenotype of the XRCC1 protein, resulting in deficient DNA repair.

11. *XRCC1* Gene Polymorphisms and DNA Damage in Peripheral Blood Lymphocytes of PVC Industry Workers Occupationally Exposed to Vinyl Chloride Monomer

K. Shakila, Sheela Priyadarshinee, V. Thambidurai

Post Graduate and Research Department of Zoology,
Government Arts College (Autonomous) Coimbatore-641 018, Tamil Nadu.

Keywords *: Chromosomal aberration, Micronuclei, Karyotype.*

Poly vinyl chloride (PVC) is the third most widely used thermoplastic polymer. The present study identified the XRCC1 genotype of the subjects and further attempted to associate the polymorphisms with the DNA damage (chromosomal aberrations and micronuclei frequency) observed. The study population consisted of 15 experimental subjects exposed to VCM and 15 control subjects unexposed to VCM .The blood samples from subjects were used for karyotype analysis, micronuclei study and genotype analysis. The present investigations has revealed that the DNA damage as measured by chromosomal aberrations and micronuclei were more pronounced in the Group II VCM exposed subjects (>than 30 years of age) as compared to the Group I VCM exposed subjects (< then 30 years of age). The investigation has revealed that the total structural chromosomal aberrations are higher in Group II as compared to Group I. The study also reveals that the frequency of micronuclei is higher in the exposed subjects as compared to non-exposed subjects of both Groups (I and II). The study showed that the three polymorphisms of the XRCC1 gene exon 10 codon 10 the wild type genotype Arg was the most frequent in both Groups (I and II). The present study has revealed that the frequency of chromosomal aberrations and micronuclei is highest in subjects with the Gln allele in both the Groups. The XRCC1 399 Gln allele is associated with reduced DNA repair function.

12. Cloning, Sequencing and Characterization of Putative Exonuclease Gene of Mycobacteriophage L1

Amit Kumar Singh, Soumyadeep Sahu, Bipasa Das, Chayan Banerjee, Nandan Kumar Jana*

Department of Biotechnology, Heritage Institute of Technology,
Anandpur, Chowbaga Road, Kolkata-700 107
***Email** : nandanjana@gmail.com

Keywords *: L1 phage, L5 phage, Mycobacteriophage, Putative, Nuclease, pNA1.*

L1 is a temperate mycobacteriophage which bears close homology with L5 phage, *In-silico* analysis of gp4 of L5, suggests its nuclease activity. Using this analysis PCR primers were designed and the putative nuclease gene of L1 phage was amplified by PCR. The amplified product was cloned into pET28a (+) vector resulting plasmid pNA1 294 bp EcoR1/Hind III PCR product of L1 phage in pET28a (+) and clone was confirmed by colony PCR. The significance of this plasmid pNA1 is that it can be used to study *in vivo* and *in vitro* expression of L1 nuclease protein. This property of L1 phage can be used for developing gene transfer system for detailed genetic study of *M. tuberculosis*.

13. Study on Polymorphism of RANTES in Systemic Lupus Erythematosus Patients of North Indian Population

Leishangthem Bidyalaxmi Devi[1], Ajay Wanchu[2], Aman Sharma[2], Archana Bhatnagar[1]

[1]Department of Biochemistry, Basic Medical Sciences Block,
Panjab University, Chandigarh-160 014.
[2]Department of Internal Medicine,
Postgraduate Institute of Medical Education and Research,
Chandigarh-160 014.

Keywords *: RANTES, Polymorphism, Systemic lupus erythematosus.*

Systemic lupus erythematosus (SLE), a chronic and complex autoimmune disease is influenced by genetic factors. RANTES (regulated on activation, normal T cell expressed and secreted) a proinflammatory chemokine plays important role

in attraction and recruitment of various cells to the site of inflammation. The polymorphism of RANTES-403 and RANTES-28A was studied in 30 patients and 30 healthy controls. PCR-RFLP performed showed 28C/C and the 403G/G genotypes in both patients and controls. With the constraint of sample size, RANTES polymorphism does not appear to be associated with SLE in North Indian population. This needs to be confirmed with larger samples.

14. Genetic Diversity Analysis of Black Gram (*Vigna mungo*) Accessions Using Molecular Approaches

Priya Srivastava* and Anjana Pandey

Nanotechnology and Molecular Biology Lab, Centre For Biotechnology,
University of Allahabad, Allahabad-211 002, U.P.
***Email** : priya.srivastava7@rediffmail.com

Keywords : *Molecular marker, Genetic diversity, Genotypes, Random Amplified Polymorphic DNA (RAPD), Polymorphisms.*

The molecular marker is a useful tool for assessing genetic variations and resolving cultivar identities. Information on genetic diversity and relationships among urd bean (*Vigna mungo*) genotypes is currently very limited. The objective of this study was to evaluate the genetic polymorphisms and identities of 8 traditional and Indian cultivars of black gram using the random amplified polymorphic DNA technique. Forty-five decamer-primers could generate a total of 140 RAPD fragments, of which 72 or 51.4 % were polymorphic. The number of amplification products produced by each primer varied from 3 to 14 with an average of 8.3 bands per primer. The size of amplified fragments was ranged from 50 to 3000 bp. The cluster analysis had placed most of the cultivars into a close relation showing a high level of genetic relatedness. The information generated from this study can be used to maximize selection of diverse parents and broaden the germplasm base in the future of black gram breeding programs. This study identified diverse genotypes like LBG-623 and UH-28 for use in hybridization program for black gram. RAPD markers are useful in the assessment of black gram diversity, the detection of duplicate sample in germplasm collection, and the selection of a core collection to enhance the efficiency of germplasm management for use in black gram breeding and conservation programs. The genetic diversity obtained in this study might be useful in future strategies for evolution of desired genotypes.

ii. HEALTH AND DISEASES

15. Alterations of Glycan Branching and Differential Expression of Sialic Acid on Alpha Fetoprotein Among Hepatitis Patients

Goutam Mandal[1], Urmimala Chatterjee[2*], Yogesh K Chawla[3], Bishnu Pada Chatterjee[1#]

[1]School of Management and Sciences, West Bengal University of Technology, Salt Lake, Kolkata-700 064,
[2]Department of Anatomy, Physiology and Biochemistry, Swedish University of Agriculture Science, Uppsala
[3]Department of Hepatology, Post Graduate Institute of Medical Education and Research Chandigarh-160 012

Keywords : *Alpha fetoprotein, Aleuria aurantia lectin, Fucosylation, Liver cirrhosis, Liver biopsy.*

The level of serum glycoproteins and their glycosylation pattern alter in liver diseases. Some of them especially alpha fetoprotein (AFP) serve as useful biomarkers for HCC. The present investigation showed high level of AFP in hepatitis B cirrhosis (HBV-LC) and hepatitis C cirrhosis (HCV-LC) patients as compared to controls. The differential expression of sialic acid linkage was observed in chronic hepatitis B (HBV-CH) and HCV-LC by ELISA; the former bound strongly with *Sambucus nigra* agglutinin (SNA, sp. NeuAcá 2,6-), whereas HCV-LC reacted preferably with *Maackia amurensis* agglutinin (MAA, sp. NeuAcα2,3-). There was significantly high glycan branching in HBV-LC an HCV-LC as illustrated by Concanavalin A. Enhanced fucosylation was observed in HBV-LC and HCV-LC patients by using fucose binding *Aleuria aurantia* lectin. It is concluded that the changes in concentration of AFP and change in glycosylation pattern have a prognostic value and it could be possible that AFP can aid in diagnosis of hepatitic diseases besides clinical examination and routine laboratory investigation.

16. Diosgenin : a Plant Steroid Induced Apoptosis in hep2 Human Laryngeal Carcinoma Cells

Shahira Rahamath and Mirunalini Sankaran*
Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu
***Email** : mirunasankar@gmail.com

Keywords : *Diosgenin, Apoptosis, Hep2 cells, MTT, ROS.*

Carcinoma is a major public health burden in all countries. Out of all carcinomas laryngeal carcinoma accounts for 25% of head and neck carcinoma and 2% of all human malignancies. In this study, we made an attempt to investigate the anticancer effect of diosgenin on Hep2 cell line. Diosgenin is a steroidal sapogenin with estrogenic and anti tumor properties. Cell viability was assessed via an MTT assay. Apoptosis was investigated in terms of acridine orange/ethidium bromide dual staining method and DNA fragmentation. Intracellular ROS generation was estimated spectrophotometrically. Diosgenin inhibited Hep2 cell growth and the IC_{50} cytotoxic dose of diosgenin showed, typical characteristics of apoptosis including the morphological changes and DNA fragmentation. Moreover, increased ROS generation was also noted in the diosgenin treated Hep2 cells. Our data demonstrates that diosgenin is a potent inhibitor of Hep2 carcinoma cells, by growth inhibition, induction of apoptosis and enhanced ROS generation.

17. Presence of Bacterial and Viral Association is Critical in Gastric Lesions and Atrophy

Babul Banerjee[1*], Prabir Banerjee[2], Sandip Banerjee[3] and Subrata Banerjee[4]

[1]Forensic Toxicology Department,
Forensic Science Laboratory, Kolkata-700 037
[2] Gastroenterology Department Calcutta Medical College and Hospital,
Kolkata-700 073
[3] Biochemistry Wing, Ucm Sskm Hospital Ipgme and R Kolkata-700 020
[4] Biophysics Department, Saha Institute of Nuclear Physics,
Salt Lake, Kolkata-700 091
***Email** : Bblb88@hotmail.com

Keywords *: Gastric carcinoma, EBV, H. pylori, GI bleeding, Atrophy, Gastropathy.*

Gastric mucosal toxicity is one of the most common problems worldwide. Although different studies have found an association between *Helicobacter pylori* infection and the development of gastric cancer; many aspects of this relation remain uncertain. The Epstein-Barr Virus is detected in a substantial subgroup of gastric adenocarcinomas worldwide. Review of literature reported that the EBV-positive gastric carcinomas carry distinct genomic aberrations. In the present study, a large cohort of EBV-positive and EBV-negative gastric adenocarcinomas has been

analyzed for their clinicopathologic features to determine whether they constitute a different clinical entity. The gastric mucosal tissue and blood samples were collected after routine tests and Panendoscopy. The gastric mucosa in patients who experience a reactive gastropathy demonstrates a broad spectrum of intense hemorrhages, erosions, ulcers. Erosions and ulcers are frequently multiple, and the base of these lesions often stains dark brown possibly owing to exposure to acid. The detection of *Helicobacter pylori* in Gastric atrophy is very critical. Most gastric cancer arises on ground of gastric atrophy and extent of atrophy increases the risk. The *H. pylori* infection is crucial and early detection-eradication are extremely essential for safety. Thus the gastric EBV acts as an alarming cofactor in GI lesions. It needs special attention for diagnosis. Presence of both HBP and EBV increase the risk of worsening the GI damaging events. Gastric toxicity matrices and atrophy in acute GI bleeding case appears very critical and risky. This pilot study builds a cornerstone for the future medical research advancement.

18. Berberine Prevents Formation of Azoxymethane Induced Colonic Preneoplastic Lesions in Rats

C. J. Thirupurasundari[1*], R. Padmini[2] and S. Niranjali Devaraj[3]

[1]Department. of Bioinformatics, SRM University, Chennai
[2]Department. of Biochemistry, Vel's Univerisity, Chennai
[3]Department. of Biochemistry, University of Madras, Chennai
***Email** : nirasundari@gmail.com

Keywords *: Aberrant crypts, Azoxymethane, Berberine.*

We investigated the antineoplastic effects of berberine, an isoquinoline alkaloid, on azoxymethane induced colonic preneoplastic lesions in rats. Berberine retards the growth of aberrant crypts, modulates the levels of tumor markers, decreased the expression of tumor progression locus-2 and restored the gap junction communication protein – connexin 43, that are critical determinants of invasive neoplasm. Berberine abated the formation of mucin-depleted foci as observed in AOM induced colon carcinogenesis. The AOM induced expression of ERK proteins were reverting back to normal levels with berberine treatment. Our results suggest that berberine is a potent antineoplastic agent in colon carcinogenesis.

19. Reversible Effects of Longterm Exercise Training on Lipid Metabolic Profiles in Aged Rats

P. Sudarsanam[1], K.R. Shanmugam[1,2] and K. Sathyavelu Reddy[1*]

[1]Division of Molecular Biology and Exercise Physiology, Department of Zoology, Sri Venkateswara University, Tirupati-517 502, (AP)

[2]Department of Nutrition and Dietetics, Faculty of Allied and Health Sciences, University of Kebangsaan Malaysia, Jalan Raja Muda Aziz, 50300 Kuala Lumpur, Malaysia.

***Email** : ksreddy2008@hotmail.com

Keywords *: Exercise, Aging, Rats.*

The present study was designed to investigate the role of exercise training on lipid metabolic profiles in the kidney tissue of two different age groups of rats. The age-matched Wistar strain male albino rats were evenly divided into 4 groups, normal control young, normal control old, exercise trained young and exercise trained old rats. Exercise training was given to the rats for 2 months. After completion of 2 months exercise training, the kidney tissue was isolated and assayed for the lipid metabolic profiles like, total cholesterol (TC), triglycerides (TG), phospholipids (PL). In old rats significant elevation of TC, TG and PL levels were observed than that of control rats. In both the age groups, exercise training significantly reversed the above metabolic profiles to normal levels due to proper utilization of lipid. This study, clearly showed that exercise may alter the age related accumulated lipid metabolic profiles and their metabolic products in the body. Hence, exercise may have beneficial to the aged rats.

20. Pulmonary Function Studies in Hyperthyroid Females with Goiter-Before and After Thyroid Surgery

G. Sugapriya

Department of Physiology, SRM Medical College Hospital & Research Centre, Potheri, Kattankulathur-603 203

Email : msmanimd@yahoo.co.in

Keywords *: Pulmonary function tests, Upper airway obstruction, Hyperthyroid.*

Fifty hyperthyroid female age group between 25 - 40 years with goiter posted for thyroid surgery were selected from department of surgery. Graphic recording of

airflow during maximal inspiration and expiration at different lung volumes were carried out in patients undergoing surgery for goiter. The same was documented one month later to find whether there is any improvement in flow rates after surgery. On analysis of the data the subjects were categorized into groups. The mean and standard deviation were calculated for all measured parameters. The significance of difference in the values were analysed using students paired 't' test before and after one month of surgery and their probability was estimated. Following thyroid surgery the changes in the early and mid airway bronchus and bronchioles were noted and the results indicated that the mechanical compression was due to enlarged thyroid.

21. Effect of *Cynometra Travancorica* on Estradiol Induced Keratinizing Metaplasia on Rat Uterus

N. Sasidharan[1], A. P. Shahid[1], Jose Padikkala[2], T. D. Babu[2], Salini[2] and Austin[2]

[1]NTFP Department Kerala Forest Research Institute,
Peechi 680 653, Kerala
[2]Amala Cancer Research Centre, Amala Nagar,
Thrissur 680 555, Kerala

Keywords : *Cynometra travancorica, Keratinization.*

Cynometra travancorica belongs to the family *Caesalpiniaceae* and used as substitute of *Saraca asoca* for treating menorrhagia. The medicinal properties of this substitute are not established well. Antiestrogenic activity of *C. travancorica* methanolic extract treated rat was analysed by radioimmunoassay. Results showed that *C. travancorica* possesses significant antiestrogenic activity. The estrogen level in the control group was 112 U/L while the level reduced to 56 U/L in *C. travancorica* treated group. The effect of extract on estradiol induced keratinization on rat uterus endometrum epithelium was also evaluated histopathologically. Oral administration of the extract was found to be effective in preventing keratinisation. On the days 16, 24 and 30 of treatment, the uterus of estradiol treated group developed a prominent layer of keratinised epithelial cells at the endometrium while in extract treated group it remained normal as same to that of control.

22. Role of Free Radicals and Reactive Oxygen Species in Oral Cancer Patients and their Suppression by Antioxidants

R. S. Singh[1], Anupam Porwal, [1] and S. K. Awasthi[2]

[1]Department of Biotechnology, Brahmanand PG College, Kanpur

[2]Institute of Life Sciences, CSJM University, Kanpur

Keywords : *Free radicals, Antioxidants, Oral cancer, MDA, Reactive oxygen species.*

In the last decade there has been a rapid progress in the understanding of the actual nature and chemistry of reactive oxygen species (ROS) at the cellular and molecular level. Free radicals have been implicated to play a pivotal role in the genesis of various oral cancers. All the major classes of biomolecules attacked by free radicals but lipids are most susceptible. Cell membranes are rich source of polyunsaturated fatty acids (PUFAs) which are readily attacked by oxidized radicals. The free radicals cause oxidative destruction of polyunsaturated fatty acid known as lipid peroxidation. It is particularly damaging because it proceeds as self perpeuting chain reaction, which may result in more free radical generation. Free radicals are indirectly measured by the level of MDA (malonaldehyde). Keeping in view such consideration, this study has been designed to monitor the role and activities of ROS and some significant stress markers such as MDA and SOD/catalase as enzymatic antioxidants in oral cancer patients. In this study we took 20 patients of oral cancer and 10 healthy controls. It was found that MDA level in healthy persons (1.426 nmoles/ml blood) was less than that in malignant persons (2.571 nmoles/ml blood). This indicated that the MDA level in malignant persons was in general significantly higher than that in healthy persons. In this study we observed that SOD level in healthy persons was 488.7504 U/g Hb as compared to that in malignant persons (149.0533 U/g Hb). It was found that catalase level in healthy persons (769.3072 µmole H_2O_2 reduced/mg protein) was higher than that in malignant person (277.2894 µmole H_2O_2 reduced/mg protein). This indicated that SOD and catalase levels in malignant persons were significantly less than that in healthy persons. The present study revealed certain aspect of free radical and antioxidants in the oral cancer patients.

23. Identification of Low Abundant Glycoproteins by 2D Gel Electrophoresis Using Albumin Depletion Kits

Sagarika Biswas, Saugata Roy, Shabir Hussain, Hasi Das*
Institute of Genomics and Integrative Biology, Delhi
***Email** : hdas@igib.res.in

Keywords : *MALDI-TOF MS, 2DE, Albumin depletion, Rheumatoid arthritis.*

Depletion of albumin and IgG in serum followed by protein separation by two-dimensional gel electrophoresis increases the probability of detection of the lower abundant proteins. In the present study low abundant proteins present in plasma and synovial fluid of rheumatoid arthritis (RA) was identified by 2D gel elctrophoresis. Various commercially available kits were used to deplete albumin and IgG from plasma and synovial fluid of RA patients. The differential proteome analysis of these samples were carried out by 2D gel electrophoresis followed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF MS). The gel profiles showed significant removal of albumin and IgG from plasma samples when viewed against gels of non-albumin depleted samples. Number of distinct protein spots were visualized and identified by MALDI-TOF MS. Removal of IgG/albumin was more efficient using Merck's kit followed by Pierce, Sigma and Biorad kits.

24. Initial Microbial Colonization in the Alimentary Tract of a New-born Baby in Different Modes of Parturition

I. Joseph and A. J. A. Ranjit Singh
Department of Advanced Zoology and Biotechnology,
Sri Paramakalyani College, Alwarkurichi-627 412, (TN).

Keywords : *Lactobacillus, New-born, Probiotic, Immune system, Nosocomial infection, Caesarian, Normal child birth.*

The intestinal microflora is a positive health asset that crucially influences the normal structural and functional development of the mucosal immune system. In the new born, the colonization of microbes in the alimentary system starts as soon as the womb comes out. A study on the early colonized bacterial species in a new born

will help to understand the immuno protective functions in the new born. The initial colonization of bacterial population in the alimentary tract of a new-born baby varied according to the mode of child birth. In the alimentary tract of the normally delivered baby, beneficial probiotic microbial invasion in the intestinal region was high and such microbes were mostly from the mother. In a surgically (Caesarian) delivered baby, the alimentary tract received mostly non beneficial microbes from the environment and hence such babies were susceptible to infection.

25. *In Vitro* Effect of Different Serum Concentration on Amniotic Fluid Derived Stem Cells in Second Gestational Age of Buffalo (*Bubalus Bubalis*)

Kapil Dev, Sanjeev Kumar Gautam*, Shiv Kumar Giri, Anil Kumar and Anita Yadav

Department of Biotechnology, Kurukshetra University,
Kurukshetra-136 119 (HR)
***Email** : sgautam@kuk.ac.in

Keywords : *Amniotic fluid stem cells, Serum, Proliferation rate.*

There are number of factors such as temperature, pH of the medium and various medium constituents that influence the proliferation rate of amniotic fluid stem (AFS) cells. But serum concentration plays an important role as attachment factors on proliferation of AFS. Our results showed that the rate of attachment, cell proliferation and cells numbers changed with the change in serum concentration. The serum concentration between 1-7% lead to low attachment rate with slow increase in cell numbers but as serum concentration increased (8-16%), the rate of attachment increased with increase in cells numbers too. In the present study, we also observed that the proliferation rate increased on increasing serum concentration upto 16%, there was maximum number of attached cells with higher proliferation rate and maximum viability. These results indicated that serum concentration in the culture medium has a significant role on attachment as well as on growth kinetics of AFS cells.

26. Comparative Effects of Fried Oils and Extracted Oils from Fried Products on Alterations in Brain Function in Experimental Animals

Chinu Chacko and T. Rajamohan

Department of Biochemistry, University of Kerala,
Thiruvananthapuram, Kerala-695 581

Keywords : *Fried products, Oils, Brain, Antioxidant status, Membrane integrity, Neurotransmitters.*

Oils extracted from banana chips fried in coconut oil and sunflower oil and fried oils left after frying were used in the study. Chemical analysis of oil samples showed significant levels of degradation products in fried oil groups compared to extracted oil groups while coconut oil significantly resisted the alterations. Male Sprague Dawley rats were fed with oils at 10% along with synthetic diet for 60 days. Administration of fried oils resulted in significant depletion of antioxidant status in fried oils fed groups and coconut oil fed group showed enhanced levels. Significant increase in the levels of peroxidation products were noted in fried oil groups but least in coconut oil fed group. Membrane bound enzymes and mono amine oxidase activities were also depressed in fried oil fed groups but least in coconut oil fed group.

27. Suppression of Pro-inflammatory Cytokines, Reactive Oxygen and Nitrogen Species by A*rnica Montana* Extract in Collagen-Induced Arthritis Rat Model

Shikha Sharma,[1] Debasis Sahu,[2] H. R. Das [2] and Deepak Sharma [1*]

[1]School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067
[2] Proteomics and Structural Biology Division, Institute of Genomics and Integrative Biology, Mall Road, Delhi-110 007
***Email**: dpak57@hotmail.com

Keywords : *Arnica montana, Collagen-induced arthritis, Cytokines, Oxidative stress.*

The present study aimed to investigate the anti-rheumatic potential of *Arnica montana* methanolic extract (AMME) against both inflammation and oxidative stress in collagen-induced arthritis rat model. Rats were immunized with porcine type II collagen and treated orally with AMME (150 mg/kg body weight/day) for 15 days. Treatment with AMME and indomethacin significantly suppressed the arthritic

symptoms as evidenced by histology and radiography. Moreover, levels of inflammatory mediators (nitric oxide, TNF-á, IL-1â, IL-6) and antioxidant enzymes were also reduced to basal limits in AMME-treated rats. In conclusion, it could be suggested that AMME might be effective for the treatment of inflammatory arthritis.

28. Oxdative Stress Mediates Caspase Dependent Cell Death in Tributyltin Treated Rat Thymocytes

Monika Gupta and Shashi Khandelwal

Immunotoxicology Lab, Indian Institute of Toxicology Research, Lucknow

Keywords : *Tributyltin, Capase, Thymocytes.*

Tributylin (TBT) is a classical immunotoxic agent and it causes immunosuppression and thymic atrophy. We report oxidative stress and apoptotic markers in TBTC induced toxicity in rat thymocytes. Addition of N-acetylcysteine attenuated apoptosis inhibited ROS generation while, buthionine sulfoximine enhanced both ROS production and the degree of apoptosis. Involvement of caspases is also evident by the use of capase inhibitors. Our study demonstrates that ROS critically modulates the downstream events, including mitochondrial dysfunction and nuclear condensaton in TBT induced immunotoxicity, TBTC at 0.3 M concentration, TBT failed to alter the proliferative activity of T cells. Detailed study of apoptotic molecular events pertaining to immune cells highlights the risks involved in the usage of this global toxicant.

29. Status of Cell Cycle Regulatory Proteins in Cultured Mammalian Liver Epithelial Cells after Exposure to Isocyanates

Hariom Panwar[1], Alok Kumar Ahirwar[2], Pradyumaa Kumar Mishra[3] and Sunita Banerjee[1]

[1]Department of Zoology, Dr. HS Gour University, Sagar-470 003
[2]Department of Biotechnology, Dr. HS Gour University, Sagar-470 003
[3]Division of Translational Research, Tata Memorial Centre, Navi Mumbai

Keywords : *Liver, Carcinogenesis, Cell cycle, Methyl isocyanate.*

Liver is often exposed to plethora of chemical toxins. Owing to its profound physiological role and central function in metabolism and homeostasis, pertinent

succession of cell cycle in liver epithelial cells is of prime importance to maintain cellular proliferation. There has been an extensive research focus in the vicinity of cell cycle regulation and relation that exists between cellular proliferation and liver diseases, Isocyanates, a group of highly reactive important low molecular weight chemical entity (-N-C-O) with diverse industrial usage have drawn significant attention in the recent past as they bind with DNA to produce toxicogenomic effects. Moreover, health complications resulting from occupational and accidental exposures of these compounds are yet elusive. Study was carried out to assess the cell cycle response of methyl isocyanate on cultured liver cells. Study was conducted at different time intervals using N-succinimidyl N-methylcarbonate, a surrogate chemical to methyl isocyanate. The role of different cell cycle modulatory proteins cyclin A, E and Cdk2; tumor suppressor protiens p53, p21 and GADD-45 were evaluated through immuno-blotting. We observed reduction in expression pattern of tumor suppressor proteins by nearly 15% and nearly 90% over-expression of cyclins and Cdk2 providing evidence for cell cycle disregulation in the treated cells and appraising oncogenic potential of methyl isocyanate.

30. Cytotoxic Effects of *Andrographis Paniculata* Extract on Ehrlich Ascites and THP-1 Cell Lines

Rahul Raghavan TV* and Joseph Madassery
Department of Biotechnology, University of Calicut,
Malappuram (Dist), Kerala-673 635
***Email**: rahulraghavantv@gmail.com

Keywords *: EC-effective concentration, MTT assay.*

Andrographis paniculata is a medicinal plant that is used in Ayurvedic system of medicine in India and in some other countries for various medicinal applications. The plant extract was reported to have anticancer properties and one of its main consituents found to be andrographolide, a diterpene lactone. The cytotoxic effect of this plant extract in various solvent systems was examined using Ehrlich Ascites and THP-1 cell lines by MTT assay. The cytotoxic activity of ethylacetate extract of this plant obtained at 37° C exhibited EC value 10 µg/ml and 50 µg/ml on Ehrlich and THP-1 cell lines respectively. The cytotoxicity could be detected only at high concentration (EC 200 µg/ml) of the plant extract prepared in chloroform, petroleum either and methanol. However, the plant extract obtained in various solvent systems by Soxhlation showed the activity only at higher concentration (EC 180 ug/ml). The above results indicate that many of the cytotoxic component present in *A.paniculate* may be heat labile.

31. Lipid Analysis in the Serum and Muscle Tissue of ***Duchenne*** and ***Becker*** Muscular Dystrophies (DMD and BMD) Patients

Niraj Kumar Srivastava [1, 2*] and Deepak Sharma [2]

[1]Department of Neurology, Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow-226 014

[2]School of Life Sciences, Jawaharlal Nehru University, New Delhi-110 067.

***Email** : nirajsuprabhat@gmail.com

Keywords *: DMD, BMD, Lipids and NMR spectroscopy.*

Mutation in dystrophin gene produces two phenotypes such as in Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD). Present study investigates proton nuclear magnetic resonance spectroscopy based analysis of lipids in the serum and muscle tissue extract of patients with DMD and BMD. Results showed that in serum of both DMD and BMD patients levels of triglycerides, phospholipids and free cholesterol were found to be significantly increased. Unlike, in muscle tissue extract of both patients only triglycerides were found significantly increased. Unlike, in muscle tissue extract of both patients only triglycerides were found significantly increased. Otherwise cholesterol level was found significantly elevated in DMD and phospholipids was significantly declined in BMD. These observations suggest the perturbation of lipid metabolism in these patients.

32. Metabolomic Approach for the Patients with ***Duchenne*** and ***Becker*** Muscular Dystrophies (DMD and BMD)

Niraj Kumar Srivastava [1, 2*] and Deepak Sharma [2]

[1]Department of Neurology, Sanjay Gandhi Post-Graduate Institute of Medical Sciences, Lucknow-226 014

[2]School of Life Sciences, Jawaharlal Nehru University, New Delhi-110 067

***Email** : nirajsuprabhat@gmail.com

Keywords *: DMD, BMD, Metabonomics, NMR spectroscopy and Metabolites.*

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are believed to be due to defect in the dystrophin gene. In the present study

proton nuclear magnetic resonance spectroscopy based analysis of metabolites in the serum and muscle tissue extract of patients with DMD and BMD were performed. Serum showed the significant elevation of branched chain amino acids, acetate and glutamine/glutamate in DMD/BMD, while tyrosine was found to be elevated in BMD patients. In muscle tissue extract the significant decline in branched chain amino acids, glutamine/glutamate, alanine, acetate, glucose and fumarate in DMD/BMD except elevation of histidine was observed, such analysis showed the metabolic perturbation in these patients.

33. Anti-inflammatory Effect of Wuramin on Collagen Induced Arthritis in Rats

Debasis Sahu, Ashish Saroha, Hemlata Gautam, Nandini Verma, Saugata Roy and Hasi R. Das*

Division of Proteomis and Structural Biology, Institute of Genomics and Integrative Biology, Mall Road, Delhi-110 007.

***Email** : hdas@igib.res.in

Keywords : *Suramin; Antioxidant, Anti-inflammatory, Collagen-induced arthritis, 2DE, MALDI-TOF MS.*

The present study was aimed to determine the efficacy of suramin against collagen induced arthritis (CIA) in female Wistar rats. *In-vitro* assays in cell free system showed significant (p=0.05) free radical scavenging activities of suramin. The suppression of inflammation in CIA was observed on suramin treatment as evidenced by decreased arthritic score, joint damage and reduced levels of proinflammatory cytokines in comparison to CIA rats without treatment. Similarly, *in-vivo* anti-oxidative effects of suramin were found to be significant (p=0.05) in tissues like liver, spleen, kidneys, joints and plasma. Comparative proteomic analysis of the total joint tissue proteins and plasma glycoproteins using 2-dimensional gel electrophoresis (2DE) showed many differentially expressed proteins in CIA rats which were recovered to normalcy after suramin treatment. These spots were identified by matrix assisted laser desorption and ionization – time of flight mass spectrometry (MALDI-TOF MS). All these observations suggest that besides anti-inflammatory activity suramin acts as antioxidant as well in CIA rats.

34. Evidence of *Wolbachia* Symbiosis in *Aedes* Mosquitoes from Karnataka, India

H. Ravikumar, N. Ramachandraswamy and H. P. Puttaraju

Department of Sericulture and Biological Sciences, School of Natural Science, Jnanabharathi Campus, Bangalore University, Bangalore-560 056
Department of Biochemistry, Central College Campus, Bangalore University, Bangalore-560 001

Keywords *: Wolbachia, wsp, Mosquitoes, PCR.*

Wolbachia are a group of obligate intracellular maternally inherited bacteria that have been found in several arthropod groups. These endosymbionts behave as a reproductive parasite by manipulating host reproductive machinery to enhance their vertical transmission. The reproductive system modification and cytoplasmic incompatibility, have received much attention for their role in applied strategies targeting economically important insect pests and disease vectors. We present here data from molecular studies to provide evidence for *Wolbachia* symbiosis in *Aedes* mosquito species such as *Ae. aegypti, Ae. albopictus* and *Ae. vitatus* collected from different locations of Karnataka, India which involve in the transmission of pathogens. The results revealed that *Wolbachia* AB super group infections were found only in *Ae. albopictus* mosquitoes. The prevalence of these endosymbionts in *Aedes albopictus* will provide the fundamental implication for devising control strategies. Consequently, it is necessary to assess the prevalence of the endosymbionts in important mosquito vectors from different geographical locations of India.

35. A laboratory *in-Vitro* Assessment of Present Status on Effectiveness of Fluoroquinolones Therapy in Urinary Tract Infection

Diganta Dey, Sudarshan Ray, Abhijit Banerjee, Parbati Banerjee and Ratnamala Ray

Department of Microbiology,
Ashok Laboratory Clinical Testing Centre Pvt. Ltd., Kolkata, West Bengal

Keywords : *Fluoroquinolones, Clinical isolates, Urinary tract infection, Antibiotic susceptibility testing, ESBL producers.*

A total of 98 Gram negative clinical isolates were analyzed for their resistance pattern against fluoroquinolones and screened for Extended Spectrum â- Lactamase

(ESBL), using disc diffusion method and double disc synergy test respectively as per CLSI recommendation. MIC was calculated using commercially available software Osiris. Among the 98 urinary isolates, 41 were *E.coli,* 29 were *Klebsiella* spp., 17 were *Pseudomonas* spp. and 11 were *Acinetobacter* spp. Of *E. coli* and *Klebsiella* spp. 61% and 52% respectively were found ESBL positive, 78% *E.coli* were resistant to Ciprofloxacin. Except *Acinetobacter* spp., all other strains were highly resistant to Ofloxacin. *Klebsiella* spp. were sensitive to all of the tested quinolones, but *E.coli* had shown greater resistance to all of the quinolones except Levofloxacin and Gatifloxacin. The study revealed the increased trend of ESBL producing organisms create infection of the urinary tract. Most of the urinary isolates became resistant to second generation quinolones may be due to their long-term use in society. Among the third generation quinolones Levofloxacin and Gatifloxacin were more potent than Sparfloxacin. There was no significant correlation between ESBL production and fluoroquinolone resistance. These resistant strains more likely to be the resultant of antibiotic pressure in our microenvironment i.e. in the body of gram population.

36. Histopathological Changes Induced in Liver by Long-term Exposure to Sublethal Concentrations of Copper Sulphate in the African Catfish, *Clarias Gariepinus* (Burchell, 1822)

Adil A. Wani, M. Sikdar-Bar*, H. A. Khan and P. A. Pervaiz

Department of Zoology, Dr. H. S. Gour University, Sagar (M.P)-470 003

***Email** : malvikabar@hotmail.com

Keywords *: Copper sulphate, Clarias gariepinus, Histopathology.*

The metals are discharged into aquatic bodies from many sources, often at sublethal concentrations. The hazardous effect of sublethal concentrations (2 and 5 mg^{-1}) of copper sulphate on liver histology of African catfish, *Clarias gariepinus* (weight and length 100-110g and 18-20 cm, respectively) was analyzed histologically after 30 and 60 days of exposure. The control group was also maintained simultaneously. The liver of control group showed normal histo-architecture, while as copper sulphate exposed fish showed cytoplasmic vacuolation, nuclear degeneration, focal areas of necrosis, hypertrophy of hepatocytes, pycnotic nuclei haemorrhage and haemolysis due to rupture of blood vessels. This research reveals that copper sulphate has a deleterious impact on liver histopathology of *Clarias gariepinus*, which could be a suitable biomarker for environmental contaminations.

37. Evaluation of Anti-Proliferative Activity of Pavonia Zeylanica, Cav. on Mammalian Cancer Cells

Shwetha Karanth, Ravishankar, H. G. Surabhi Shrivastava, Naidu R. and G, Panduranga Murthy

Centre for Shridevi Research Foundation - CSRF & Dept. of Biotechnology,
Shridevi Institute of Engineering & Technology,
Sira Road, Tamkur-572 106

Keywords *: Paronia zeylanica Cav. Phytochemical analysis, Antimicrobial activity, Antioxidant activity, Anti proliferative activity, MCF-7 cell lines.*

Many traditional healing plants successfully passed several hundred years of empirical testing against specific diseases and thereby demonstrating that they are well tolerated in humans. Although quite a few ethno-pharmacological plants are applied against a variety of conditions there are still numerous plants that have not been cross-tested in diseases apart from the tradtional applications. Herein we demonstrate the anti-proliferative potential of *Paronia zeylanica* used by the Seliga tribes of BR hills Karnataka, India against tumors, used as purgative and with vermifungal effects. Different parts of the plants were collected, shade dried and extracted with six solvents of increasing polarity. Preliminary qualitative phytochemical analysis showed presence of proteins in ethanolic extract alone and carbohydrates in all the extract. Ethanolic extracts showed positive tests for phenols, flavonoids, terpenoids, tannis and alkaloids. Aqueous extracts was positive for Alkaloids and terpenoids. The antimicrobial activity tested for the different solvent leaf extracts against *Shigella*, *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aueroginosa* and *Escherichia coli*. The ethanolic leaf extract showed good results against *Shigelia* sps. and *S. typhi* in ethanolic and aqueous extracts and the zone of inhibition was found to be 1.0 cm. The total antioxidant activity of ethanolic leaf extracts was evaluated by ABTS method and IC50 value was found to be 7±0.74 µg/ml. Anti proliferative activity was evaluated against MCF-7 cell lines (Mammarian cancer cells) for the inhibition of proliferation and the induction of cell death as hallmark endpoints to measure the efficiency of anti-cancer drugs. Here, it was found that there is a presence of bio-active compound that is arresting the proliferation and growth of these cancerous cells.

38. Diagnosis of Different Hepatitis by Alteration of Glycosylation Exemplified by Concanavalin A Lectin and High Performance Anion Exchange Chromatography

Shuvendu Singha[1], Goutam Mandal[1], Rina Ghosh[2] and Bishnu Pada Chatterje[1]

[1]Department of Natural Sciences, School of Management and Science, West Bengal University of Technology, Salt lake, Kolkata-700 064
[2]Department of Chemistry, Jadavpur University, Kolkata-700 032

Keywords : *Glycosylation, Liver cirrhosis, Hepatitis, HPAEC.*

Changes in N-linked glycosylation of different serum glycoproteins are known to occur during infection and progression of various diseases including different kinds of hepatitis namely chronic hepatitis B, hepatitis B induced liver cirrhosis, hepatitis C induced liver cirrhosis and alcoholic liver cirrhosis. Sera from ten each hepatitis patients were allowed to pass through ConA–Sepharose column. The column bound fractions were subjected to 12% SDS-PAGE when some common and uncommon bands in different hepatitis patients' sera appeared. The bands of molecular weight 25 and 75 kDa appeared in HBV-CH, HBV-LC and ALC respectively were cut, destained, dried and subjected to deglycosylation by PNGaseF. The individual monosaccharides constituting the oligosaccharide chain was analyzed by HPAEC. The variation in the quantity of monosaccharides may give an insight in to the diagnosis of different hepatitis**.**

39. Molecular Fragment Concept Analysis (molfr.ca) for Oral Bioavailability in p38 MAP Kinase Inhibitor

Vijay Rathod, G. Rajkiran, Nitu Bansal, Abhay T. Sangamwar*

National Institute of Pharmaceutical Education and Research (NIPER),
Sector-67,
S. A. S. Nagar, Punjab-160 062
***Email**: abhays@niper.ac.in

Keywords *: Pharmacokinetics, Hologram QSAR.*

Molecular fragment concept analysis (molfr.ca) an adaptation of sub structural fragment concept analysis from structural molecular composition is introduced for

the estimation of the bioavailability of p38 map kinase inhibitors. The Molecular fragment concept analysis (molfr.ca), the propensity of compounds for bioavailability, in the context of oral bioavailability specific sub structural fragments can be defined and applied to retrieve compounds with the derived bioavailability rule "4-7"a count of sub structural fragments. This rule has been validated using in-house p38 map kinase inhibitor data and applied prospectively to uncover bioavailability of p38 map kinase inhibitor in clinical trial; in addition the role of discriminating fragments in bioavailability recognition was interrogated using available structural data, providing an insight into their effect on oral bioavailability.

40. Immunopathogenesis of Pristane Induced Lupus in Balb/c Mice

Ujla Minhas[1], Ranjana Minz[2], Radhika Srinivasan[3], Archana Bhatnagar[1]
[1]Department of Biochemistry, Panjab University, Chandigarh
[2]Department of Immunolopathology, PGIMER, Chandigarh
[3]Department of Cytology & Gynaecological Pathology, PGIMER, Chandigarh

Keywords *: Systemic lupus erythematosus (SLE), Glomerulonephritis, Autoantibodies, Peritoneal fluid.*

Systemic lupus erythematosus (SLE) is a systemic autoimmune syndrome defined by clinical and serological features, including arthritis, glomerulonephritis and autoantibodies against cytoplasmic and nuclear antigens etc. Pristane (2, 6, 10, 14-tetramethylpentadecane) induced model of SLE was explored for uncovering the pathogenesis of SLE. Cytokines, NO and ROS were investigated in serum and peritoneal fluid. Tissues like liver, lung and spleen were explored for histopathological changes. Results showed major alterations in cytokines, NO and ROS levels along with histopathological changes.

41. *Lactobacillus Casei* Reduces the Inflammatory Reaction and Bone Damage Associated with Collagen Induced Arthritis (CIA) by Suppressing the Pro-inflammatory Cytokines

Sarika Amdekar and Vinod Singh
Department of Microbiology, Barkatullah University,
Hoshangabad Road, Habibganj, Bhopal-462 026, Madhya Pradesh

Keywords : *Coxygenase-2, Proinflammatory cytokines, Lactobacillus casei.*

This study evaluated the therapeutic efficacy of *Lactobacillus casei* in treating rheumatoid arthritis using collagen induced arthritis (CIA) animal model. Joint damage in rheumatoid arthritis is mediated through immunological mechanism. Balance between pro-Inflammatory and anti-Inflammatory cytokines level is one of the factor which is responsible for the progression of arthritis. Healthy female Wistar rats (weight- 180-200 gm) were included in this study. Induction of arthritis and oral adminstartion of *L. casei* was started on same day. Indomethacin was used as standard reference drug. Serum level of IL-6, α- TNF and IL-10 and compared with normal healthy control. Four point arthritis indexes were also assessed at the end of week for 28 days. Histopathological analysis of knee joint was also done to assess the histopathological symptoms. *L. casei* treated rats showed normal histopathology without any synovial infiltration, pannus formation, cartilage and bone destruction. Arthritis score was also lower for the group orally administered with *L. casei*. *L. casei* administration significantly decreased the pro-Inflammatory cytokines (IL-6 and TNF-α) and increased the anti-inflammatory cytokines (IL-10). Present study suggests that *L. casei* has potent antiarthritic effect in CIA induced arthritis model. Inhibition of COX-2 via inhibiting the pro-inflammatory cytokines is an understanding of the complex interactions involved in these pathways. This might allow the development of novel therapeutic strategies for rheumatoid arthritis.

iii. BIOTECHNOLOGY

42. Molecular Characterization and Expression of *Mips* Gene in Developing Seeds of *Glycine Max*

Awadhesh Kumar, Sweta Kumari, Monica Jolly and Archana Sachdev
Division of Biochemistry, Indian Agricultural Research Institute, New Delhi

Keywords *: Soybean, MIPS, phytate, developing seeds, expression analysis.*

Phytic acid (*myo*-inositol 1,2,3,4,5,6 hexakisphosphate) is a phoshphorylated derivative of *myo*-inositol, which functions as a major storage form of phosphorous in plant seeds. MIPS (*myo*- inositol-1-phosphate synthase) plays a major role catalyzing the first step in phytic acid biosynthesis. In the present study, a genomic sequence of *MIPS* from soybean (*Glycine max* var. Pusa16) was amplified using long PCR. Cloning and characterization of the genomic sequence revealed a total length of 2608 bp containing 9 introns interrupting 10 exons. The transcribed sequence of the gene had an expected open reading frame of 1533 nt to encode for

510 amino acids. Strong homology with the previously reported gDNA (*MIPS*), as revealed in the BlastN search also indicated a high degree sequence similarity to *Phaseolus vulgaris, Vigna radiata, Cicer arietinum and Medicago truncatula MIPS*. The maximum level of phytate content observed in mature soybean seeds was 1.9%. MIPS expression by RT-PCR and Northern analysis revealed increased transcript levels during early stages of seed development reaching to a maximum at 6-8 mm seed size. At least 4 copies of the gene were detected during Southern analysis of the genomic DNA.

43. *In Vitro* Effect of Different Photoperiods on *Trichoderma Harzianum* for its Growth Rhythm and Commercial Applications

Manojkumar Shinde[1] and Dnyaneshwar Waghchoure[2*]

[1]New Arts, Commerce and Science College, Ahmednagar

[2]Ashwamedh Agritech and Farm Solution Systems, Tal Kopargaon, Ahmedngar.

***Email :** ashwamedhagri@yahoo.co.in .

Keywords : *Biocontrol, Growth rhythm, Photoperiod, Mycelium, Sporulation.*

Trichoderma harzianum is a known biocontrol agent for the management of root knot nematodes. It rapidly multiplies and covers the roots and helps in root formation. *In Vitro* conditions, studies were done on growth rhythm in order to understand the best growth response of *Trichoderma* against different photoperiods. The experiment was carried out at 25±2º C and relative humidity 48±3. It was observed that in artificial photoperiod sporulation was excellent. The photoperiod exposure can be given to the cultures in order to get more growth at commercial level.

44. Standardization of pH and Light Intensity for the Biomass Production of *Spirulina Maxima*

J. P. Pandey [1*], A. Tiwari [1] and P. S. Bisen[2]

[1]Department of Biotechnology, Govt. T.R.S. College, Rewa (M.P.),

[2]Bisen Biotech and Biopharma Pvt. Ltd. Gwalior (M.P.)

***Email** : japy25@gmail.com

Keywords : *Biomass, pH, Light Intensity, Chlorophyll, Protein, Spirulina maxima.*

Spirulina maxima is known to be useful to man in virtually all aspects of life including health, food and cosmetics. Through manipulating environmental condition of the algal growth, one can modify the biomass production. In the present investigation the production of *Spirulina maxima* was optimized in terms of biomass and metabolites. The dry weight of *Spirulina maxima* was 0.73 g/500ml and protein and chlorophyll content were 63.8% and 13.1 mg/gm respectively at pH 9. At 5 Klux light intensity the dry weight of *Spirulina maxima* was 0.72 g/500 ml while protein content and chlorophyll were 64.2% and 9.5 mg/gm respectively.

45. Total Phenolic Contents in *Simarouba Glauca DC*

Manaswi Patil, Madhuri Zende, C. J. Thirupurasundari

Department of Bioinformatics, School of Bioengineering, SRM University, Kattankulathur, Chennai (T.N.)

Keywords *: Phenolics, Simarouba, Medicine, Bark, Petioles.*

Simarouba glauca (edible oil seed bearing tree), has been used as natural medicine in tropics. It has large quantity of phenolic compounds apart from quassin, malic acid, gallic acid and mineral salts. High quantity of phenolic constituents account for medicinal properties of plants. Present investigation was undertaken to study phenolic contents in leaves, petioles, seed kernels, bark and stem and its comparison was done in young parts with older parts of *S. glauca*. Ascending phenolic content value was observed in one month old plant when compared with one year old plant parts. This information can be utilised by plant breeders for variety improvement and by the pharmacists for development of new herbal medicines.

46. *In Vitro* Cytotoxic and Antioxidant Activity of *Acorus Calamus* Rhizhome

Sinto Antony[1], Nevin K.G.[2]

[1]Department of Biotechnology,
Sree Narayana Guru Institute of Science and Technology,
North Paravoor, Ernakulam, Kerala.
[2]Department of Biochemistry,
Sree Narayana Guru Institute of Science and Technology,
North Paravoor, Ernakulam, Kerala,

Keywords : *Natural products, Acorus calamus, Antioxidants, Cytotoxicity.*

Natural products of plant origin and numerous non-nutritive dietary constituents have been shown to play a significant role in cancer chemoprevention. *Acorus calamus* L (Acoraceae family), commonly known as "vayambu" is a semiaquatic herb with creeping rhizomes and sword shaped long leaves found throughout India near marshy places, river banks and lakes. The plant is considered to have many pharmacological properties. The present study was designed to evaluate the phytochemical analysis for phenolic compounds, flavonoids in the water extract of the *Acorus calamus* rhizhome. The water extract was also tested for its cytotoxic activity on Daltons lymphoma ascites (DLA) cells as well as the antiradical scavenging activity. The water extract of *A. calamus* showed significant cytoxic activity against DLA cells (LD_{50}- 100 μg). The extract also showed significant *in vitro* antioxidant activity. In conclusion, the phytochemicals present in the water extract of *A. calamus* can be developed as a potential anti cancer drug by virtue of its cytotoxic and antioxidant activity.

47. Screening of Wood Degrading Fungal Samples Collected from Marine Sources of Machilipatnam, (AP) and Production of Laccase from Novel Fungi

N. Vijaya Sree[1*], P. Udaya Sri, Y. V. V. Aswani Kumar and N. Rama Rao[2]
Department of Biotechnology, Acharya Nagarjuna University,
Guntur-522 510, (AP)
[2]Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur-34, (AP)
***Email** : vijaya_1410@yahoo.com

Keywords : *Recombinant laccase, Wood degrading fungi.*

Wood degrading fungi and their enzymes find potential applications in the pulp and paper industry, detoxification of environmental pollutants, decolorization of dyes, cosmetics (including hair dyes), food and beverages, clarification/stabilization of fruit juices, clinical diagnostics, enzymatic conversion of chemical intermediates and upgradation of animal feeds. One of the most promising groups of enzyme, laccases has many biotechnological applications, including decontaminating phenol-polluted systems. Belonging to the class of phenoloxidases, laccases catalyze the polymerization of several phenolic substances to polymeric products. In addition, they transform lignin and lignin-related compounds, showing very broad substrate specificity. Laccases are used in a variety of processes, such as bioremediation, bio-bleaching of denims, in processing of beverages, as bio-analytical tool in biosensors to estimate the quantity of phenols in natural juices or the presence of other enzymes. The laccases from novel wood degrading fungi are in control of environmental pollution, and the possibility for their use in large scale operations were explored.

48. Characterization of Bio-Active Compound in Endophytic Fungi *Curvularia* Isolated from *Cynodon Dactylon*

Indumathi S., Soundhari C. and Gayathri S.
Valliammal College for Women, E-9, Anna Nagar East,
Chennai-600 102.

Keywords *: Bioactive compound, Endophytic, Curvularia.*

Fungal plant pathogen, which infect all major crops, are a threat to global food security. Investigation of endophytic microorganisms have been recently intensified due to potentialities of them in the production of bioactive compound. Endophytic fungi *Curvularia* was isolated from *Cynodon dactylon* and bioactive compound was identified, purified by column and thin layer chromatography. The compound was identified by NMR, IR and mass spectrometry and was tested against various bacterial plant and human pathogens. The compound was found to be active against *Salmonella typhi.* After toxicological studies the compound may be used for commercial purpose.

49. Comparision of Pesticide Biomineralising Ability of the Selected Fungal Species in Cotton Soils of Andhra Pradesh, India

P. Udaya Sri*, N. Vijaya Sree, K. M. Subbu Rathinam and K. R. S. Sambasiva Rao
Department of Biotechnology,
Acharya Nagarjuna University, Guntur-522 510, (AP)
Department of Zoology, Rajah Serfoji Govt.College,
Thanjavur-613 005, (TN)
***Email** : pudayasri@gmail.com

Keywords *: Pollution, Organophosphate pesticide, Mineralization, Biocleaners.*

Various kinds of pesticides have been used in cotton production to increase yield and farm income. The indiscriminate use of pesticides is causing negative effects on the environment and human health. In the present study, an attempt has been made to isolate the fungal species capable of degrading organophosphorus pesticides that are widely sprayed on cotton crop and develop a model for the control of pesticide pollution. The comparative ability of the fungal species *Rhizopus microsporus* and *Trichoderma viride* to degrade the organophosphorus pesticide,

Chlorpyriphos was studied in shake flask cultures. The fungal species grew successfully in the culture media treated with the different doses of insecticides (10, 25, 50 and 100 µg), but the growth rate varied with the insecticide doses. Different parameters like concentration of pesticides, duration of culture, temperature, medium, and pH were also optimized. The study has elucidated that *Rhizopus microsporus* and *Trichoderma viride* can be very effectively used as biodegrading agents for the degradation of the pesticides and will be an economically effective microbial process which is an alternative for the control of environmental pollution.

50. Efficacy of Phosphate Solubilizing Microorganisms on Germination, Growth and Phosphorus Uptake of Wheat (*Triticum Aestivum*)

P. B. Patil, G. V. Naiknaware, S. D. Mahajan, D. K. Hawaldar and V. V. Patil

Department of Biotechnology, COCSIT College, Latur, Maharashtra.

Keywords : *Phosphate solublizing bacteria, Wheat, Biofertilizer.*

Phosphate solubilizing microorganisms used as biofertilizer, they enhance phosphorus availability to plant by mineralizing organic phosphorus in soil by solubilizing precipitated phosphate. Several phosphate solubilizing bacteria occur in soil, usually their number not high, therefore inoculation of plant by a target microorganism at much high concentration to take advantage of the property of phosphate solubilization for plant yield enhancement. In the present research work we studied efficacy of phosphate solubilizing microorganisms on germination, growth and phosphorus uptake of wheat (*Triticum aestivum*) and we found that phosphorus solubilizing bacteria mainly *Bacillus polymyxa*, *pseudomonas cepacia* and *Bacillus subtilis* were very effective for increasing the plant available phosphate in soil as well as the growth and yield of wheat (*Triticum aestivum*).

51. Production of Citric Acid by *Aspergillus Niger* Using Cane Molasses and Cheese Whey in Submerged Fermentation

A. A. Bhairappa, P. D. Bansode and N. B. Patkar

Rajarshi Shri Shahu Mahavidyalaya,
Department of Biotechnology, Latur, Maharashtra

Keywords *: Citric acid, Aspergillus niger, Cane molasses, Cheese, Whey.*

Citric acid production by *Aspergillus niger* using cane molasses and cheese whey in surface culture process was studied. Citric acid having great industrial importance is used as a flavoring agent. This was found that cane molasses was most favorable medium for citric acid production. In cane molasses the citric acid production (29.38%) was highest after 144 h of incubation. Highest biomass of *Aspergillus niger* was produced after 48 h of incubation in both the culture media. In general, there was an increase in citric acid production and decrease in sugar concentration as time progressed.

52. *In Vitro* Plant Propagation of *Trichosanthes Dioica* Roxb.cv. Hilli from Nodal Explants

Rashmi Komal* and Ashoke Kumar Gupta

Tissue Culture Laboratory, Department of Botany,
Patna University, Patna-06, Bihar.
***Email** : rashmi0911@gmail.com

Keywords *: Plant propagation, Shoot multiplication, Trichosanthes dioica Roxb. cv. hilli.*

An efficient protocol was developed for *in vitro* plant propagation from nodal explants of *Trichosanthes dioica* Roxb. cv. hilli on semisolid MS basal medium supplemented with different concentrations of BAP and kinetin. Different nodal segments were used as explants, but the best performance was shown by the lower nodes. Among the various concentrations of growth regulators used MS + 2.0 mg/ l BAP showed highest percentage of shoot induction (92.74%) and maximum number of shoots per explants (4.00 ± 0.74) from nodal explants after four-five weeks of culture. The elongated shoots rooted within seven to eight days in half strength MS supplemented with 1.0 mg/l IBA. IBA proved to be better than IAA for efficient rooting. The *in vitro* raised plantlets successfully established in earthen pots filled with soil, sand and vermicompost mixture.

53. Isolation and Purification of Polyphenol Oxidase from Various Plant Sources and its Use in Decolourization of Industrial Dyes

P. S. Solanki, P.V. Khakke, S. P. Bobade, S. M. Karhade, A. B. Jadhav and S. N. Harke
MGM's Institute of Bioscience and Technology, Aurangabad.

Keywords *: Reactive dyes, Polyphenol oxidase, Bioremediation, Waste water treatment.*

Reactive dyes are important chemical pollutants from various industries. Polyphenol oxidase (PPO) shows the activity of decolourization of various industrial dyes such as malachite green dye. Polyphenol oxidase is isolated from locally available plant sources such as cabbage, locally available brinjal, wild brinjal, banana peel, banana pulp, potato, apple, coriander and mango. The enzyme activity is evaluated using catechol as substrate. The enzyme is partially purified by ammonium sulphate precipitation (70%). The decolourization of these phenolic compounds by PPO was evaluated and confirmed by using U.V. spectroscopy. The ability of PPO enzyme to decolorize various industrial dyes confirmed that enzyme had remarkable potential for its application in bioremediation and waste water treatment especially in detoxification of phenolic waste.

54. Alteration in Structure and Biodegradability of Lignocelluloses in *Imperata Cylindrica* by *Daedaleopsis Confragosa*

Amee Padhiar, Susy Albert and Dhara Gandhi
Department of Botany, Faculty of Science,
The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat

Keywords *: Lignocellulose, Daedaleopsis confragosa, Imperata cylindrical, SEM, Light microscopy.*

The white rot fungus *Daedaleopsis confragosa* was evaluated for its ability to delignify *Imperata cylindrica*, a very common unpalatable grass growing in the water-logged areas. The compositional and structural alterations in the plant cell walls affected by the fungi were determined. Light microscopic studies supported with scanning electron microscopic studies indicated that the silica bodies remain unaffected by the fungal attack. Weight loss experiment showed 60% lignin loss in seven weeks time. The study revealed *Daedaleopsis confragosa* has the potential to delignify and modify cell walls of *Imperata cylindrica* making it suitable for paper industry.

55. Effect of *Alternaria* Leaf Blight on Seed Germination and Seedling Vigour of Sunflower in Rohilkhand Region

Vikas V. Patel*, Reetu Gangwar and U.S. Mishra

Phytopathology Lab. Department of Botany. Bareilly College, Bareilly, M.J.P., Rohilkhand University, Bareilly-243 006

***Email**: vpversatile@gmail.com

Keywords : *Alternaria leaf blight, Sunflower, Seed germination, Seedling vigour.*

Alternaria leaf blight of sunflower is a very common and destructive disease in Rohilkhand region. Despite the rapidly growing cultivation of sunflower spread of the crop in India, the productivity of crop is shown to decrease in recent years due to the susceptibility of plants to the fungal diseases. The symptoms of *Alternaria* leaf blight appeared in the month of March in the form of characteristic small circular, brown patches on the surface of leaves that grew in size and coalesced to cover the entire surface of leaves producing blight symptoms. Marked blight symptoms were seen in the head (capitulum) of heavily infected plants in which seeds were also infected with *Alternaria helianthi.* Naturally infected seeds with *A. helianthi* and those inoculated experimentally showed 38.6% and 23.0% reduction respectively in germination. Shoot and root length of seedlings were also significantly reduced in both cases. There was a marked increase in number of seedlings showing blight incidence with increase in spore load of *A. helianthi* on seeds. Biocontrol of *Alternaria* blight by selected natural herbal plant extracts *in-vitro* has been observed and recommended for use to the farmers.

56. *Burkholderia Caribensis sp. nov.* (GU372342) Isolated from Acidic Lowland Rice Agro-Ecosystems of South Assam, India.

[1]B. Deb Roy[*], [2]S. Nath, [2] B. Deb and [1] G.D. Sharma

[1]Microbiology Laboratory, Department of Life Science, Assam Central University, Silchar-788 011, Assam

[2]Microbiology Laboratory, Department of Botany and Bioinformatics Centre, Gurucharan College, Silchar-788 004, Assam.

***Email** : b_deb_roy@sify.com

Keywords : *16S-rRNA sequenc, Biofertilizer, Burkholderia caribensis str. SDSA-I10/1 (GU372342), Diazotroph, Nitrogenase activity, Rainfed lowland.*

Six isolates of *Burkholderia sp.* were obtained from rhizosphere soils of rice grown in acidic lowlands of South Assam. Among the identified *Burkholderia* isolates, SDSA-I10/1 showed higher nitrogen fixing ability and these were selected for 16S rRNA sequencing. The isolate SDSA-I10/1 showed its highest resemblance to *Burkholderia caribensis* MWAP84 (Y17011) and it is identified as *Burkholderia caribensis* str. SDSA-I10/1 (GU372342) of the class â-proteobacteria. Inoculation of *Burkholderia caribensis* (GU372342) improved the growth and yield of rice significantly over uninoculated control plants and it may be used as indigenous microbial inoculant for intensive rice cropping.

57. Xylanase Production Under Solid State Fermentation and their Sequential Treatment in Biobleaching of Kraft Pulp

Lalit Kumar[1], Vijay Kumar Gupta[1*], Abha Sharma[2], Sushil Nagar[1], Vasanta Vadde Thakur[3], RK Jain[3] and Ramesh Chander Kuhad[2]

[1]Department of Biochemistry, Kurukshetra University, Kurukshetra-136 119 (HR)
[2]Departemnt of Microbiology, University of Delhi, South Campus, New Delhi
[3]Central Pulp and Paper Research Institute, Saharanpur (UP)
***Email** : vkgupta59@rediffmail.com

Keywords : *Xylanase, Bacillus subtilis, Kraft pulp.*

Biobleaching and bioprocessing of pulp involve the use of xylanase enzymes. Once modified, hemicellulose is removed by xylanase, the lignin layer is thus easily available for penetration and degradative action. In the present work, cellulase free xylanase was produced under solid state fermentation conditions from *Bacillus subtilis* ASH, which was isolated from decaying wood. The strain was grown and maintained on xylan-agar containing (g/l): Xylan 5.0, peptone 5.0, beef extract 3.0, NaCl 5.0 (pH 7.0). Untreated and xylanase treated pulp was characterized for kappa number and brightness. Treatment of kraft pulp with xylanase (20 IU/g) resulted in a brightness gain of 1.8 units and reduction in chlorine requirement upto 15 %. The xylanase production has been scaled up to 2 Kg level. The treatment of kraft pulp with sequential action of xylanase and laccase also resulted in ClO_2 savings and a brightness improvement.

58. Production of *Fusarium Axysporum* Apore in Solid State Fermentation and Efficacy as a Biocontrol Agent Against *Parthenium* Rag Weed

Sunita Farkya

Department of Education in Science and Mathematics,
Regional Institute of Education, Ajmer

Keywords : *Fusarium oxysporum, Solid state fermentation.*

Production of *Fusarium oxysporum* spores following solid state fermentation (SSF) strategy was studied with respect to biological control of *Parthenium* reg weed. The commercial development of a biocontrol fungus requires availability of a low cost fermentation method. Fermentation process should be selected to trigger the production of stable, durable and virulent propagules. Spores produced in submerged fermentation are known to be more sensitive, unstable and less virulent than propagules produced in an aerial environment. Appropriate fermentation technology needs to be selected for viable and highly virulent fungal propagules. The cost effectiveness of these biological products is closely related to the efficacy of the fermentation methods and the type, quality and quantity of propagules produced. SSF was performed using ton different organic wastes. Maximum sporulation 7.7x10 spores/ml was observed on wheat bran followed by host tissue. Spores obtained from various organic wastes germinated within 24 h. Virulence of fungus was greatly affected by SSF of various organic wastes. Spores obtained from SSF of host tissue were highly virulent and could initiate disease epidemics in pot experiment and 98.5% control of *Parthenium* rag weed was obtained within 12 days.

59. Fermentative Hydrogen Production from the Fermentation of Sugarcane Bagasse Hydrolyasate by Lab Isolate *Citrobacter* CDN1

Pallavi Sinha and Anjana Pandey

Nanotechnology and Molecular Biology Lab, Centre for Biotechnology,
University of Allahabad, Allahabad- 211 002

Keywords *: Fermentation, Biological hydrogen production, Sugarcane bagasse, Hemicellulose, hydrolysis.*

In the present study we exploited the SCB hemicellulose hydrolysate to produce hydrogen by an efficient hydrogen producing lab isolate *Citrobacter* CDN1

strain isolated from the cow dung. Sugarcane bagasse was hydrolysed with sulphuric acid to yield glucose and xylose. In the hydrolysate 1% malt extract and 0.4% yeast extract was mixed. This media was used for fermentative hydrogen production by isolate. Effect of amount of sulphuric acid on hydrolysis of bagasse, effect of initial pH, effect of innoculum volume and innoculum age have been studied and it was found that use of bagasse will not only be useful for hydrogen production but also for waste management.

60. Effect of Plant Essential Oils on Biofilm Formation and Adherence of Multidrug Resistant *Staphylococcus Aureus*

H. Jayalekshmi, P. Dhanya Sree, Bipin G. Nair

Amrita School of Biotechnology,
Amrita Vishwavidyapeetham,
Kollam, Kerala-690 525

Keywords *: Biofilm, Plant essential oils, Staphylococcus aureus.*

Biofilms are microbial communities of surface attached cells embedded in a self-produce extracellular polymeric matrix. Inhibition of biofilm provides a method of controlling the effect of pathogenic bacteria without strong selection for drug resistance. Although the antimicrobial properties of essential oils are extensively studied, the anti biofilm activities are not well studied. The primary objective of this study was to investigate essential oils viz. walnut oil *(Juglans regia)* eucalyptus oil *(Eucalyptus polybractea*) garlic oil (*Allium sativum*) nutmeg oil (*Myristica fragrans*) and black pepper oil (*Piper nigrum)* for inhibition of growth and biofilm in a multi drug resistant strain of *S. aureus*. Using microtitre plate system with crystal violet staining was employed to test the effect of essential oils on biofilm formation and adherence. Eucalyptus oil, nutmeg oil and garlic oil showed significant dose dependent reduction in the biofilm formation. These results validated the efficacy of essential oils as antibiofilm agents and an important step towards the development of new antibiofilm drug.

61. Ethylamine Induced Tall Mutants in Jute (*Corchorus Olitorius* L.)

P. K. Ghosh[1*] and A. Chatterjee[2]

[1]CSB, CSR71T1, Berhampore-742 101, Murshidabad, West Bengal
[2]Centre of Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, 35, Ballygunge Circular Road, Calcutta-700 019
***Email** : paritosh.ghosh@rediffmail.com

Keywords *: Corchorus olitorius L., Tall mutant, Chromosome.*

Presoaked seeds of jute (*Corchorus olitorius* L., Variety JRO-632) were treated with 1% ethylamine for 6 h. Tall mutants were screened in M3 in contrast to the normal plants. Tall mutants otherwise looked normal excepting the nature of palmate leaf habit. A number of yield component growth parameters were recorded like plant height, basal diameter, plant spread, root length, pod per plant, seeds per pod, pod length/breadth ratio, number of primary branches per plant, number of secondary branches per plant, leaf angle, branching angle, first flowering date, 100% flowering date, total duration % of pollen sterility and weight of 100 seeds which were found to vary from the control plant. Chromosome analysis revealed a number of aberrations like stickiness, fragmentation, clumping, polyploidy, and laggard and bridge formation etc. at very low frequency. This tall mutant plant gave more fiber yield than the control plants with superior quality.

62. Ethylmethane Sulphonate Induced Bushy Head Mutants in Jute (*Corchorus Olitorius* L. Variety Jro-632)

P. K. Ghosh[1] and A. Chatterjee[2]

[1]CSB, CSR&T1, Berhampore-742101, W.B.
[2]Centre of Advanced Study in Cell and Chromosome Research,
Department of Botany, University of Calcutta,
35, Ballygunge Circular Road, Kolkata 700047

Keywords *: Corchorus olitorious L., Bushy head mutant, Ethylmethane sulphonate.*

Presoaked seeds of jute (Corchorus olitorius L. Variety JRO-632) were treated with 0.5% ethylmethane sulphonate (EMS) for 24 hours. Bushy head mutants were screened in M3 in contrast to the normal plants. Bushy head mutants otherwise looked normal excepting the nature of tall habit. A number of yield

component growth parameters were recorded like plant height, basal diameter, plant spread, root length, pod per plant, seeds per pod, pod length/breadth ratio, number of primary branches, per plant, number of secondary branches per plant, leaf angle, branching angle, first flowering date, 100% flowering date, total duration, % of pollen sterility and weight of 100 seeds which were found to vary from the control plant. Chromosome analysis revealed a number of aberrations like stickiness, fragmentation, clumping, polyploidy, and laggard and bridge formation at very low frequency. These early flowering mutant plants gave more fiber yield than the control plants with superior quality. Multiple cropping has been possible with the availability of irrigation water and a number of early maturing varieties have been introduced in case of various other crops. There should be a suitable bushy head variety of jute also to be best fitted in the multiple cropping patterns. With this objective the work on induction of mutation with chemical mutagen EMS was initiated.

63. Identification and Characterization of a Bacterial Agonist in the Development of Fern Leafspot Disease

Dipavali Acharya, Parijat Sil, Rishita Baishya, Shriya Saha, Shreyshi Majumder,

Debasmita Das and Anup Kumar Mitra*

Department of Microbiology, St. Xavier's College, Kolkata-700 016

***Email :** drakmitra01@hotmail.com

Keywords : *Adiantum spp, Bevibacillus, Alternaria.*

The Chandigarh-Shimla Highway NH-22 is a busy highway, because of some industrial area situated in Zala, Solan at a distance of 45 km from Chandigarh. Majority of vehicles emit sulphur dioxide, carbon monoxide and unburnt particulate hydrocarbon. The road side cryptogams are prone to injury from particulates. The road side ditches are filled with water from the rain fed streams. The *Adiantum spp*. growing in this area show a characteristic leaf spot disease which is more pronounced in areas near the ditch. The casual fungus was *Alternaria spp*. that sporulates profusely leading to the development of the disease. The disease is favoured by warm and windy weather under moist conditions. In heavily infected leaves an additional species of bacteria was isolated and characterized to be gram negative, catalase positive, oxidase negative and also urease negative. The molecular characterization by 16s DNA denoted the bacteria to be *Baciillus borstelensis* strain *JBE00014* (Gene Bank Accession Number : FJ982663.1) based on nucleotide

homology and phylogenetic analysis. Two separate fragments with forward and reverse primer analyzed the 1415 base pairs or the 16s rDNA and on the basis of 114 BLAST hits, the organism was found to be *Bevibacillus borstplensis* strain JBE0014, having 99% similarity with *Bacillus spp.* R-30914. The presence of the bacteria increases the percentage germination of the conidia of *Alternaria* and thereby it is indicated that there is an agonistic relationship between the two organisms.

64. Morphological, Biochemical Characterization and Molecular Cataloging of a Few Edible Mushroom Varieties to Assess Variation in Properties and Commercial Importance

Ishita Mukherjee, Aditya Bhattacharya, Sitabja Mukhrjee, Sounak Kundu, Sandeep Kaur Matharu, Sreya Das and Sadeshna Shyam Choudhury

Department of Microbiology,
St. Xavier's College, Kolkata-700 016

Keywords *: Mushroom, Antioxidant properties, Biochemical characterization.*

Edible mushrooms mostly belong to Basidiomycotina, which have important biotechnological, environmental and medical applications. Morphological studies performed do not shed much light on varietal specificity but helps in species characterization to some extent. A biochemical study was done with the help of a few enzyme assays - tyrosinase B-D-glucosidase (for absorptive nutrition), superoxide dismutase, catalase, peroxidase (for enzymatic antioxidant power), lipid peroxidation and UV absorption compounds assays (role of non-enzymatic antioxidants). The tests were performed with extracts prepared from the pileus of *Agaricus bisporus, Pleurotus sajoroju, Calocybe indica* and two of its hybrid varieties (collected from Bose Farm, Kolkata). Morphologically, the mushroom cultivars varied considerably with the pileus diameter, width and stipe length being maximum for *C. indica* (A) (10-15*2-3*6-8 cm) followed by PCH9(C), PCH3(B), *P. sajor*- caju(E), *A. bisporus* (D) (1-4*1-2*5 cm). Mushroom browning (a result of tyrosinase activity) thus correlates to the shelf life of a mushroom; it was found to be maximum for *C. indica PCH9* variety (0.0172 ml/ug) while minimum for *A. bisporas* (0.165) ml/ug) B-D glucosidase assay (relates to the nutritive value which was found to be maximum (0.223 ml/mg/ug) for *P. sajor -caju* and minimum for *C. indica PCH3* variety (0.013 ml/µg). The maximum and minimum activities in case of S.O.D., catalase, lipid peroxidation (mustard oil and chicken liver) were shown by *P. sajor*

-*caju* and *C. indica* respectively, which proves that these mushrooms are a valuable source of biologically active compounds with potential for protecting cellular DNA from oxidative damage. Though the lipid peroxidation value E was higher but the antioxidant potential assay through percentage of DPPH reduction (including both enzymatic and non-enzymatic antioxidants) is highest for E (60%) which was also confirmed by assays of catalase, peroxidase, superoxide dismutase, UV absorbing compounds. So the higher lipid peroxidation value may be due to higher peroxidant molecules like lipoxygenase enzyme in E. The molelcular analysis study showed that B and C were closely related, whereas A and D were also related but E clustered separately thus determining the genetic relatedness among the common edible mushroom cultivars and the new hybrid developed; which also allowed us to catalog the new hybrid varieties. Eventually we noted that among the hybrid varieties *Calacybe indica PCH9* has considerable commercial importance though *Pleurotus* - *sajor caju* is the best among the general cultivars. The biochemical parameters concluded that the hybrid varieties of *C. indica* are best among all the varieties in terms of shelf life but *P. sajar-caju* had the highest antioxidative potential and nutritional quality.

65. Seasonal Variations and Biodiversity Indices of Phytoplankton in Harsool-Savangi Dam, District Aurangabad, India

S .E. Shinde[1*]. P. R. More[1], R. Y. Bhandare[1], T. S. Pathan[2] and D. L. Sonawane[1]

[1]Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431 004

[2]Department of Zoology, Kalikadevi Arts, Commerce and Science College, Shirur (Ka) Tq. Shirur dist. Beed

***Email :** sunilshinde1004@rediffmail.com.

Keywords : *Phytoplankton, Seasonal variations, Biodiversity indices, Percentage wise compositions, Harsool-Savangi dam.*

The present study deals with seasonal variations and biodiversity indices of zooplankton during January 2008 to December 2008 in Harsool-Savangi dam, Aurangabad [M.S] India. Total 35 taxa recorded were 15 *Chlorophyceae*, 7 *Bacillariophyceae*, 7 *Cyanophyceae* and 6 *Euglenophyceae*. Present study revealed that percentage wise compositions, biodiversity indices and maximum population density of zooplankton was recorded at north site in summer and minimum

population density was recorded at south site in monsoon. In present study, some pollution indicating species were found like *Navicula, Nitzschia, Oscillatoria, Anabaena, Nostoc* and *Euglena* according to Palmer's index.

66. Prospecting of Genes for Oil Biosynthesis Pathway from *Jatropha Curcas*

Purushothaman Natarajan and Parani Madasamy

Genomics Laboratory, Department of Genetic Engineering, SRM University, Kattankulathur-603 203, Tamil Nadu

Keywords : *Jatropha curcas, EST, Gene discovery, Normalized cDNA library, Annotation, Oil biosynthesis pathway, DNA sequencing.*

Jatropha curcas L is promoted as an important non-edible biodiesel crop worldwide. Jatropha oil, which is a triacylglycerol, can be directly blended with petro-diesel or transesterified with alcohols and used as biodiesel. Genetic improvement in *Jatropha* is needed to increase the seed yield, oil content, drought and pest resistance, and to modify oil composition so that it becomes a technically and economically preferred source for biodiesel production. However, genetic improvement efforts in *Jatropha* could not take advantage of genetic engineering methods due to lack of cloned genes from this species. To overcome this hurdle, we have initiated the current gene discovery project with an objective of discovering as many functional genes as possible from *Jatropha* by large scale EST sequencing and analysis. A normalized and full-length enriched cDNA library was constructed from developing seeds of *Jatropha curcas* L. Size of the library was about 1 x 10^6 clones and average insert size was 2.1 kb. Totally 12,084 high quality ESTs with average read length of 576 bp were generated. Contig analysis of these ESTs showed 2,258 contigs and 4,751 singletons. Contig size ranged from 2-23 and there were 7,333 ESTs in the contigs. This resulted in 7,009 unigenes (58%) which were annotated and functionally categorized. From the present study, we have isolated 42 potential oil biosynthesis genes essential for the manipulation of oil biosynthesis pathway in *Jatropha*. All of these 42 genes are being fully sequenced and we are pleased to share that among these genes nearly 30 genes are not cloned anywhere else from *Jatropha curcas* L. Furthermore, we have done *de novo* sequencing and transcriptome analysis using 454 GS FLX titanium platform and normalized cDNA from five major tissues of *Jatropha* to discover more genes for oil biosynthesis pathway and to generate more transcriptomic resource.

iv. MICROBIAL TECHNOLOGY

67. Exploration of antioxidant properties of tunic extracts from two marine Ascidians, *Phallushia nigra* and *Phallushia arabica*

S. Mohamed Hussain, P. Sivaperumal and G. Ananthan*
CAS in Marine Biology, Faculty of Marine Sciences,
Annamalai University, Parangipettai
***Email** : casananthan@yahoo.co.in

Keywords : Antioxidant, Ascidians, Reducing power, Ascorbic acid.

In vitro antioxidant activity of crude methanol extracts from two selected marine ascidians were evaluated. Each assay of both extracts was conducted at various concentrations in triplicate (40, 80,120 and160 ìg/ml) using ascorbic acid as standard. All assays revealed that, methanol extracts of *Phallusia nigra* and *P. arabica* exhibited higher antioxidant activity compared to standard. Maximum activity was observed at 160 ìg/ml in all assays. Total antioxidant assay of *P. nigra and P. arabica* were determined (140 ìg/ml and 85 ìg/ml), followed by reducing power (38 ìg/ml and 12 ìg/ml) respectively, by plotting standard graph and the percentage IC 50 value of hydrogen peroxide radical scavenging assays were observed to be 60% in *P. nigra* and 63.56% in *P. arabica*. Antioxidant activity of ascidians extracts were increased with increasing concentration.

68. Antifungal Activity of *Terminalia Chebula* and *Terminalia Catappa* on Two Dermatophytes

C. Soundhari* and S. Rajarajan,
P.G. and Research Department of Microbiology & Biotechnology,
Presidency College, Chennai (TN)
***Email** : souviabe@gmail.com

***Keywords :** Medicinal plants, Tinea corporis, MIC and MFC, Antifungal drugs.*

Tinea corporis is a superficial fungal infection presenting erythematous scaling patches with central clearing caused by Dermatophyte. The present study evaluated the *in vitro* fungicidal activity of lyophilized, aqueous and ethanolic extract of *T. chebula* and *T. cattappa* on tinea corporis causing fungi by

determining MIC and MBC and compared with standard drugs. The MFC of aqueous and ethanolic extracts of *T. chebula* on *T. mentagrophytes* and *T. rubrum* were 12.5 µg/ml and 6.25 µg/ml, whereas MFC of aqueous and ethanolic extracts of *T. catappa* was 12.5 µg/ml for *T. mentagrophytes* and 6.25 µg/ml for *T. rubrum*. The efficacy of lyophilized extract of *Terminalia* species was nearly equal to that of the standard antifungal drugs.

69. Isolation, Characterization and Production of IAA by *Pseudomonas Fluorescens*

Inamdar Taukin Nasir

New Arts, Commerce and Science College,
Ahmednagar College, Ahmednagar-414 001
Email : tanu2429@yahoo.co.in

Keywords : *Indole-3-acetic acid, PGPR, Pseudomonas fluorescens, Rhizoshere, Tryptophan.*

In present studies, attempts were made to isolate, characterize and produce IAA from *Pseudomonas fluorescens* from rhizospheric soil and to enhance the production by providing a precursor molecule-Tryptophan. The isolate used was obtained from rhizospheric soil sample of Peucedanum *gravedens* on King's B medium and various biochemical tests were performed. For extraction and purification, paper chromatography was performed. Root elongation assay was performed by inoculating seeds of jowar, *Brassicca*, wheat on King's B medium. It was concluded that PGPR *Pseudomonas fluorescens* is important in plant growth as it produces IAA which is important phytohormone and regulates the plant growth.

70. Molecular Identification of Salt Tolerant Microbes Using 16s rRNA Gene Sequence from East Coast of India

B. K. Behera, M. P. Brahmane, Priyanka Das, Amrita Chanda and Avijit Patra

Central Inland Fisheries Research Institute,
Barrackpore, Kolkata-700 120

Keywords *: Salt tolerant microbes, 16s rRNA gene sequence.*

The rRNA genes are highly conserved in the bacteria and other organisms. Consequently, characterization of 16s rRNA gene is now well established as a standard method for the identification of species, genera and families of bacteria. In this study, same technique has been used to identify salt tolerant marine microbes from East Coast of India. The water, soil and sediment samples were collected from 15 different sampling sites from 3 regions of east coast of India; viz. Bakhali, Frasergunj, Kakdwip, Sagar Island, Namkhana, Haldia, Digha in West Bengal, Chandipur, Puri, Chandrabhaga, Chilka in Orissa and Visakhapattanam, Yanam, Kakinada, Rajamundry in Andhra Pradesh. A total of 120 microbes were isolated from the collected samples. All these collected microbial isolates were subjected to 10%, 15%, 20% and 25% NaCl concentrations for salt stress physiological study. In this study, 80 microbial isolates were found resistant to 10-25% NaCl concentrations. Out of that, molecular typing for eight microbial isolates was carried out by 16S rRNA gene sequence analysis. The bacterial isolates identified were *Bacillus cereus, Pseudomonas aeruginosa NO 5, Staphylococcus haemolyticus, Shewanella sp.62, Staphylococcus pasteuri strain BQN3N-02d, Staphylococcus saprophyticus, Vibrio alginolyticus* and *Vibrio sp. K380.*

71. Screening of Dietary Substances : New Blockade Strategy on Quorum Sensing

M. Dhaneesha, D. Arathy Pai and H. Jayalakshmi

Amrita School of Biotechnology,
Amrita Vishwavidyapeetham, Kollam-690 525

Keywords *: Quorum quenching, Fruit extracts, Chromobacterium.*

The antimicrobial properties of dietary phytochemicals is well known, where as the ability as quorum sensing modulators is less studied. The objective of this investigation was to determine the quorum sensing inhibition activity of common fruit extracts, viz., *Ananas cosmosus, Citrus sinensis, Vitis Concord Seedless, Punica granatum, Phyllanthus emblica, Anacardium occidentale, Lycopersicon esculentum, Malus domestica, Vitis muscat and Vitis vinifera*. The bacterial strain used was *Chromobacterium violaceum* MTCC 2656. Loss of purple pigment in *Chromobacterium violaceum* is indicative of quorum sensing inhibition by the fruit extract introduced. The experiments were done in triplicate using standard methods. Six fruit extracts, viz., *Ananas cosmosus, Citrus sinensis, Vitis Concord Seedless, Punica granatum, Lycopersicon esculentum* and *Vitis muscat* expressed quorum

quenching activity. *Phyllanthus emblica* showed antimicrobial activity. Quantitative assessment of pigment inhibition indicated that the quorum quenching activity of the fruits was concentration dependent. *Citrus sinensis* showed lowest quorum quenching activity. The molecules within the extracts that are involved in the inhibition of quorum sensing and the mechanism of quorum quenching are to be studied as a future prospect in order to exploit the wide possibilities of quorum quenching as a possible future treatment scenario for infections caused by bacteria which regulate pathogenocity by means of quorum sensing.

72. Isolation, Identification and Antimicrobial Susceptibility Evaluation of Microbes Isolated from Biofilm on Urinary Catheters

S. Balasubramanian[1], K. Chairman[2], K. Jothivel[1], M. Kannan[3] and A. J. A. Ranjit Singh[2]

[1]Department of Microbiology,
Thanthai Hans Roever College, Perambalur.
[2]Department of Advanced Zoology and Biotechnology,
Sri Paramakalyani College, Alwarkurichi.
[3]Department of Microbiology, VHNS College,
Virudhunagar, Tamil Nadu.

Keywords : *Proteus mirabilis, Biofilm, Antimicrobial activity.*

Bacterial species colonize in dwelling catheters as biofilm induce complications in patients care. Forty five catheters used by patients visiting a hospital were collected and screened for biofilm formation. Catheters that had been used over 25 months had a heavy biofilm matrix. From the biofilm matrix seven species of microbes were isolated. The predominant bacteria seen in catheters were *E. coli*, (27 %) *P. mirabilis* (20 %) and *S. epidermis*,(18 %). The biomass of microbes associated with the biofilm was estimated. The mean dry weight of biomass of bacteria associated with a catheter that was used for over a month time was in the range 2.5±0.04 g – 3.1 ± 0.6 g. *P. mirabilis* normally colonizes the catheter after long-term usage. But it was found to colonize the microtitre plate to attain a peak growth at 84 h. *P. mirabilis* isolated from the biofilm was able to tolerate the antibiotics tetracycline, penicillin, kanamycin and gentamycin at a dose level of 20 µg/ml. The study indicated that the catheter has to be replaced if biofilm formation is noticed.

73. Study of Marine Microbial Diversity, Isolation, Screening of Antimicrobial Activity and its Potential Applications

Tushar Kumar Verma*, Mihirjyoti Pathak, Kumud Ranjan and D. Sankari
Department of Biotechnology, SRM University, Chennai- 603203
***Email**: tush.20.11@gmail.com

Keywords : Marine microbial diversity, Antimicrobial activity.

Collection of soil from coastal area reveals that variety of marine micro-organisms isolated have potential applications in industrial and scientific research. Three different sources of rhizoidal soils, one from terrestrial and two from intertidal zone containing microbes were collected and serially diluted and plated on PDA, NAM, King's B and SCA media upto 10^{-6} dilution. Variety of useful micro-organisms of *Pseudomonas spp.*, *Actinomycetes spp.*, and *Aspergillus spp.* were isolated. These organisms showed potential applications. The 16S rRNA sequencing is in process for strain identification. Certain *Pseudomonas spp.* produce fluorescence colonies as well red and green colored pigments in serial dilution 10^{-5} in Kings B media. Ethyl acetate crude extract of *Actinomycetes spp.* 10^{-3} in SCA media and *Pseudomonas spp.* showed antimicrobial activity against various plant and human pathogens. Lipase, tannase, protease, gelatinase and amylase were also produced which have potential applications.

74. Production and Characterization of Polyhydroxybutyrate from Molasses Produced by *Bacillus Megaterium* NCIM 2326 and its Applications

Anil Kumar Vemu, Sisir Kumar Barik and Saravanan M
Department of Biotechnology, Faculty of Science and Humanities,
SRM University, Kattankulathur-603 203

***Keywords** : Polyhydroxybutyrate (PHB), Bacillus megaterium.*

Production of PHB was studied by using *Bacillus megaterium* (NCIM 2326) with different concentrations of sugar cane molasses (2%-5%). The process of fermentation was maintained in aerobic conditions, inoculated organisms utilize sucrose as the carbon source. During the fermentation, organisms produce PHA synthase as the key enzyme used for PHB synthesis and accumulation intracellularly. Maximum production was obtained with 2% molasses from *Bacillus megaterium*

(46% w/w) when compared to other *Bacillus sps*. The present study reveals that *Bacillus megaterium* gives higher yield of PHA by utilizing low concentration of molasses. From the study we concluded that PHA production is possible from low cost molasses. Furthermore, the synthesized PHA was characterized by NMR, UV spectroscopy and SEM for further applications.

75. Isolation of Hyper Poly-β-Hydroxybutyrate Producing Bacteria from Soil Samples

G. S. Yadav[1], Arpana Mittal[1], Versa Goyal[1], Anita Yadav[2] and N. K. Aggarwal[1]

[1]Department of Microbiology, Kururkshetra University, Kururkshetra.
[2] Department of Biotechnology, Kurukshetra University, Kurukshetra.

Keywords *: Bacteria, PHB production, Isolation, Screening, Soil, Nile blue A, Sudan black B.*

Bacterial isolates from different locations in Haryana were screened for poly–â- hydroxybutyrate (PHB) production. Many microbes stored PHB as reserve food material. A total of 310 microbes were isolated. Initial screening was done by staining with Sudan black B to detect cellular inclusions. For secondary screening, staining with Nile Blue A was performed. After primary and secondary screening, total 29 isolates were found to be PHB positive. PHB extracted from positive isolates was confirmed by method of PHB estimation of Law and Slepecky. In the Law and Slepecky method, PHB was dissolved in sulphuric acid and incubation was done at 100°C for 10 minute in water bath to convert the PHB into crotonic acid. After cooling, OD was measured using spectrophotometer at 235 nm and concentration of PHB was calculated with the help of standard graph made using commercial PHB.

76. Effect of Supplementary Carbon and Nitrogen Sources on Amylase Production by *Trichoderma Viridae* Using Corn Cob Residue as Substrate in Solid State Fermentation

P. Lalitha, K. Shravya, K. Arunalakshmi

GITAM University, Rushikonda, Vishakapatnam-530 045

Keywords *: Amylase, Trichoderma viridae, Supplementary carbon and nitrogen sources, Solid state fermentation.*

The effect of combinatorial effect of supplementary carbon and nitrogen sources (carbon sources – glucose, fructose, maltose and sucrose; nitrogen sources – ammonium nitrate, sodium nitrate, yeast extract and peptone) in the production of amylases by *Trichoderma viridae* was studied in solid state fermentation using corn cob as the substrate. Results showed the potential of solid state systems when the combination of fructose and sodium nitrate as supplementary carbon and nitrogen sources respectively.

77. Comparative Study of Physicochemical Characterization of Soil from Arid or Semi-arid Region of Rajasthan

Deeksha Gaur, P. K. Jain, V. K. Gupta and R. K. Gaur

Department of Biotechnology,
MITS University, Lakshmangarh,
Sikar-332 311, Rajasthan.

Keywords *: Physico-chemical, TDS, EC, WHC, Arid region, Semi-arid region.*

Soil physicochemical characters determined the types of plants or microorganisms in that particular soil. The physicochemical properties of soil includes its physical and chemical characterstics like pH, electrical conductivity (EC), total dissolved solids (TDS), humidity, moisture content, water holding capacity (WHC), organic carbon, chloride ion and bicarbonate. In the present investigation we conducted a comparative study between arid (Bikaner and Churu) and semi-arid regions (Udaipur and Ajmer) of Rajasthan. In this study we analyzed different characteristics of soil. From the results we concluded that arid region contains pH range in between 8.05-8.15, moisture content 0.58-0.65 %, TDS range 0.07-0.10, EC 0.11-0.15 and WHC 10.78-11.50 %. In semi arid region the pH range was observed from 7.60-7.75, moisture content was 2-4 %, TDS 0.35-0.50, EC 0.30-0.75 and WHC 25-35 %. We also observed high concentration of organic carbon, bicarbonate and chloride ions in semi-arid region than arid region. The soil of arid region was sandy whereas the soil of semi-arid region had clay. These studies revealed that mineral and water levels in semi-arid region are higher than that in arid region to suggest the soil of semi-arid region has been more suitable for plant growth.

78. Purification and Characterization of Chitosanase from *Paecilomyces Lilacinus*

Sikta Kar, Goutam Mandal and Bishnu Pada Chatterjee

Department of Natural Sciences, School of Management and Science,
West Bengal University of Technology,
Salt lake, Kolkata-700 064

Keywords : *Paecilomyces lilacinus, Chitosanase, Chitin, Chitosan, Chitooligosaccharides, Glucosamine.*

Chitosanase, a useful enzyme, degrades chitosan and produces chitooligosaccharides. The aim of this study was to purify the chitosanase enzyme from soil fungus, *Paecilomyces lilacinus* for applications in various fields. The enzyme was released extracellularly from the *P. lilacinus* fungus in M 9-chitosan agar medium and the crude protein in the culture filtrate was precipitated by 80% ammonium sulfate. The protein was dissolved in 20 mM acetate buffer (pH 5.6), dialyzed against the same buffer and injected into Resource Q anion-exchange column in FPLC system. The active fractions were estimated for chitosanase activity. The specific activity of the purified enzyme was 1050 U/mg with a purification fold of 95. The molecular mass of the purified enzyme was estimated to be 40 kDa by SDS-PAGE and its absolute mass was 39.6 kDa by ESI-MS-Q-TOF analysis. Chitosanase showed highest activity at pH 6 at 50°C. It showed antimicrobial effect, being maximum against *Staphylococcus aureus* followed by *Bacillus subtilis* and *Pseudomonas aeruginosa* and least against *Escherichia coli*. Chitooligosaccharides isolated from chitosan showed maximum antimicrobial effect against *S. aureus* in comparison to *P. aeruginosa.* Chitooligomeric mixtures separated by HPLC produced high amount of *N*-acetyl glucosamine (monomer) at different time intervals with the production of low amount of dimer in 7 h.

79. Isolation and Characterization of Amylase Producing *Bacillus Coagulans* from Coastal Waters of Bay of Bengal, Visakhapatnam

K. Suribabu* and T. Lalitha Govardhan

PG Department Microbiology and Research Centre,
Dr. Lankapalli Bullayya Postgraduate College, Visakhapatnam-530 013 (AP)
***Email** : ksuribabu_sda@yahoo.mail.com

Keywords *: Amylase enzyme, Morphological, Biochemical characterisation.*

As the marine waters are the treasures of enzyme producing organisms, investigations were undertaken to isolate and identify the extracellular amylase producing bacteria capable of digesting starch. Isolation of *Bacillus coagulans* that produce amylase has been reported first time from coastal waters of Bay of Bengal at Gangavaram beach, Visakhapatnam. *Bacillus coagulans* was isolated by culturing on starch-agar media, the halozone of amylase activity was observed by pouring iodine on the overnight incubated culture petriplate colony, morphological and biochemical characteristics of isolate were analysed as per Bergey's manual of Determinative Bacteriology. The characteristics of the isolate would be discussed keeping the view of enzyme applications like use in clothing and dishwasher detergents to dissolve starch from fabrics and dishes and in various industries.

80. Isolation of Lipolytic Fungi and Enhancing their Extracellular Lipase Production Using Waste Oil Cakes

Saidani V.H. Satyanarayana and R. Gyana Prasuna

Department of Microbiology, GIS, GITAM University,
Visakhapatnam-530 045, A.P.

Keywords : *Lipase, Lipolytic fungi, Mucor, Rhizopus, Oil cakes.*

Lipases are the hydrolytic enzymes produced by various microorganisms such as bacteria (*Pseudomonas fluorescence*) and fungi (*Aspergillus niger, Candida, Geotrichum candidum, Humicola lanuginosa, Mucor, Rhizopus, Pencillium*). We used fungal species such as *Rhizopus* and *Mucor* to study the lipase production. As a substrate for growth, instead of costly ingredients, we have tried growth of the above organisms and lipase production using agro wastes like oil cakes (coconut, palmoil and ground oil cakes) as substrates. Highest growth of *Rhizopus* was found on ground nut oil cake, when used at a concentration of 4%, whereas *Mucor* showed best growth on coconut oil cake when used at a concentration of 4%. The organism showed lipase production when grown on these agro wastes. The lipase production of both isolates *Rhizopus* and *Mucor* was highest on palmoil cake at concentration of 4%. On the basis of our results we can suggest use of these agro wastes either alone or with standard media for bio mass production to be used further for lipase production.

81. Biochemical Studies on the Polychaete, *Nereis* sp, in Relation to Maturity Stages

S. Elayaraja*, P. Murugesan, Sunil Kumar Sahu, S. Muthuvelu and P. Mayavu

Centre of Advanced Study in Marine Biology,
Faculty of Marine Sciences,
Annamalai University,
Parangipettai-608 502, Tamil Nadu
***Email** : enzymesarathi@rediffmail.com

Keywords : *Polychaete, Proximate composition, Oocyte diameter, Maturity and size group.*

In the present study, proximate composition (protein, lipid, carbohydrate and moisture content) of *Nereis* sp. was determined in relation to oocyte diameter/size groups by using the standard protocols. After identification, the worms were segregated into four different size groups as group I (4.0 to 9.0 cm), group II (9.1 to 13.0 cm), group III (13.1 to 16.0 cm) and group IV (16.0cm and above). Based on the oocyte diameter they were further classified as immature (4.0 to 9.0 cm), early maturing (9.1 to 13.0 cm), late maturing (13.1 to 16.0 cm) and matured (16.0 cm and above). Proximate composition showed considerable variations between size groups. The amount of protein was found to be maximum in size group I (74.47±1.08%) and minimum in group IV (49.3±0.85%). The lipid content from size group II (14.53±1.21%) to size group III (19.46±0.69%) increased gradually. The amount of carbohydrate was also found to be very less in all the size groups. Their levels were 2.16±0.07%, 2.14±0.02%, 2.12±0.06% and 2.11±0.16% in groups I, II, III and IV respectively. Oocyte diameter in different size groups revealed very interesting results that with the increase in maturity stages/size groups the oocyte diameter also increased. The oocytes were found in the minimum size range of 9.48 µm to a maximum of 325.82 µm. From the above study it is undoubtedly clear that immature (size groups I and II) polychaetes were much better live feed than the mature (size group III and IV) ones with respect to biochemical compositions.

82. Effect of Gallotannin Concentration on the Bioconversion Percentage of Tannic Acid to Gallic acid by *Penicillium Claviforme* PM – 19

Priti Mishra[1]*, S. R. Padmadeo[1], B. K. Mishra[2] and P. K. Singh[3]

[1]Department of Biochemistry, Patna University, Patna – 800 005
[2]Department of Botany, B.S.College, Danapur – 800 012
[3]Department of Botany, R.S.College, Tarapur, Munger
***Email** : prity_mishra@yahoo.co.in

Keywords : *Tanneries, Tannase, Gallic acid, Percent conversion, Bioremediation, Penicillium claviforme.*

Kanpur is one of the largest industrial metropolises of U.P. having population of more than two million. About 480 leather industries are situated in the Jajmau area along the river Ganga at the downstream of the city. The tannery effluents are flown in the river Ganga. These effluents are highly toxic and contain tannin in addition to chloride, sulphide, chromium, etc. In the present work tannin tolerant fungal strain, *Penicillium claviforme PM – 19* has been isolated from tannery soil and evaluated for its tannase activity. The strain has the potentiality to produce the enzyme Tannin Acy1 Hydrolase (E.C.3.1.1.20) in presence of the substrate tannin as carbon source in the medium. Minimal medium containing 0.2% tannic acid as the sole carbon source was used for the isolation of the fungal strain. Tannase is an inducible and regulatory enzyme. With the increase in the concentration of tannic acid in the culture broth the reduction of the enzyme tannase by the *P. claviforme PM – 19* strain is accordingly enhanced. The enzyme tannase (TAH) catalyses the hydrolysis of ester and depside bonds of hydrolysable tannins to liberate gallic acid and glucose. Gallic acid has undisputed commercial importance. It is a phenolic compound (3,4,5, trihydroxy benzoic acid) and is used mainly in pharmaceutical industries for manufacturing trimethoprim. The bioconversion percentage of tannic acid to gallic acid increases with the increase in TA concentration in the medium up to 100 $mgm1^{-1}$. At this concentration the percent conversion is 84. Any further increase in gallotannin concentration resulted in the decline of percent conversion. This may be either due to toxic nature of gallotannin or due to end product repression. High tannin tolerant *Penicillium claviforme PM –19* strains may be developed either through isolation and selection or through genetic manipulation and they may be used in the biotechnological processes like industrial production of gallic acid from the hydrolysable tannins. They can also be used for bioremediation of tannins from tannery effluents and tannin contaminated soils. Immobilization of TAH has added benefit.

83. ABSTRACT WITHDRAWN

84 Fermentative Production and Characterization of Methionine by Bacteria Isolated from Soil

Richa Karmakar*, Deependra Kumar Ban

Department of Food Technology and Biochemical Engineering,

Jadavpur University,

Kolkata-700 032

***Email** : richa1503@gmail.com

Keywords *: Methionine production, Escherichia coli.*

Methionine is an essential amino acid that helps the body process and eliminates fat. It is essential for the formation of healthy collagen used to form skin, nails, and connective tissue, and helps to reduce the level of inflammatory histamines in the body. It cannot be synthesized by human so it has to be consumed from external source. Methionine is produced chemically from acrolein, methyl mercaptan and hydrogen cyanide. But now a days, there is a great interest in replacing the existing chemical production by a biotechnological process. Chemical process uses many hazardous chemical and it also produces substantial wastes. So an effective biotechnological process is desirable to avoid all the above mentioned disadvantages. The purpose of this research was isolating the native strains of microorganism secreting extra cellular methionine in soil of Kolkata (West Bengal, India). From all the tested cultures, *Escherichia coli* was selected for the study. Optimum production of methionine from *Escherichia coli* was 2.195 mg/ml. at pH 7.5 and temperature 35°C. This strain produced methionine with characteristics suitable for application in food and pharmaceutical industries.

85. Cold-active Bacterial Lipase in Detergent : An Energy Saving and Low Pollution Disposal Additive

Deependra Kumar Ban

Department of Food Technology and Biochemical Engineering,

Jadavpur University, Kolkata-700 032

West Bengal

Email : deependra@yahoo.com

Keywords *: Bacillus spharicus, Cold active lipase, Oil, Detergent.*

Oil stains are inevitable in our daily life and its removal by lipase added detergent is attractive alternative. Application of potential cold active lipase in detergent formulation is energy saving and low pollution disposal additive. Bacteria *Bacillus spharcus* isolated from western Himalaya soil secrete extracellular cold active lipase with optimum activity 2.51U/ml, at pH 8.0 and temperature 15°C. This lipase added to different detergent formulations (7 mg/ ml) available in market and washing performance of composition was done at same pH and temperature as mentioned above. Optimum brightness 65% and whiteness 61% produced with kitchen waste oil stained cloths, while with used engine waste oil stained cloths, brightness 56% and whiteness 50% achieved when enzyme (2.51 U/ml) and detergent ratio in 1.2 in 100 ml distilled water was added.

86. Potential Zinc Solubilizing Bacteria Isolated from Mixed Sewage of the Eastern Wetlands of Kolkata

Amrita Mukherjee, Rubina Islem, Sayantan Bose, Sandeep Kaur Matharu, Sitabja Mukherjee, Aditya Bhattacharya and Madhumita Maitra*

Department of Microbiology, St. Xavier's College (Kolkata),
30 Mother Teresa Sarani, Kolkata-700 016 W.B.
***Email** : msirkar_70@hotmail.com

Keywords *: Pseudomonas pseudoalcaligenes, Metal tolerant bacteria, Gram negative cocco-bacilli, Coliform origin, Zinc solubilized, Probiotic effect, Degrade oil spill, Bioremediation.*

The eastern wetlands of Kolkata has been a reservoir of some of the most unique metal tolerant bacteria that have inexplicable bioremediation potential in immobilizing radionuclides and toxic metals, in the degradation of oil, as a biobleaching agent and in chelating zinc in living systems. Four bacterial strains were isolated from mixed sewage and were designated as L4(1), L4(2), L4(3) and L4(4). Gram staining was performed to determine the grain nature and morphology of the strains. All were found to be gram negative cocco-bacilli in character. The IMVIC biochemical tests were performed for the characterization of the stains. Further the L4(4) strain was found to grow on Eosin Methylene Blue agar medium, thereby indicating it to be of coliform origin. Different concentration of zinc solutions like 100 ppm, 500 ppm and 1000 ppm were prepared in which each of the four strains were inoculated and incubated at 37 °C for 4 days. The bacterial growth was measured spectrophotometrically at 610 nm. Atomic absorption spectroscopy analysis was performed to determine the uptake of zinc from the medium. Maximum uptake at 1000 ppm concentration of zinc was observed by stain L4 (4) (954.7 mg/1) and least by strain L4 (1) (896 mg/1). At 500 ppm concentration of zinc, maximum uptake was observed for strain L4 (3) (464.7 mg/ 1) and minimum for strain L4 (4) (442/7 mg/1). The L4 (4) was identified to be *Escharichia coli* and L4 (3) having maximum uptake at 500 ppm concentration, was identified with the help of 16s rDNA analysis and was identified to be *Pseudomonas perudadcoligenes*. This organism acts as a potential source of zinc for fishes thereby acting as a probiotic. We project this organism for future

treatment of marine oil spillage and it can be considered as one stop remedy for different kinds of marine pollution.

87. Comparison of Resistivity of the Two Commercially Available Probiotic Organisms, Saccharomyces Boulardii and Lactobacillus sp Against Commonly used Antibiotics and the Effect of Honey in the Enhancement of the Growth of these Organisms

Suchismita Dey, Parineeta Majumder, Moumita Basu, Monideepa Shah, Sunendita Banerjee and Kasturi Sarkar

Keywords *: Probiotics, Prebiotics, Streptomycin, Tetracycline.*

The aim of the current study was to compare the beneficial effects of the probiotic yeast *Saccharomyces boulardii* with the well known probiotic bacteria, *Lactobacillus sp.* Both of these microgranisms have been found to possess the ability to produce certain biomolecules that have an overall beneficial effect on the human digestive tract and thereby help in enhance good health. These organisms have been termed as probiotics and are being widely studied. For the last few years, probiotic organisms are available in market in liyophilized form where three to four species of probiotic organisms are combined together. Doctors are prescribing probiotic organisms in various diseases mainly in colon associated disorders. Probiotics are also taken with antibiotics to maintain the normal flora of intestine. In this experiment, we tried to find out the resistivity of the above said two probiotics against a number of popular antibiotics viz. amoxicillin, tetracycline and streptomycin. We performed disk diffusion test and tested the growth of the microorganisms in presence of a range of concentrations of these antibiotics by means of turbidimetric assay to examine the effects of the antibiotics on both the microorganisms. It was found from the results that *Saccharomyces boudardii* could resist the growth inhibitory effect of the antibiotics more than lactobacilli in general, which was indicated by the denser growth in the turbidometric assay and their similar zones of inhibition in the disk diffusion test. Among the different antibiotics, streptomycin was able to inhibit the growth of lactobacilli the least while tetracycline was able to do the same for *Saccharomyces boularudi,* which were indicated by the smallest zones of inhibition for the respective antibiotics in comparison to those obtained in case

of the other antibiotics tested. Turbidimetric assay also supports the data. Results indicated that *Saccharomyces boulardii* in general, is less inbibited by the antibiotics than lactobacilli and the effect is most pronounced against tetracycline, the broad spectrum antibiotic, a protein synthesis inhibitor that is frequently used in the treatment of many bacterial infections. Another aspect of our study was to evaluate the effect of prebiotic molecules on the growth of lactobacilli and *Saccharomyces boulardii*. Prebiotics are small oligosaccharides which are fermented by the probiotics and thus provide energy for quick growth of the probiotics. We used honey as the prebiotic since it is full of small oligosaccharides. It was found that in the presence of honey (of density 1.26 g/ml), growth of both the probiotics increased and the increase was 27% and 83% for lactobacilli land *Succharomyces boulardii* respectively. In conclusion we can say that *Saccharomyces boulardii* is a better choice as a probiotic when taken together with antibiotic since the yeast remains almost uninhibited in the presence of antibiotics and that consumption of honey along with probiotics can develop the overall beneficial effect of these organisms, thus improving human health.

v. DRUG DEVELOPMENT

88. Synthesis and Anticancer Activity of New Tetrazole Derivatives from Baylis-Hillman Allyl Amines

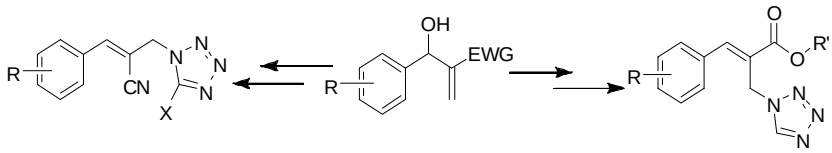
CH. N. S. Sai Pavan Kumar,[1] Dusmant Kumar Parida,[2] Anil Kumar Kota[2] and Vaidya Jayathirtha Rao[1,2]*

[1]Organic Chemistry Division II,
Indian Institute of Chemical Technology, Hyderabad-500 607
[2]National Institute of Pharmaceutical Education and Research,
Balanagar, Hyderabad-500 037
***Email** : jrao@iict.res.in

Keywords *: Anticancer, Tetrazole, Allylamine.*

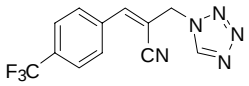
Cancer chemotherapy has entered a new era of molecularly targeted therapeutics, which is highly selective and not associated with the serious toxicities of conventional cytotoxic drugs. Tetrazoles and their derivatives possess diverse range of biological activities such as antiviral, antibacterial, antifungal, antiallergic,

anticonvulsant, anti-inflammatory, anticancer and as angiotensin-II inhibitors. In drug design, tetrazoles are regarded as an isoster for the carboxylate group. In continuation of our interest on the applications of Baylis-Hillman chemistry, herein



R = H, Me, OMe, NO_2, Br, Cl, F, CF_3, naphthyl R' = Me, Et X = H, Me

EWG = COOMe, COOEt, CN



IC_{50} = 1.6 µM against Hep G2 liver carcinoma cell line

we described the efficient synthesis of new tetrazoles from BH allyl amines. These compounds were evaluated for *in vitro* anticancer activity against five cell lines. Most of the compounds exhibited good anticancer activity in ìM concentration out of 16 compounds synthesized and screened.

89. Evaluation of Antidiabetic Effect of *Trigonella Foenum-graecum* and *Momordica Charantia* Seed Extract on Alloxan Induced Diabetic *Rats*

Bhagyashri. D. Patil[1], S. S. Kshirsagar[2]

[1] New Arts, Commerce and Science College, Ahmednagar,
[2]Walchand College of Arts and Science, Solapur, Maharashtra
***Email :** bhagya4research@rediffmail.com

***Keywords** : Antidiabetic effect, Trigonella foenum-graecum, Momordica charantia.*

The medicinal plants are listed in various indigenous systems such as Siddha (600), Ayurveda (700) and Amchi (600), Unani (700), and Allopathy (30 plant species) for ailments. Even today, majority of the medicines are prepared from the plant and animal products, minerals and metals etc. Major pharmaceutical industries depend on the plant products for the preparation of Ayurvedic medicines. Diabetes mellitus (DM) is in the top 5 of the most significant diseases in the developed world, and is gaining significance in other regions. Present number of diabetics worldwide

is 171 million and this is likely to increase to 340 million or more by the year 2030. Diabetes mellitus is a primary disorder of carbohydrate metabolism, which generally involves absolute or relative insulin deficiency and/or insulin resistance and ultimately leads to hyperglycemia. A number of Indian medicinal plants are listed for curing diabetic disease. In the present investigation an attempt has been made to evaluate the anti-diabetic effect of *Trigonella foenum-graecum* and *Momordica charantia* seed extract on alloxan induced diabetic rats.

90. In Vitro Antibacterial Activity of Lyophilized Ethanolic Extract of Fruit Rind from Terminalia Chebula L. on Two Pathogenic Bacteria from Subgingival Plaques

Shanthi. G* and S. Rajarajan

P.G. and Research Department of Microbiology and Biotechnology,
Presidency College (Autonomous), Chennai, (TN)
***Email** : shanthism1116@gmail.com

Keywords *: Antibiotic, Oral anaerobe; Subgingivitis, Terminalia chebula.*

The fruit rind of *Terminalia chebula* has been an ingredient of many of the Siddha and Ayurvedic preparations in tooth powder and traditional drugs. The present study is on the antibacterial activity of ripe fruit rind of *Terminalia chebula. L* for two sub gingival pathogenic bacteria *(Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis).* The MIC and MBC of Seitz filtered lyophilized ethanol extract for both the anaerobic bacteria were estimated at 12.38 µg/ml. This study revealed that results interpret the chebulic myrobalan possess a broad spectrum of antibacterial activity and hence can be considered as potential plant part for further investigation in making it into a drug for the treatment of sub gingivitis.

91. Acceptability and Effectiveness of *Aegle Marmelos* (Bael Fruit) in Management of Constipation

Sana Salman and Neelam Kumari*

Department of Nutrition, Isabella Thoburn College, Lucknow
***Email :** neelamkumari080@gmail.com

Keywords *: Constipation, Aegle marmelos, Bael squash, Bael fruit biscuits.*

Digestive disorders are among the most common problems in health care. Approximately 30-40% of adults have frequent indigestion. Constipation is the most common physiological disorder of the intestine. Ripe bael fruit is regarded as best of all the natural laxatives, as it cleans and tones up the intestine. There are several reasons of constipation but lack of fiber in the diet is one the commonest causes of it. In the present study, *Aegle marmelos* (bael fruit) based product (a) bale fruit squash and (b) bael fruit biscuits were prepared and tried to assess its role in curing constipation in patients. For the present study 50 individuals of age group 10-35 years including both male and female children and adults were selected and were divided in to three groups viz. group I, II and III. Group I was consisted of 20 individual and treated as control and fed on normal diet whereas group II and III were treated as experimental groups and were fed on bael squash with normal diets and bael fruit biscuits with normal diet respectively. The present study showed that Bael squash was more effective than bael fruit biscuits.

92. Typical Anti-Cancer Studies of Andrographolide from *Andrographis Paniculata* Leaves

Sumanjali A, Seema Chaitanya, Ch Srinivasa Rao and B Meena V*

Centre for Biotechnology, Department of Chemical Engineering, AUCE (A), Andhra University, Visakhapatnam

***Email** : meena_sekhar09@yahoo.co.in

Keywords *: Andrographolide, Andrographis paniculata, Anti-cancer.*

Andrographolide is the main diterpenoid lactone contained in the leaves of *Andrographis paniculata.* This bioactive component has multifunctional medicinal properties such as activity against fever, dysentery, diarrhoea, inflammation, and sore throat as well as immune disorder. The objective of this work was to study the effect of polarity and Hildebrand solubility parameter of solvents in the extraction of andrographolide from *A. paniculata* and to develop a mathematical model to quantitatively describe the extraction phenomena. The extraction was carried out by employing various organic solvents and their mixtures with water as solvents using standard soxhlet method. Five grams of ground-dried *A. paniculata* leaves was extracted using 1.00 x 10-4 m3 of solvent for 80 minutes. The standard soxhlet extraction method was conducted using methanol, ethanol, ethyl acetate and water at different extraction times to verify the mathematical model proposed in this work. Methanol was found to be the best solvent for the extraction of andrographolide from

A. paniculata. The extracted compound was subjected on cell cycle and was determined using FACS and western blot analysis of cell cycle proteins. Hollow fibre assay was used to determine if the compounds had the same effect on the cell cycle *in vitro* and *in vivo*. Our results from the *in vitro* and *in vivo* experiments show that the compound andrographolide block the cell cycle at the G0-G1 phase through the induction of the cell cycle inhibitor.

93. Effect of Natural Flavonoid Coumarin on Cytotoxicity, Lipid Peroxidation and Antioxidant Status in Hep 2 Cancer Cell Line

Manimozhi Jalagopal and Mirunalini Sankaran*
Department of Biochemisty and Biotechnology,
Annamalai University, Annamalai Nagar (TN)
***Email** : mirunasankar@gmail.com

Keywords *: Coumarin, Cytotoxicity, Hep 2 cancer cell lines, Antioxidants, Lipid peroxidation.*

This study was designed to evaluate the anticancer effects of coumarin by MTT assay, lipid peroxidation markers and antioxidants status against Hep 2 cancer cells. Human epidermoid larynx carcinoma cancer cells (Hep 2 cells) were treated with different concentrations of coumarin (1000, 500, 250, 125, 62.5, 31.25, 10, 5, 2.5 ìg/ml) and its cytotoxic effect was measured by MTT assay. Our present investigation showed that coumarin decreased cell viability with an IC_{50} value of 62.5 µg/ml. The IC_{50} was determined by dose response curve by plotting the graph of concentration versus % cell viability. Hep 2 cancer cells showed decreased levels of lipid peroxidation with increased activities of enzymatic antioxidants (SOD, CAT and GPx). Among the various doses of coumarin (62.5, 125, 250 µg/ml), 250 µg/ml dose significantly decreased lipid peroxidation and increased antioxidant activities. On the basis of these findings, coumarin may be considered to have potential therapeutic potential in human Hep 2 cancer cells.

94. TRPV1 Mediated Analgesic Activity of *Solanoglycosydane*

Monjur Ahmed Laskar*, M. Dutta Choudhury, Pankaj Chetia and Shuvasish Choudhury
Bioinformatics Centre, Assam University,
Silchar-788 011, Assam

***Email :** monjurbioinfo@gmail.com

Keywords *: Solanoglycosydane, FLEXX, Analgesics, TRPV1.*

Solanoglycosydane, an active compound isolated from the tender fruits of the plant *Solanum torvum* Swartz, was screened to study its molecular mechanism using FLEXX. Solanoglycosydane showed greater binding affinity towards TRPV1, which is a prime target for the development of novel pain reducers, (analgesics).

95. Antibacterial Activity of Liquid Soaps Against Daily Encountered Bacteria

Prajakta Prakash Yarte, Swati Vishwanath Surwase, Ashwini Sanjay Gade, Amruta Chandrakant Athawale, Rupali Ramayya Swami and Namdev Parkar,

Rajarshi Shahu Mahavidyalay, Latur (Maharashtra)

Keywords *: Liquid soaps, Antibacterial activity, Daily encountered bacteria.*

This study aims to check antibacterial activity of liquid soaps viz.. Dettol, Savlon, Lifebuoy and Lux against daily encountered bacteria such as, *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermis* and *Pseudomonas aeruginosa*. Minimum inhibitory concentration and minimum bactericidal concentration activity of selected strains was determined by performing arithmetic dilution and spread plate method. From results, it was found that antibacterial soaps (Dettol and Savlon) were more efficient as compared to beauty soaps (Lux and Lifebuoy). Most resistant bacterium to liquid soaps was *Pseudomonas aeruginosa* and most sensitive was *Staphylacoccus epidermis*. Hence, gram positive bacteria are killed at low concentration than gram negative bacteria.

96. Antitumor studies of *Solanum xanthocarpum,* Schrad and Wendle

K. S. Sudha and Jose Padikkala

Amala Cancer Research Centre, Thrissur-680 555

Keywords *: Cyclophosphamide, Cytotoxicity, Solanum xanthocarpum.*

Antitumor activities of methanolic extract of *Solanum xanthocarpum*, Schrad and Wendle were studied. The antitumor activity of the extract was determined by

using DLA cell line induced solid tumor and EAC cell lines induced ascites tumor model in mice and compared with standard anticancer drug cyclophosphamide. The treatment with methanolic extract of *Solanum xanthocarpum* (50 mg/kg and 100 mg/kg body weight) significantly reduced the volume of solid tumor developed and increased the body weight of ascites tumor model. The life span of treated animals was increased up to 67.78%. The results were more significant in mice treated with 100 mg/kg body weight. This indicated the antitumor properties of *Solanum xanthocarpum* suggesting its potential use as an anticancer agent.

97. Therapeutic Lead Molecules Against *Pseudomonas Aeruginosa*

T. Tamilvanan, A. Ahameethunisa, P. M. Shanooba and Waheeta Hopper

Department of Bioinformatics,
School of Bioengineering, SRM University,
Kattankulathur, Tamil Nadu-603 203

Keywords *: Pseudomonas aeruginosa, Computer-aided drug design, LpxC, Piperine, Agar susceptibility test.*

Pseudomonas aeruginosa, an opportunistic pathogen, resistant to most antibiotics, causes life-threatening infection in individuals with compromised immune systems. To identify therapeutic lead molecules against *Pseudomonas aeruginosa*, UDP-(3-*O*-(*R*-3-hydroxymyristoyl))-*N*-acetylglucosamine deacetylase (LpxC), essential for pathogenesis of the organism was selected as the target. A database of the compounds was screened against the target using Arguslab. The best ligands were docked using Autodock. Piperine, one of the natural compounds screened, showed good docking score. *In vitro* inhibition of the growth of *P. aeruginosa* ATCC 27853 by piperine was carried out. Minimum inhibitory concentration of piperine against *P. aeruginosa* was determined as 64 µg/ml. The *in silico* and *in vitro* screening suggest that piperine could be used as a therapeutic compound for *Pseudomonas* infections.

98. Antimicrobial peptides: An overview

Mohd Anis*, Lav Kumar, Neelgagan Singh, Parvez Ahmad, Shubra Guha and U. S. Gupta

Department of Zoology,
Dr. H. S. Gour University, Sagar, M.P.
*__Email__ : anisurehman1982@gmail.com

Keywords *: Antimicrobial peptides, Defensin, Cecropin, Thanatin, Drosomycin.*

The increase of microbial resistance has led to continuing efforts for finding newer and more effective drugs. Antimicrobial peptides are generally found in animals, plants and microorganisms and are of great interest in medicine, pharmacology and food industry. These peptides are capable of inhibiting pathogenic microorganisms while causing no harm to host cells. The defensins are peptides found in granules in the polymorphonuclear neutrophils and are responsible for defense against organisms. Several animal defensins like dermaseptin, antileukoprotease, protegrin, and others have efficacy tested and have been shown to be effective against bacteria, fungi and protists. There are also specific defensins from invertebrates e.g., drosomycin in drosophila, heliomycin and thionins in plants. The aim of present work was to compile a comprehensive bibliographic review of the diverse potentially antimicrobial peptides in an effort to systematize the current knowledge on these substances as a contribution for further researches.

99. Wheatgrass Juice Therapy : Benefits and Healing Properties

P. S. Solanki, P. V. Khakke, S. M. Karhade, B. D. Rachetti, A. B. Jadhav and S. N. Harke

MGM's Institute of Bioscience and Technology, Aurangabad.

Keywords : *Wheatgrass juice, Staphylococcus aureus, Triticum aestivum, AMES test.*

Medicinal plants and traditional medicines have been used by tribal communities and ancient civilizations for thousands of years. Recently modern medicine system has looked back at medicinal plants and herbs for healing. Wheatgrass is a food prepared from the cotyledons of the common wheat plant (*Triticum aestivum*). It provides chlorophyll, amino-acids, vitamins, minerals and enzymes. We can grow and juice wheatgrass at our homes as it is useful for providing supplemental nutrition for having unique curative properties. Wheatgrass grown indoor in trays for 10 days, harvested and dehydrated at low temperature had healing property and its daily intake also increased the haemoglobin content in the blood. We checked an antimicrobial activity of extract from wheatgrass by using *Staphylococcus aureus*. We determined chemical composition such as protein, iron and reducing power of wheat grass. We had also checked the antimutagenic activity of wheatgrass by

AMES test.

100. Amelioration of Cisplatin Induced Nephrotoxicity by PTY - A Herbal Preparation

Santosh Nagwani and Yamini B. Tripathi
Department of Medicinal Chemistry, Institute of Medical Sciences,
Banaras Hindu University, Varanasi-221 005

Keywords : *Ayurveda, Anti-oxidant, Food supplements, Herbal, Nephroprotective.*

Cisplatin, a drug for cancer treatment, is associated with severe nephrotoxicity that limits its clinical use. Cisplatin involves enhanced oxidative stress, mitochondrial dysfunction and death of tubular cells. Nephroprotective role of PTY, prepared from methanolic extract of tubers of *Pueraria tuberose* D.C., has been studied. PTY was orally given to rats in different doses for seven consecutive days, along with cisplatin (8 mg/kg B.W., i.p.) on 4th day. PTY significantly prevented the rise in serum creatinine, blood urea nitrogen. It prevented the decline in glutathione content, activities of SOD and catalase. It also prevented DNA damage, tubular swelling, cellular necrosis and protein cast deposition as compared to experimental control group in kidney. These changes were restored to near normal levels by PTY in dose of 40 mg/100 g B.W. Thus, it is proposed that the PTY possesses dose-dependent protective effect against cisplatin induced kidney damages, primarily through its free radical scavenging property.

101. Screening and Evaluation of Antioxidant Properties of Some Legume Seeds for their Antioxidant Enzymes viz., Catalase, Superoxide Dismutase and Glutathione Peroxidase

Nirankush V*. Khubalkar, Deepti Mahule and Ashwini Banginwar
Ramabai Barlingay School of Biotechnology,
L.A.D. College for Women, Nagpur
***Email** : nirankushk@gmail.com

Keywords : *Reactive oxygen species (ROS), Free radicals, pH, Temperature, Oxidative*

Stress, Aging, Antioxidant enzymes.

Organisms produce reactive oxygen species (ROS) also known as free radicals like OH^-, O^-_2, NO etc., which if left unchecked, would seriously affect an organism's viability. The higher amounts of ROS lead to oxidative stress that plays a role in the aging process and in number of human disease states. Antioxidants can be synthesized *in vivo* or taken in the form of diet. The present work was aimed at the evaluation of some commonly consumed legumes i.e., *Arachis hypogea, Vigna radiata, Vigna aconitifolia* for antioxidant properties of the enzymes which catalyze free radical quenching reactions. Samples were assayed for the enzyme activity at different pH and temperature.

102. Antimicrobial Activity of *Phoenix Dactylifera* Seeds

Sanjukta Chatterji, Surekha Shukla, Deepak K. Yadav, Vijay Bahadur, Prashant K. Rai, Shikha Mehta and Geeta Watal*

Alternative Therapeutics Unit, Drug Development Division, Medicinal Research Lab, Department of Chemistry, University of Allahabad, Allahabad–211 002

***Email** : geetawatal@rediffmail.com

Keywords *: P. dactylifera, Antibacterial, E. coli, Gram-negative.*

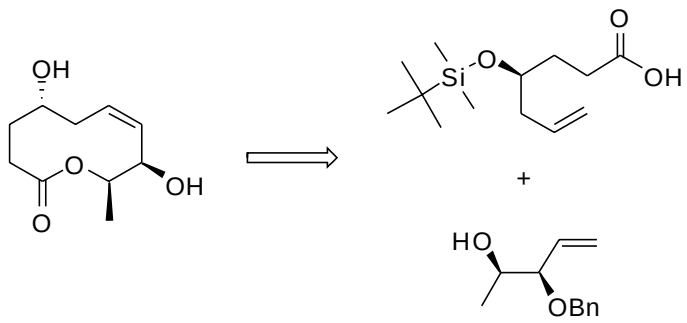
The present study was performed to evaluate the antibacterial activity of *Phoenix dactylifera* L. seeds against two gram-positive bacterial strains viz. *Staphylococcus aureus* and *Enterococcus faecalis* and three gram-negative bacterial strains viz. *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The aqueous extract of *P. dactylifera* was shown to possess the inhibiting activity (MIC ~ 0.15 mg/L) at very low concentration against *E. coli* in comparison to other strains. The next higher concentration of extract showed inhibitory activity (MIC ~ 0.3 mg/L) against *P. aeruginosa* only. Finally, the highest concentration with inhibiting activity (MIC ~ 0.6 mg/L) was active against the rest of the strains viz. *S. aureus, K. pneumoniae* and *E. faecalis*. Among all the five bacterial strains tested, *E. coli* was found to be the most sensitive to the aqueous extract of *Phoenix dactylifera* seeds and the order of sensitivity for all the strains was as follows: *E. coli* > *P. aeruginosa* > *S. aureus* / *K. pneumoniae* / *E. faecalis.*

vi. TECHNIQUES

103. Stereoselective Synthesis of Stagonolide-G

CH. N. S. Sai Pavan Kumar, S. Naveen Kumar, M. Ravinder and V. Jayathirtha Rao*

Organic Chemistry Division II, Indian Institute of Chemical Technology,



Hyderabad-500 607

***Email :** jrao@iict.res.in

Keywords *: Stereoselective, Stagonolide-G.*

Macrolides, particularly lactones with medium-sized rings (8–10 membered), have continued to attract the attention of both biologists and chemists in recent years, due to the interesting biological properties and scarce availability. Recently, three new nonenolides and Stagonolide G-I have been isolated from the solid culture of *Stagonospora cirsii*, a fungal pathogen isolated from *Cirsium arvense*. In accordance of our continuing synthetic efforts towards the molecules with intriguing characteristics and usefulness, we embarked on the stereoselective synthesis of Stagonolide-G in a convergent approach. We accomplished the synthesis in an efficient and inexpensive manner using D-mannitol as chiral pool for the construction of both the fragments.

104. Toxicity Studies to Assess the Safety of Sulphonyl Urea Derivative Loaded Polymeric Nanoparticles

U. M. Dhana Lekshmi[1], Narra Kishore[1], G. Pramod Reddy[2] and P. Neelakantareddy[1]

[1]Bio Organic Chemistry Laboratory, Central Leather Research Institute,
(CSIR) Adyar, Chennai–600 020.
[2]Siddha Central Research Institute,
Arumbakkam, Chennai–600 106

Keywords : *Nanoparticle, Toxicity.*

To our knowledge, no such polymeric nanoparticle formulation toxicity study has been reported for oral use. The oral route of drug administration is generally preferred because of its versatility, safety and relative patient comfort. Hence, there is an outstanding need of research for polymeric nanoparticles to find whether they are stable for prolonged shelf life, and yet have no toxicity when administered orally. To assess the toxicities of sulphonyl urea derivative loaded polymeric nanoparticle systematically and to observe the toxic effects of nanoparticles on the functions of various tissues and organs in rats, the rats were randomly divided into 5 different groups. Variation in protein, carbohydrate and fat metabolic profiles of the rat exposed to nanoparticle were studied and the results did not reveal any toxic signs. The activities of SGOT/ SGPT and ACP/ ALP levels in serum, brain, kidney, liver and spleen were also not significantly different when compared to controls. Histopathological effects of these nanoparticles were studied and the results clearly evidenced its safety.

105. A Novel and Rapid Enzyme-Linked Immunosorbent Assay Procedure by Applying Mild Pressure

Dileep Kumar Kannoujia and Pradip Nahar
Institute of Genomics and Integrative Biology (CSIR),
Mall Road, Delhi–110 007

Keywords : *Pressure, Enzyme-linked immunosorbent assay, Activated surface.*

The enzyme-linked immunosorbent assay (ELISA) is a very useful technique for clinical diagnostics, detection of biothreat agents, high-throughput screening of drugs and drug targets and biomedical research. Here, we demonstrate an ELISA procedure for the detection of an antigen or antibody, in which ELISA steps are carried out by applying pressure instead of conventional thermal incubation. Pressure-mediated ELISA (PELISA), carried out in 1 h showed more than a 2-fold increase in ELISA value than the control experiment carried out at the same time and temperature without applying pressure. Estimation of total IgE by the 1-h

PELISA method gives similar ELISA value to that obtained by conventional ELISA carried out in about 18 h. Since PELISA is sensitive, specific and reproducible, it could be an excellent alternative to conventional ELISA procedures.

106. Development of an Assay for Testing Quality of a Live Attenuated Vaccine : Standardization of Polymerase Chain Reaction for the Detection of Porcine Circovirus Contamination

Deepak Kumar and Nagendra R. Hegde*
Bharat Biotech Foundation, Hyderabad.
***Email** : hegden@bharatbiotechfoundation.org

***Keywords** : Porcine circovirus, PCR, Vaccine.*

The current vaccine safety and quality control measures are very stringent. Presence of contaminating virus or DNA, and any extraneous material, in live vaccine is either a quality problem for the manufacturer. A recent publication reported the presence of adventitious virus DNA sequences in 5 out of 8 live vaccines screened. We conducted preliminary research work to ensure that the rotavirus vaccine produced by Bharat Biotech International Ltd. (BBIL) was free from porcine circoviruses. Cell bank, vaccine virus banks, additives used in large-scale production, vaccine bulk, and formulated material were tested and found to be negative for the presence of porcine circoviruses by PCR.

107. Decolorization of Azo Dye by Free and Immobilized Enzymes of *Pleurotus Citrinopileatus*

M.P. Singh*, Sanjay Kumar Vishwakarma and Abhishek Kumar Srivastava
Department of Biotechnology, VBS Purvanchal University,
Jaunpur-222 001, U.P.
***Email** : mpsingh.16@gmail.com

***Keywords** : Azo dye, White rot fungi, Immobilized enzymes.*

Azo dye (trypan blue, acid black) removal was studied by extracellular enzymes of white rot fungi *Pleurotus citrinopileatus*. Laccases, lignin peroxidases, manganese dependent peroxidases, polyphenol oxidases and other enzymes are extracellular enzymes produced by white rot fungi. These enzymes were extracted from 12 day old culture of *Pleurotus citrinopileatus* and their performances were

evaluated in both free and immobilized form. In aqueous phase, these extracellular enzymes showed decolorizing ability. Studies were further carried out to understand the process parameters such as pH and dye concentrations during enzyme- mediated dye degradation process. Experimental data revealed that dye concentration and pH play a significant role on the overall enzyme- mediated reaction. Calcium alginate immobilized extracellular enzymes of *P. citrinopileatus* showed effective performance compared to free enzymes.

108. Simple High-Cell Density Fed-Batch Technique for High-Level Recombinant Protein Production with *Pichia Pastoris* : Application to Intracellular Production of Hepatitis B Surface Antigen

Anjugam. R. and Nandini Ravi

St. Joseph's College of Engineering, Chennai

Keywords *: Fed batch. Recombinant protein, Pichia pastoris, Hepatitis B.*

Hepatitis B is a serious global public health concern. Though a safe and efficacious recombinant vaccine is available, its use in several resource-poor countries is limited by cost. We have investigated the production of Hepatitis B virus surface antigen (HBsAg) using the yeast *Pichia pastoris* GS115 by inserting the *HBsAg* gene into the alcohol oxidase 1 locus. Large-scale production was optimized by developing a simple fed-batch process leading to enhanced product titers. Cells were first grown rapidly to high-cell density in a batch process using a simple defined medium with low salt and high glycerol concentrations. Induction of recombinant product synthesis was carried out using rather drastic conditions, namely through the addition of methanol to a final concentration of 6 g L^{-1}. This methanol concentration was kept constant for the remainder of the cultivation through continuous methanol feeding based on the *on-line* signal of a flame ionization detector employed as methanol analyzer in the off-gas stream. Using this robust feeding protocol, maximum concentration of ~7 g HBsAg per liter culture broth were obtained. The amount of soluble HBsAg, competent for assembly into characteristic virus-like particles (VLPs), an attribute critical to its immunogenicity and efficacy as a hepatitis B vaccine, reached 2.3 g per liter of culture broth. In comparison to the highest yields reported so far, our simple cultivation process resulted in a ~7 fold enhancement in total HBsAg production with more than 30% of soluble protein competent for assembly into VLPs. This work opens up the possibility of significantly reducing the cost of vaccine production with implications for expanding hepatitis B vaccination

in resource-poor countries.

109. Dihydrogen Reduction of Organic Substrates by Using zsm-5 Anchored pd (ii) Complexes as Catalyst

Priyanka Sagar* and Rohit Kumar

Department of Chemistry,
SSJ, Campus, Kumaun University, Almora-263 601, Uttrakhand.
***Email** : sagarpriyanka_2006@yahoo.co.in

Keywords : *Pd (II) complexes, ZSM-5, Nitrocompounds, Alkene, Alkyne, Catalytic hydrogenation.*

The HZSM-5 was used to immobilize the homogeneous Pd (II) complexes of S-triazine derivatives and anthranilic acid. They were found very efficient towards the catalytic hydrogenation of alkenes, alkynes, nitrocompounds, benzaldehyde and benzil at 25^0C and 1.38 $x10^3$ KNm^{-2} pressure of molecular hydrogen. At this temperature and pressure of molecular hydrogen, ZSM-5 anchored Pd (II) complexes could be used repeatedly. DMF-toluene (1 : 2) mixed solvent medium was found suitable for these complexes. Diminished catalytic activity was not observed even after 15-20 repeated catalytic runs. This indicated that zero (almost negligible) leaching out phenomenon of the metal or metal complexes. Immobilized Pd (II) complexes were found more active, stable, thermo potent, eco-friendly and industrially applicable as compared to its homogeneous counterpart.

110. *De Novo* Sequencing of β-Lactoglobulin Tryptic Peptides Using CID/ ETD Based MS/MS Fragmentation

A. C. Rohit and H. S. Aparna

Department of Studies in Biotechnology, Manasagangotri,
University of Mysore, Mysore-570 006, Karnataka.

Keywords : *Lipocalin, Bubalus bubalis, Colostrum,* β*-lactoglobulin, Tandem mass spectrometry.*

β-lactoglobulin (β-LG), a core member of lipocalin family is abundant in whey

of ruminants and has attracted considerable attention as a rich source of bioactive peptides involved in diverse biological functions. β-lactoglobulin, an important whey protein of milk has drawn considerable attention as a rich source of bioactive peptides involved in diverse biological functions. The β-lactoglobulin isolated from buffalo colostrum (BLG-col) was used for *de novo* sequencing using tandem mass spectrometry. The tryptic peptides separated by LC-MS/MS workflow were computed integrating CID and ETD supported with ion trap to uncover sequence diversity in â-lactoglobulin of early and mature milk of buffalo and bovine. The MS/MS fragmentation of selected peptides P1 to P8 was acquired. A stretch of eight amino acids detected by CID for P1 corresponds to N-terminal sequence of BLG-col. Consequently, 14 amino acid sequence derived from both CID and ETD for P6 represents C-terminus of the protein, which is in compliance to buffalo milk but has variation at terminal amino acid to bovine β-lactoglobulin. The amino acid sequence derived for P3, P4 and P7 were denoted as hypocholesterolemic peptides (NCBI-PSE 141). At position 74 in P4, the presence of methionine instead of glutamine explicit discrete differences from that of buffalo and bovine milk β-lactoglobulin. The sequence of P2, P5, and P8 showed homology to both bovine and buffalo milk β-lactoglobulin. Further, *de novo* sequence of BLG-col was compared and analyzed with buffalo and bovine milk β-lactoglobulin employing CLUSTAL, which revealed inter and intra-species sequence diversity. In addition, molecular model for BLG-col was constructed using automated template-based protein modeling.

111. Masked Sulfhydryl Groups at the Active Site of Goat Brain Cathepsin L

Ramesh C Kamboj[1], Ashwani Mittal[2] and Hari Singh[2*]

[1]Department of Chemistry Kurukshetra University,
Kurukshetra, Haryana–136 119
[2]Department of Biochemistry, Kurukshetra University,
Kurukshetra, Haryana–136 119

Keywords : *Goat brain cathepsin L, Cathepsin B, Organomercurial affinity chromatography.*

Cathepsin L (EC 3.4.22.15), an intracellular lysosomal thiol protease has high

degradative activity on cellular and matrix proteins and thus assumes significance vis-à-vis other intracellular proteases in overall lysosomal protein catabolism. Cathepsin L is also known to be involved in several diseased conditions. During purification of cathepsin L when partially purified (25,000 Da mol wt) fraction from Sepahdex G-100 gel filtration column containing both cathepsin B and cathepsin L was applied on an organomercurial affinity column designed by coupling p-aminophenylmercuric acetate to CNBr-activated Sepharose 4B, it was observed that only cathepsin B (EC 3.4.22.1) was bound to the affinity column. Cathepsin L was eluted unretarded. However, when the unbound fraction was incubated with 0.2 M $HgCl_2$ for 24 h at 4°C, a mercaptide derivative of the enzyme was obtained. When this form was reduced with 10 mM cysteine at 37°C for 10 min followed by subsequent removal of cysteine over Sephadex G-50, and when reapplied to the affinity column, surprisingly it was observed that cathepsin L was bound to the column. With the action of $HgCl_2$, the masked thiol groups on the enzyme active site were exposed. This latent nature of thiol groups in cathepsin L was also confirmed by the observation that the activity of purified cathepsin L in presence of 4M urea increased by 3-fold in contrast to cathepsin B activity which was completely abolished.

112. Mitochondrial DNA Detection at the D-310 Region by COLD-PCR in Cancerous Tissue

Jagadish Hansa[1], Rosy Mondal[1], Ravi Kannan[1] and Sankar Kumar Ghosh[2]*

[1]Biotechnology Department, Assam University, Silchar-788 011,
[2]Cachar Cancer Hospital & Research Centre, Meherpur, Assam
***Email** : drsankarghosh@gmail.com

Keywords : *mtDNA, D-loop, D-310, COLD-PCR, Somatic mutation, Head & neck cancer.*

Mitochondrial DNA mutations occur in a wide variety of degenerative diseases, sudden infant death, ageing and cancer. Many of the somatic mtDNA mutations in human cancers are located in the displacement loop (D-loop) and particularly at polycytidine stretch (C-tract) referred as D310. Most of the somatic

alterations found in tumors showed deletion/insertions of I- or 2-bp generating D310 variants identical to constitutive polymorphisms. To ensure highest sensitivity in mutation detection and overcome from the polymorphism, we adapted COLD-PCR method followed by DNA sequencing. After analysis of all the sequences for C-tract alteration, we got some severe changes in the region by means of deletions and insertions. Though the region has polymorphism about 6-9C in first stretches of sequence in D-310 but the sequence of C-tract has found 30% changes in all cases of head and neck cancerous patient.

113. Molecular Characterization of Honeybee Species Through RAPD-PCR

Bharat Neekhra*, Divya Pandey, Vivek Malik and Subodh Kumar Jain

Molecular Biology Laboratory, Department of Biotechnology,
Dr. Hari Singh Gour Vishvavidyalaya, Sagar-470 003 MP
***Email** : neekharji@gmail.com

Keywords *: RAPD, PCR, Primers, Honey bee, Biomarker characterization.*

The random amplified polymorphic DNA (RAPD) analysis of the genomic DNA for two species of honeybee *Apis dorsata* and *Apis florea* (Order: Hymenoptera) have been subjected to PCR using eight decamer oligonucleotide primers P1 (GATGACCGCC), P2 GGCACCATIC), P3 (GGCACGTAAC), P4 (GGCATGACCT), P5 (GGGTAACGCC), P6 (GGTGCGCCTT), P7 (GTCAGAGTCG) and P10 (GTGCCCGATG). All of them produced discrete bands of various size revealing genetic similarity as well as differentiation between two species of honeybee considered under study. With primer PI (GATGACCGCC), a fragment of 900 base pairs was observed in both the species while, with primer P4 (GGCATGACCT), band having 450 bp was observed. These bands can be considered as diagnostic bands for the genus *Apis*. All the other primers also produced speices specific bands. RAPD markers thus can be successfully applied in honeybees for the study of biomarker characterization and evolutionary relationships. The data acquired out of this study can be used for identification and classification of other social insects.

114. Measurement and Analysis of Heart Rate Using Fuzzy Logic

N. Thanigasalam Kailash Krishnan and C. Vaithilingam*

Department of EEE, SRM University-603 203

Keywords : *Wrist mounted heart rate measurement, Auto-watch, Fuzzy logic, Piezoelectric sensors.*

The constant monitoring and analysis of heart rate of an individual can help in maintaining a record of his physical condition over a period of time. This data helps the individual to predict impending ailment, also, to regulate his method of training to improve fitness. The device being proposed aims to serve the above mentioned purpose.

A wrist mounted heart rate measuring device capable of continuous measurement of pulse (using piezoelectric sensor) and storage of the same for further analysis (using Fuzzy logic) designed. This entire setup is integrated on to a digital watch circuit modified so as to make it self-charging.

115. Study of Alum-Albumin Interaction in a High Temperature System

S. P. Subhathirai, S. P. Sangamithirai, Nagendra Kumar and D. John Thiruvadigal

Department of Nanotechnology, SRM University,
SRM Nagar, Kattankulathur-603 203, Kancheepuram Dist, Tamil Nadu.

Keywords : *Alum, Egg white, Siddha medicine, Recrystallization.*

Potassium aluminum sulphate with the formula $KAl(SO_4)_2.12H_2O$, commonly known as alum possess enormous medicinal properties when combined with certain substances have largely remained unexplored. The present study analyzes the consequences that happen when alum is treated with the egg white and the resultant end product is used as a traditional siddha medicine. To begin with the alum was purified by recrystallization process in a super saturated solution. Subsequently the purified crystals were then grinded with egg white. The resulting substance was heated at a very high temperature for several hours. Finally the end product was analyzed for its size range, crystallization pattern and chemical composition using various analytical methods.

116. PCR Based Detection of Fumonisin Producing Strains of *Fusarium*

Verticillioides and Gene Related to Toxin Production

V. Karthikeyan[1*], P. P. Karthikeyan[2], S. Patharjan[3], P. Saravanakumar[4], M. Siva[1], P. S. Aruna Bhavani[1], D. Spadaro[5], M. Lodovica Gullino[5], A. Garibaldi[5] and P. Palani[1]

[1]CAS in Botany University of Madras, Guindy Campus, Chennai, TN,
[2]Department of Bioinformatics, SRM University, Kattankulathur, TN,
[3]PRIST University, Centre for research and Development, Thanjavur, TN,
[4]Deartment of Environmental and Herbal Science,
Tamil University, Thanjavur, TN,
[5]Centre of Competence for the Innovation in the Agro-environmental Sector,
Agroinnova, Via Leonardo Da Vinci 44, 10095 Grugliasco - (Torino) – Italy.
[*]**Email** : karthickeyanv@yahoo.com

Keywords *: Fumonisin, F. Verticillioides, Polyketide synthase, FUM 5.*

Contamination of rice and rice-based products with fumonisins poses a threat to agriculture and food safety worldwide. Fumonisins are mycotoxins produced by both rice and maize pathogen *Gibberella fujikuroi* mating population A; anamorph *Fusarium verticillioides* (Sacc.) and several related species. In this work eighty two strains of *Fusarium* species collected from infected rice samples were subjected to PCR assay to discriminate fumonisin producing and nonproducing strains with Inter Generic Spacer region of rDNA coding units specific primer named as VERTF-1/ VERTF-2 were used. Twenty one isolates of *F. verticillioides* scored positive for VERTF-1/ VERTF-2 pair of primers proves to be potential fumonisin production and 25 isolates were scored negative. Specific primers for polyketide synthase gene FUM1-(previously FUM5) were used to amplify DNA fragments of all 82 strains. This resulted in positive signals in 21 strains of *F. verticillioides.* Both the primers confirmed the identification of fumonisin producing *F. verticillioides* strains and mycotoxin responsible gene associated with rice tissues.

117. Multi Dimensional Strategy to Study Proteome Dynamics in Developing Cotton Fibers

Saravanan Kumar, Deepak Patil and Vanga Siva Reddy
International Centre for Genetic Engineering and Biotechnology,
Aruna Asaf Ali Marg, New Delhi-110 067.

Keywords : *Cotton fiber, Gossypium hirsutum, 2D-polyacrylamide gel electrophoresis, ion exchange and hydrophobic chromatography, isobaric tags, proteome.*

Cotton is the most predominant source of natural textile fiber and also considered as a oil crop. Cotton fibers are seed trichomes that develop from the epidermal layer of the ovule. The development of fiber is a complex and highly regulated biological process. Various approaches have been employed to understand this process at the genome level which may be of great importance to breeders and biologists. Proteomics is one of the promising approaches used to understand this complex biological process. Conventional approaches based on 2D-PAGE followed by mass spectrometry has been used to understand the cotton fiber development, but this approach suffers from several drawbacks such as suppression of low abundant proteins, low molecular mass proteins, gel to gel variation etc. In order to identify the complexity of cotton fiber proteome due to the extensiveness and dynamic nature of the proteins involved and also to address the disadvantages of conventional approaches, we have developed a multi dimensional approach involving ion exchange, hydrophobic chromatography and isobaric tags followed by mass spectrometry. We present our data on the extensiveness of fiber proteome that has not been reported so far and differential expression of various proteins during the fiber cell initiation, elongation and maturation phases.

118. New Findings of Four Selecractinian Corals from Great Nicobar Island

Tamal Mandal[1], C. Raghunathan[1] and Ramakrishna[2]

[1]Zoological Survey of India, Andaman and Nicobar Regional Centre,
National Coral Leaf Research Institute, Haddo, Port Blair-744 102
[2]Zoological Survey of India,
Promi Vigyan Bhawan, M. Block, New Alipore, Kolkata-700 053
***Email** : t_genetics@yahoo.com

Keywords : *Coroporidae, Fungidae, Faridae, Dendrophyindae, Great Nicobar, Biosphere Reserve.*

Seleractinian corals are the most promising part of marine ecosystem of

Andaman and Nicobar Islands. *Aeropara desodviji* (Walface 1994) *Fungie talvanmesis* (Hoeksema& Dai, 1991) and *Playguna ryakyuensis* (Yabe and Sagiyama, 1936) and *Tulasenea famikuard* Wells, 1982) are the four species represented the families such as *Acroporidae*, *Fungidae*. Favildae and Dendrophyllidae recorded from Great Nicobar Island as new findings. The occurrence of these four species was observed in various sites of Great Nicobar Biosphere such as Kondal island, Lakshman beach and Jogindenagar beach. This paper deals with the morphological characters of these species for their proper identification.

119. Analysis of the Sodium and Potassium Content of Three Fresh Water Gastroped Molluscan Muscles of India

Madan Mohan Palit
N. D. College, Howrah-711 101

Keywords *: Flame Photometer, P globesa, Fila globeaa.*

The dried tissue was pulverised in a mortar. The sodium and potassium contents were measured with flame photometer (ESL-Evans Electro Solaniur Ltd, England), following the method of Vermadkia and Woodbury (1962). The sodium and potassium contents were measured from the photometric readings and calculated from the standard curves. The result shows remarkable variation in the sodium contents of the three fresh water species. The sodium content of the pedalmiscles of *P. globosa* and *Y. bengalensis* was less when compared with that of lyeneac. Further in *V. bengalensis* the sodium content was lowest among the three fresh water species of gastrapod mollusc. Regarding the potassium content *Y. bengalensis* showed the lowest amount. *P. glabosa* showed high amount of potassium like that of *L. predosuccimea* and in both the cases the potassium content was similar. Sodium and potassium promote the contractual processes of the peal muscles of the three fresh water gastropod molluscs. Further work is in progress regarding activity of the sodium and potassium ions in the contractile machanism of the pedal muscles of these three fresh water gastropod molluscs.

120. Estimation of Various Contents in Seaweeds by Modern Method

Raman Gunjan, R. K. Lal, M. P. Sinha and A. K. Ghosh*
Department of Bio-chemistry, Patna University, Patna, Bihar
***Email** : ashisg@gmail.com

Keywords *: Seaweeds, Protein.*

Seaweeds, which have traditionally been used by the western food industry for their polysaccharide extractives -alginate, carrageenan and agar- also contain compounds with potential nutritional benefits. Seaweeds have recently been approved in France for human consumption (as vegetables and condiments), thus opening new opportunities for the food industry. These seaweed ingredients must meet industrial and technical specifications and consumer safety regulations (fluresnce L, 1999). The aim of our project was to estimate certain basic nutritional parameters i.e. protein and carbohydrate content of various collected species of seaweeds. Protein content was estimated by the Bradford method where as carbohydrate by Anthrone Method. The protein and carbohydrate content of seaweeds from different areas were estimated and compared.

121. *In Vitro* Biocompatibility Studies of Quantum Dot Tagged Polymer Beads

Ann Jasmine Jose[1*], Mark Bradley[2]

[1]Department of Chemistry, KCG College of Technology,
Karapakkam, Chennai, 600 096, India
[2]School of Chemistry, University of Edinburg, UK
***Email** : annjasmine@gmail.com

Keywords *: Nanomaterials, Quantum dots, Polymer, Biocompatibility, Cells.*

Quantum Dots are thought to have potential as novel probes for both diagnostics and therapeutic purposes. Quantum dots were incorporated into preformed nanoporous, monodisperse biocompatible polymeric beads by swelling and collapsing the bead matrix. Confocal laser scanning microscopy 3D images were obtained proving the uniform distribution of the Qdots in the bead matrix. The QD-tagged polymer beads were incubated overnight with cultured cells at 37°C. Optical sections from confocal microscopy confirmed that the beads were engulfed by the cell rather than adsorbed on the surface. They were found to be non-toxic and did not disrupt the normal cell physiology even after 7 days of incubation.

vii. BIOINFORMATICS

122. Regulation of Futile Cycles by Transcription Factors : Insights into Glycolysis Pathway

Munmun Dutta, Shalini Das Gupta, Kavya Kannan and N. Rathankar

Department. of Bioinformatics, School of Bioengineering,
SRM University, Kanchipuram district, TN-603 203

Keywords : *Futile cycles, Transcription factors, Glycolysis pathway.*

A futile or a substrate cycle occurs when two metabolic pathways run simultaneously in opposite directions and have no overall effect other than to dissipate heat energy. One such cycle in the glycolytic metabolic pathway, is a reversible process, in which the forward reaction catalyzed by phosphofructokinase (PFK1) phosphorylates fructose-6-phosphate to fructose-1,6-biphosphate, while hydrolysis of fructose-1,6-biphosphate to fructose-6-phosphate is catalyzed by Fructose-1,6-bisphosphatase (FBP1) in the reverse direction. In this paper, we attempt to determine the regulatory factors that trigger and control this futile cycle in glycolysis pathway and aim to find the aberrant levels of gene expression of these glycolytic enzymes in cancerous cells. Towards this initiative, promoter sequences that regulate the expression of pfk1 and fbp1 genes from human genome were predicted using Gene2 promoter module in the Genomatix software suite. Seven experimentally verified promoter regions, (four from PFK1 and three from FBP1) were identified. A systems approach to this data is being modeled to understand the Transcription factor regulation in these enzymes using these seven promoter regions, which will be presented in the conference.

123. Identification of Polypharmacological Targets of P38 Map Kinase Inhibitor

G. Raj Kiran, Vijay Rathod, Nitu Bansal and Abhay T. Sangamwar*

National Institute of Pharmaceutical Education and Research (NIPER),
Sector-67,
S. A. S. Nagar, Punjab-160 062
***Email :** abhays@niper.ac.in

Keywords : *Polypharmacology, Off-targets, Chemogenomics, Systems biology.*

The identification of protein drug interaction is crucial for correlating complex

modes of drug actions for clinical indications. We have used a novel computational strategy for identification of protein ligand binding profile on genome wide scale and apply it in elucidating the molecular mechanisms associated with the adverse drug effects of p38 map kinase inhibitors. P38 map kinase inhibitors are mainly used in inflammatory disorders. We have computed the off targets of p38 by taking SCIO-469, a map kinase inhibitor which is in phase-II of clinical trials and have identified a panel of off targets from the human structural genome and map those targets to biological pathways via the literature. These findings may be helpful in suggesting what adverse drug effects might be caused by an inhibitor and strategies to minimize them by fine-tuning multiple off-target interactions using single or multiple therapies. This work extends the scope of chemogenomics approaches and exemplifies the role that systems biology has in the future of drug discovery.

124. *In Silico* and Structural Studies on Juvenile Hormone Binding Protein (JHBP) of *Helicoverpa Armigera* (HUBNER) as a New Age Biopesticide

D.M. Mamatha*, M. Sreekant[1], K. Swetha Kumari [2] and K. Sreedevi[3]

[1.]Department of Seribiotechnology, Sri Padmavathi Women's University, Tirupati-517 502,

[2] Division of Bioinformatics, Department of Zoology, S.V.University, Tirupati-517 502,

[3] Department of Entomology, S.V.Agricultural College, Angra University, Tirupati-517 502

***Email** : drdmmamatha@gmail.com

Keywords : *JHBP- Juvenile hormone binding protein, Helicoverpa armigera, JH - Juvenile hormone.*

The government regulators the consumers are becoming increasingly concerned and have banned or limited the use of some hazardous pesticides looking into the overwhelming evidence about the residual toxicity of chemical pesticides, its potential risk to humans and other life forms and the environment. The world-wide deaths and chronic illnesses due to pesticide poisoning recorded to about one million per year. The economic impact of pesticides in non-target species has been estimated at approximately $8 billion annually in developing countries. In addition, due to the continuous use of chemical pesticides, the subsequent generations of the pest population show up with increased resistance. These endangering conditions throw an unending challenge to evolve new methods for biological control of pests.

However, they are not potential enough, when the threshold limits of pests exceed. Under these circumstances, there is always a need to explore new strategies to evolve potential biopesticides with increased insecticidal efficacy to combat the pest menace in an eco-friendly way. This paper deals with the physiological, structural and functional role of the JHBP in insect's growth, development and reproduction. Further, through molecular modeling, docking and site directed mutagenesis, proposed that JHBP can be a potential protein which can be targeted as a new age biopesticides.

125. Establishing an *in-Silico*-Ayurvedic Medication for Alzheimer's Disease

Sumit Raj and Pasupuleti Renuka

Department of Bioinformatics,
Sathyabama University, Chennai (TN),

Keywords *: Alzheimer's disease, Ayurvedic medication, Canscora decussate, Nardostachys jatamansi, Mucuna pruriens.*

Alzheimer's disease is an incurable, degenerative, and terminal disease. It is associated with mutations in amyloid precursor protein, presenilin 1, presenilin 2 or apolipoprotein E. The 3D structures of these proteins were designed using Homology Modeling. Active compounds of medicinal herbs – *Canscora decussate*, *Nardostachys jatamansi* and *Mucuna pruriens* were selected as these three herbs have properties of memory enhancement. Chemical structures of the active component of these three herbs were drawn using chemsketch, combined and converted to .pdb. The four proteins were successfully docked with – *Canscora decussate*, *Nardostachys jatamansi* and *Mucuna pruriens* active component combination.

126. Computational Identification of MicroRNAs and their Targets in Chickpea (*Cicer Arietinum* L.) GSS Sequences

Akan Das[1], Susheel Kumar[2] and Tapan Kumar Mondal[1, 3*]

[1]Biotechnology Laboratory, Faculty of Horticulture, Uttar Banga Krishi Viswavidyalaya, Cooch Behar-785 165, WB,
[2] Tissue Culture and Cryopreservation Unit, National Bureau of Plant Genetic Resources, Pusa, New Delhi-110 012,

[3]National Research Center of DNA Fingerprinting, National Bureau of Plant Genetic Resources, Pusa, New Delhi-110 012,
***E-mail** : mondaltk@yahoo.co.in

Keywords : *Cicer arietinum, Computational identification, Genomic survey sequence, mcroRNAs.*

MicroRNAs (miRNAs) are a class of small (~22 nucleotides), endogenous, non coding RNA molecules which directly involve in gene regulation at the post transcriptional level. High conservation of miRNAs in plant provides the basis for identification of new conserved miRNAs in other plant species through homology search. Here, previously reported plant miRNAs were BLASTed against the Genomic Survey Sequence databases of chickpea (*Cicer arietinum* L.), and through a series of filtering criteria, finally 6 miRNAs belonging to 5 miRNA families were identified, and 16 potential target genes of them were subsequently predicted in *Arabidopsis*, which encode transcription factors or enzymes participating in regulation of various biological processes. These findings lay the foundation and considerably broaden the scope of understanding the functions of miRNAs in chickpea.

127. An *in Silico* Approach to Study the Comparative Efficacy of Different Triazolopyrimidines as Potential Antimalarial Drugs

Debarati Choudhury, Nandita Chakraborty, Pankaj Chetia*, M. Dutta Choudhury and G. D. Sharma

Bioinformatics Centre, Assam University, Silchar-788 011
***E-mail :** pankaj.chetia@bioinfoaus.ac.in

Keywords *: Plasmodium falciparum, Dihydroorate dehydrogenase, Triazolopyrimidine, ADME/Tox, Mobyle@rpbs, PDB, PubChem, FlexX, Docking.*

The enzyme dihydroorate dehydrogenase(DHOD) present in the fatal malarial parasite Plasmodium falciparum that catalyses the rate limiting step of the pyrimidine salvage pathway and oxidises dihydroorate to orate, has been docked with twenty compounds with triazolopyrimidine group selected from PubChem using FlexX. The ADME/Tox screening of these 20 molecules showed no toxicity. All the compounds were found to have good affinity towards pfDHOD and capable of inhibiting the enzyme. Among these twenty compounds, compound 7, i.e. N,5-dimethyl-N-naphthalen-2-yl-[1,2,4]triazolo[1,5-a]pyrimidin-7-amine showed the highest docking score of -31.3699 Kcal/mol and compound 15, i.e. 5-methyl-N-naphthalen-1-yl-

[1,2,4]triazolo[1,5-a]pyrimidin-7-amine being the lowest, with docking score of -5.6947 Kcal/mol, which reveals the antimalarial potency of these triazolopyrimidine compounds.

128. *In Silico* Investigations on Few Members of a Plant Root Nematode from Genus *Meloidogyne*

C. Sharma[1], Sohini[2] and P. V. Arya[3]

[1]Department.of Zoology, G.D.G D. College, Mooradabad (UP)
.[2]Department of Biotechnology, Amity University, Noida (UP)
[3]Department.of Zoology, D .S. College, Delhi-110 003

Keywords *: Nematode, Meloidogyne, Phylogeny and Bioinformatics.*

Nematodes are microscopic roundworms, which attack plants. The damage they cause to plants is often subtle and is easily confused with nutrient problems. Although hundreds of different kinds of nematodes may infect plants *Meloidogyne* is one of the well known genus represent large numbers of species. Present communication deals with some species of this genus using bioinformatic tools. Sequences such as ribosomal RNA, ITS representing various species of selected genus have been retrieved. Species selected for the present *in silico* study included *Meloidogyne incognita, M. hapla, M. javanica, M. arenaria, M. chitwoodi, M. paranaensis, M.mayaguensis, M. fallax, M. enterolobii, M. graminicola, M. naasi, M. artiellia, M.minor, M. hispanica, M. floridensis, M. thailandica, M. graminis, M. exigua* and *M.ethiopica*. Sequence alignment was done using Clustal-X. Phylogenetic tree preparation was done using NJ as well UPGMA approaches which are widely used for the same purpose. The findings bring about molecular evidences of the phylogenetic relationships among the selected members of the genus *Meloidogyne*.

129. An Additive *in Silico* Approach to Proteome Projects

Bhupendra Khandelwal and P. P. Karthikeyan*
Department of Bioinformatics, SRM University,
Kattankulathur–603 203.

***Email** : karthikeyanpp@ktr.srmuniv.ac.in

Keywords : *Proteome; Sex chromosome protein; Human; Uncharacterized protein; Database.*

The proteome project is a large-scale programme dealing in an integral way with patterns of expression of proteins in biological systems, in ways that complement and extend genome projects. This *in silico* approach aids in the proteome projects by characterization of uncharacterized proteins; as it can serve as a resource for comparison of proteomes across species. As example a database of human sex chromosome's (X & Y) uncharacterized proteins is created with predicted and validated structure (3D) and functions which is freely available for download, review, refinement and update via https://sites.google.com/site/humanxchromosome/.

130. Computational Annotation of Human Chromosome-14

Anil Kumar D. V., and P. P. Karthikeyan*

Department of Bioinformatics, SRM University,
Kattankulathur–603 203.
***E-mail** : karthikeyanpp@ktr.srmuniv.ac.in

Keywords : *Human chromosome, Acrocentric, Unmodeled protein, Bioinformatics web tools, ab initio.*

Chromosome 14 is one among the five acrocentric chromosomes in the human genome with 109 million base pairs. Majority of genetic disorders like uniparental disomy were localized to chromosome 14 by linkage studies. In this work about 73 unmodeled proteins (out of total 642 proteins) from human chromosome 14 were taken from Uniprot Database. Primary structure analysis was done using bioinformatics web tools such as Protparam, Compute Pl/Mw & Radar. Secondary structures were predicted by using GORIV, HNN & SOPMA. Function prediction was achieved by BLAST, COG, PFAM and INTERPROSCAN. Based upon the homology, structures were predicted by using Swiss-model & *ab initio* structure prediction method using I-TASSER server.

131. Computational Function and Structural Annotations for Hypothetical Proteins of *Arabidopsis Thaliana* Chromosome 1

Anirban Chattopadhyay and P. P. Karthikeyan*
Department of Bioinformatics, SRM University,
Kattankulathur–603 203.
***Email** : karthikeyanpp@ktr.srmuniv.ac.in

Keywords : *Arabidopsis thaliana ; Chromosome, Hypothetical / Unknown protein; Database.*

Arabidopsis thaliana is a small plant that belongs to *Brassicaceae* family with five chromosomes and estimate of 20,000 genes. *Arabidopsis* research serves as a convenient model not only for plant biology, but for many other eukaryotes. In this view, 500 hypothetical proteins of *A. thaliana* chromosome 1 were taken from NCBI database and functions were predicted using bioinformatics web tools such as BLAST, COG, PFAM and INTERPROSCAN. Tertiary structure predictions were done using online tools such as I-TASSER (*ab initio*) and Swiss model. The datasets were made available online via http:// sites.google.com/site/arabidopsisplant/.

132. *In Silico* Annotation for Hypothetical Proteins of *Cyanothece sp.*ATCC 51142

Peeyush Sahu and P. P. Karthikeyan*
Department of Bioinformatics, SRM University,
Kattankulathur–603 203.
E-mail : *karthikeyanpp@ktr.srmuniv.ac.in

Keywords : *Cyanothece sp. ATCC 51142, Hypothetical proteins, Online database.*

Cyanothece is a single cell bacterium which works 24/7, in the sunlight it perform photosynthesis and in night it ramps up the expression of genes governing a surprising number of vital processes, including energy metabolism, nitrogen fixation and respiration. Out of the total 5,310 proteins associated, with *Cyanothece sp*. ATCC 51142, 2,569 proteins were hypothetical, of which 350 hypothetical proteins were characterized. Total 47 of these 350 hypothetical proteins were functionally predicted with 100% confidence using knowledge datasets such as Interproscan, Scanprosite, COG, BLAST & Pfam. These 47 proteins were found to be present in protein families with their domain constitution as DGAT 1, S2P-M50 metallopeptidase, CBS domain, FTSK, helicase domains, and DNAJ domain. All the datasets are available for download via: http://sites.google.com/site/cyanothece/.

133. *In Silico* Characterization of Unmodeled Proteins in Human Chromosome1

Vaibhav Singh and P. P. Karthikeyan*
Department of Bioinformatics, SRM University,
Kattankulathur–603 203.
E-mail : *karthikeyanpp@ktr.srmuniv.ac.in

Keywords : *Chromosome, In silico, Unmodelled proteins.*

Chromosome 1 is the largest human chromosome with 263 million base pairs. Identifying genes on each chromosome is useful for protein characterization through sequence based comparison methods which could provide additional information about the biochemical mechanism of proteins at atomic level. In view of this, about 187 unmodeled proteins (total 2,057) of human chromosome 1 were taken from Uniprot Database. Their Primary structures were predicted by online tools such as Protparam, Compute pI/Mw & Radar. Secondary structures were predicted by GORIV, HNN, and SOPMA. Function prediction was done using BLAST, COG, PFAM and INTERPROSCAN. Tertiary structures were predicted by I-TASSER (*ab initio*) and Swiss model (homology modeling).

134. Pharmacophore Modeling and Virtual Screening Studies to Design Some Potential Neuraminidase Inhibitors as New Leads

Vijay Singh, Pravin Shinde, Shirishkumar P and M. Elizabeth Sobhia*
Department of Pharmacoinformatics, National Institute of Pharmaceutical Education and Research (NIPER), Sector-67, S.A.S. Nagar (Mohali)-160 062
***Email :** mesophia@niper.ac.in

Keywords : *Neuraminidase, Influenza, Pharmacophore model, Virtual screening.*

Neuraminidase (NA) is a potential target to prevent infection from influenza viruses by developing efficacious therapeutic agents against them. A pharmacophore model was generated using Catalyst software for diverse set of NA inhibitors which provided a rational hypothetical picture of the primary chemical features responsible for activity. The best model so obtained consists of spatial arrangement of 5 chemical features: one hydrogen bond acceptor, one hydrogen bond donor, one hydrophobic, and two ring aromatic features. The generated and validated model was further used for virtual screening of Zinc database and 120 compounds were

obtained. The study provides useful information for developing new potentially active candidates targeting NA as anti-influenza agents.

135. Disorder Predictors and Amino Acid Bias

S. Karthigayini, H. Gomathi and N. Rathankar

Department of Bioinformatics, School of Bioengineering,
SRM University, Kattankulathur, Tamilnadu-603 203,

Keywords *: Disorders, Aminoacid bias.*

The inaccurate results of protein predictors indicate differences among the functional classes of disordered protein regions. Hence an attempt has been made to determine the amino acid bias, each predictor uses to predict, understand and classify the disordered proteins in this paper. Towards this initiative, 517 disordered proteins from DISPROT 5.1 database were used as a seed dataset. Total 433 disordered fragments of length greater than 30 residues were delineated from this dataset using Perl programs. Subjecting them to seven disorder-predictors, fragments with length greater than 20 residues and conserved in datasets predicted by both the predictor and the DISPROT dataset were only considered for further analysis. A thorough statistical analysis of the composition bias introduced by these predictors using disordered regions will be presented in the conference.

136. Inhibitors for the Multidrug Efflux Membrane Protein, MexB of *Pseudomonas Aeruginosa* – an *in Silico* Analysis

V. Aparna, N. Mohanalakshmi and Waheeta Hopper

Department of Bioinformatics, School of Bioengineering, Faculty of Engineering and Technology, SRM University, Kattankulathur, Tamilnadu-603 203.

Keywords *: Pseudomonas aeruginosa, Efflux pumps, MexB, Phenylalanine, Arginine β-naphthylamide, Virtual screening.*

Pseudomonas aeruginosa is an opportunistic pathogen, notable for multidrug resistance, mediated by low permeability outer membrane and efflux systems. MexB

is a multidrug exporter protein in *Pseudomonas*. To identify potential efflux pump inhibitors, compounds which are structurally similar to an efflux pump inhibitor; phenylalanine arginine β-naphthylamide, were taken for virtual screening against MexB protein using Schrödinger v9.0. Binding affinity and active site interaction of compounds were determined by docking studies using AutoDock v4. Molecular Dynamics Simulation of the protein-inhibitor complex was performed to study the motion of protein with respect to time. These compounds could have the potential to inhibit efflux pumps and thus provide an ideal way to combat antibiotic resistance.

137. *In Silico* Identification of Non-Synonymous SNPs in *IQGAP* Genes and their Respective miRNA Targets

Arya. A. Das, Ishangi Agarwal and Yogesh

Department of Bioinformatics, SRM University,
Kattankulathur, Tamilnadu-603 203

Keywords : *Non-synonymous SNP, SIFT, Polyphen, 5'UTR, 3'UTR, Haplotype tag SNPs TargetScan, PicTar web interface.*

In this study, the genetic variation due to non synonymous SNPs on IQGAP1, IQGAP2 and IQGAP3 which may be responsible for development of certain cancers were analyzed. SIFT and PolyPhen programs helped to analyze the nsSNPs and FastSNP, UTR scan programs were used to compute SNPs in the 5' and 3' untranslated regions. Functionally significant SNPs were predicted by FASTSNP and UTRscan algorithm. Also seen the copy number variations, expressed sequence tags and genome survey sequences of the selected genes. Results from TargetScan algorithm and PicTar web interface to find the best miRNA targets were included.

138. Novel Anti-Inflammatory Drug Leads from Marine Actinomycete *Salinispora* Species : an *in Silico* Approach

Dinesh Kumar K and Waheeta Hopper

Department of Bioinformatics, School of Bioengineering,
Faculty of Engineering and Technology, SRM University,
Kattankulathur, Tamilnadu-603 203

Keywords : *Cyclooxygenase, Salinispora, molecular docking, Glide, dynamics simulation.*

Cyclooxygenase (COX) catalyzes the biosynthesis of the prostaglandins from the substrate arachidonic acid. COX-2 discovery has helped in suppressing inflammation without side effects, by the use of the present range of NSAIDs. *Salinispora*, an actinomycetes, possesses a rich and diverse metabolome. Salinosporamide A is currently in human clinical trials for the treatment of cancer. Biological activities of certain compounds isolated from *Salinispora* remain unknown. Using Glide molecular docking (Schrondinger v9.0), the compounds from *Salinispora* were screened against the inflammatory target, COX-2, for their bioactivity prediction. Based on the Glide score and interactions with the active site residues the best compounds were selected for dynamics simulation studies. These compounds could be potential anti-inflammatory leads and need validation *in vitro* and *in vivo.*

139. Novel Protocol for Annotating Orphan Enzymes from ORENZA Database

Sunny Dasgupta and N. Rathankar Rao

Department of Bioinformatics, School of Bioengineering, SRM University,
Kattankulathur, Tamilnadu-603 203,

Keywords *: Orphan enzymes, ORENZA database.*

Despite the current availability of thousands of amino acid sequences, more than 39% of the well-defined enzyme activities (EC numbers) are not associated with sequence information in major public databases for certain species. This wide gap separating knowledge of biochemical function and sequence information is found in nearly all classes of enzymes (also known as orphan enzymes) and has been catalogued as a collection of 1525 enzyme activities in the publicly available ORENZA database. Hence, characterizing sequence information would be immensely

helpful for structural biologists to decipher the structure-function relationships. Towards this initiative, an orphan enzyme (EC: 1.1.1.16) which catalyzes the conversion of NAD+ to NADH namely galactitol-2-dehydrogenase involved in galactose metabolism was chosen. A novel protocol utilizing the motif information from galactitol-2-dehydrogenase like proteins from other genomes provided us a clue to decipher three key motifs using various sequence search protocols. Motif searches against the non-redundant protein database revealed 21 hypothetical proteins containing these motifs and hence were predicted to have galactitol-2-dehydrogenase activity. Confirmation of sequence information will be made by analyzing structural interactions with the ligand molecule and will be presented in the conference.

140. Silence Speaks : Phenotypes Affected by Synonymous Mutations

Muthangi Bindu[1] and Shrish Tiwari[2]

[1]Department of Bioinformatics, School of Bioengineering,
Faculty of Engineering and Technology,
SRM University, Kattankulathur, Chennai.
[2]Department of Bioinformatics,
Centre for Cellular and Molecular Biology, Hyderabad

Keywords *: TRPM2 gene, BPAD, Rare codons, CAT gene, Ribosome traffic, Protein misfolding, DRD2 gene, MDR1, EgFABP1.*

Synonymous mutations and substitutions affecting non coding DNA are collectively known as silent mutations. A synonymous mutation (also called a silent substitution) is the evolutionary substitution of one base for another in an exon of a gene coding for a protein, such that the amino acid sequence produced is not modified. The aim of this study was to identify synonymous mutations in the coding sequences of chromosome 21 of *Homo sapiens* which resulted a synonymous mutation 3205C>T on *TRPM2* gene, observed to be associated with bipolar affective disorder (BPAD). This method is a synonymous analysis involving rare codons as functional units of gene sequences and their association with a change in phenotype. Phenotype may constitute protein misfolding, protein structure and translation or change in mRNA stability. Similar analysis performed on *DRD2* gene of 11th

chromosome of *H. sapiens* followed silent mutation 132C>T which affects mRNA stability. In *E. coli, CAT* gene a consecutive set of synonymous mutations was observed that affect ribosome traffic and protein folding resulting in an increased aggregation of protein due to misfolding. Our results on silent mutations of *TRPM2*, *DRD2*, *MDR1* of *H. sapiens* and *CAT*, *EgFABP1* genes of *E. coli* suggested an intriguing correlation between synonymous codon changes and protein folding dynamics.

141. Phylogenetic Analysis of Some Species of Family Orchideaceae Inferred from Chloroplast mat*K* Nucleotide and Protein Sequence Data

P. B Mazumder[1], Paromita Chakraborty[1], Soumitra Nath[1 *], P. Chetia[2], M. D. Choudhury[2], G. D. Sharma[2]

[1] Department of Biotechnology, Assam University,
Silchar-788 011, Assam.
[2] Department of Life Science, Assam University,
Silchar-788 011, Assam.
***Email** : nath.soumitra1@hotmail.com

Keywords *: Chloroplast, matK, Clustal X_2, MEGA 4.1, Orchideaceae, indels, systematics, phylogeny.*

Genetic relationship between the species is valuable for genetic improvement and phylogenetic studies. This study was conducted to understand major evolutionary relationships among 74 species of *Orchideaceae* using sequence data of mat*K* obtained from GenBank. Sequence alignments showed variable numbers of indels and substitutions in variable regions. The NJ tree showed interspecies evolutionary relationship. *Corycium carnosum* and *Pterygodium alatum* showed high resemblance with distantly related species *Ceratandra.* The similarity of protein and nucleotide tree indicated no biasness of nucleotide to protein code. Overall, the results indicated mat*K* gene provides well-defined relationships; therefore can be successfully used in plant systematics.

142. Inhibitory Effects of Melatonin in Serotonin Pathway : A Systems

Approach

Swetha M, Penumetcha Sirisha and N. Rathankar
Department of Bioinformatics, SRM University,
Kattankulathur–603 203

Keywords : *Melatonin, serotonin, Inhibitors.*

Serotonin and melatonin are two widely used drugs for treatment of hypertension and cardiovascular diseases respectively. Although derived from the same precursor, the drugs have inhibitory effects on each other and thus cannot be administered together. A systems approach to study the possible receptor-drug / protein-protein interactions in the serotonin-melatonin biosynthesis pathway and its interactions with other gene products is thus essential to understand this complex feedback system. Towards this initiative, extensive literature survey was conducted to determine the possible receptor-ligand interactions in serotonin and melatonin. It was found that higher levels of serotonin, increases the production of growth hormone, while melatonin inhibited the production of these growth hormones. Thus, the critical node linking the cause and nullifying action of growth hormones by serotonin and melatonin in the melatonin biosynthesis pathway was used to create a mathematical model. A solution to this model may draw conclusions to arrive at an optimal solution to administer the drugs together, which will be discussed in detail in the conference.

143. Structural and Functional Characterization of the Cytochrome1A1 Ligand Binding Domain by Homology Modeling

Nitu Bansal, G. Raj Kiran, Vijay Rathod and Abhay T. Sangamwar*
National Institute of Pharmaceutical Education and Research (NIPER),
Sector-67, S. A. S. Nagar, Punjab-160 062
***Email** : abhays@niper.ac.in

Keywords *: CYP1A1; Homology modeling; Active site; Anticancer Drug design.*

Humans are subjected daily to ever increasing amounts of exogenous compounds. Some of them are capable of inducing cytochrome P450s, a process that allows the cell to adapt to changes in its chemical environment. One of the most widely CYP studied is CYP1A1 because it metabolizes a large number of xenobiotics to cytotoxic and or mutagenic derivatives. The structure of CYP1A1 offers a template to study structure-function relationships of alternative substrates and other cytochrome P450 family 1 members. To explore CYP1A1 as a potential target for anticancer chemotherapy homology model of CYP P450 1A1 from *Homo sapiens* was prepared, on the basis of template crystal structure of human microsomal P450 1A2 (PDB Id: 2HI4) in complex with inhibitor using modeller9v8

145. Molecular Mechanism of Cadmium Induced Testicular Injury

Smita Khanna and Shashi Khandelwal

Immunotoxicology Lab, Indian Institute of Toxicology Research
Lucknow-226001

Cadmium is a common environmental and occupational hazard and its adverse effect on reproductive organ has been well documented. The present study is planned to delineate the mechanism of Cd toxicity in rat testes. Our study shows that Cd causes apoptosis in germ sertoli cells which is governed by oxidative stress. We assayed ROS, GSH and MMP to ensure the role of oxidative stress, which was further confirmed by thiol modulators. The initial biochemical response show in sertoli germ cells was a drastic fall in MMP followed by ROS generation. The downstream events included cytochrome-c release leading to caspase-3 activation and culminating in cell death via apoptosis.

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