CSIR

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द्विवार्षिक प्रतिवेदन

biennium report

2009-2011

सूक्ष्मजीव प्रौद्योगिकी संस्थान

INSTITUTE OF MICROBIAL TECHNOLOGY

mandate

- To provide integrated research, development and design base for microbial technology.
- To undertake basic and applied research and development programs in established and newly emerging areas of relevant biotechnology including Genetic Engineering.
- To optimize the existing microbial processed currently available and in use in the country.
- To develop and maintain gene pool resources and genetic stocks of microbial cultures and other cell lines. This could also serve as a reference centre to assist other centres.
- To establish facilities for biochemical engineering, instrumentation development including microprocessor systems, a computer centre, and development of mathematical models for process parameters.
- To establish facilities for design of process equipment and bioreactors.
- To impart training in microbiology, microbial technology and biochemical engineering.
- To conduct training and refresher courses for research workers and technologists.
- To establish documentation and information retrieval and dissemination facilities and a data bank to meet the needs of the institute.
- To establish and maintain effective linkages with industry and educational institutions.
- To develop capabilities for producing design and engineering packages for industrial plants.

With
Compliments

Dr. Girish Sahni
Director,
IMTECH

द्विवार्षिक प्रतिवेदन
Biennium Report
2009-2011

IMTECH

INSTITUTE OF MICROBIAL TECHNOLOGY
Sector 39-A, Chandigarh (INDIA)

सूक्ष्मजीव प्रौद्योगिकी संस्थान
सैक्टर 39-ए, चण्डीगढ़

CSIR Tableau on Rajpath during Republic Day Parade 26 January, 2011

From Generic to Genomic Medicine

CSIR, established in 1942, is an ensemble of 37 state-of-the-art institutes involved in scientific and industrial R&D ranging from aerospace to ocean exploration, from micro-electronics to structural and environmental engineering, from smart materials to mechatronics, and from petrochemicals to healthcare.

The tableau highlights the technological contributions of CSIR in the domain of 'Affordable Healthcare'. It captures the innovative R&D contributions of CSIR focused at self-reliance and creating technological niches leading to not only building up of generic drug industry but also catalyzing new drug development in the country. Post-Independence, CSIR has to its credit 13 out of 17 new drugs and several new drug candidates developed in the country. CSIR has now built unique expertise in genomics and its R&D efforts are leading to development of predictive medicine, based on genetic constitution of an individual patient.

The tableau conveys the importance of knowledge-intensive contemporary R&D in building national capacity to deliver affordable health care for the world.

Compilation Committee:

Dibyendu Sarkar (Chairman)

Ashish (Member)

S. Kumaran (Member)

Chhering Tobden (A.O.)

Brijesh Kumar (F & A.O.)

R. Soni (Invited Member)

Garry Bedi (Convenor)

Photography & Concept: Garry Bedi

Contents

निर्देशक की कलम से.

सूक्ष्मजीव प्रौद्योगिकी संस्थान, चण्डीगढ़ के लिए वर्ष 2009–2011 का द्विवार्षिकी प्रतिवेदन प्रस्तुत करते हुए मुझे अति बड़ी प्रसन्नता हो रही है । यह रिपोर्ट इस अवधि के दौरान संस्थान की वैज्ञानिक उपलब्धियों को दर्शाने के साथ-साथ एक बहुमूल्य अवसर भी प्रदान भी करती है कि हम अपनी प्रगति को आंके तथा शोध एवं विकास की गतिविधियों को और बढाएँ ।

1984 में स्थापित, भारत की यह प्रमुख प्रयोगशाला ने सूक्ष्मजीव जैवप्रौद्योगिकी के क्षेत्र में विशिष्ट पहचान एवं प्रतिष्ठा अर्जित की है । न केवल औसत इमपैक्ट फैक्टर प्रति पेपर के मामले में, अपितु मूल शोध को समाज के लिए उपयोगी उत्पादों और प्रक्रियाओं में बदलने के क्षेत्र में आज भी इसकी गणना सीएसआईआर की पाँच शीर्ष प्रयोगशालाओं में की जाती है । वर्ष 2009, इमटैक का रजत जयंती वर्ष जिसके आयोजन 24 जनवरी, 2009 से आरम्भ होकर वर्ष भर चलते रहे । इनमें दो अंतरराष्ट्रीय सम्मेलनों की सफल मेज़बानी शामिल है, "रोगजनक सूक्ष्मजीवों का संचालन व अध्ययन " 22–24 जनवरी, 2010 तथा 'जीनोमों पर कार्य करने के कम्प्यूटर आधारित पहलू' 28–30 मार्च, 2011 जिनमें भारत व विदेश से प्रतिभागियों ने भाग लिया ।

हाल ही के वर्षो में इमटैक द्वारा राष्ट्रीय महत्व की गई महत्वपूर्ण परियोजनाएँ प्रारम्भ की गई हैं । इनमें से कुछेक की प्रकृति बहुविषयक है जिसके लिए संस्थान के अंदर ही विभिन्न कार्यक्षेत्रों से विशेषज्ञों को एक सुगठित टीम के रूप में कार्य करना होगा । इमटैक के सभी शोध समूहों द्वारा किया जा रहा उत्कृष्ट कार्य 191 प्रकाशनों में प्रतिबिम्बित हो रहा है जोकि प्रतिष्ठित अंतरराष्ट्रीय जर्नलों में प्रकाशित हुए हैं इसके साथ ही 20 पेटेंट फाइल किए गए जिनमें से भारत एवं विदेश के मिलाकर, 16 प्रदान किए गए हैं ।

यह अभिलिखित करते हुए बड़ा हर्ष हो रहा है कि वैज्ञानिक हस्तांतरण के लिए तथा औद्योगिक विकास के मोर्चे पर यह अवधि बहुत महत्वपूर्ण रही है । प्रौद्योगिकियों के लाइसेंसीकरण में हुई प्रगति में हमने मील पत्थर स्थापित किए । 10 जुलाई, 2009 को रिकम्बिनेट स्ट्रेप्टोकाइनेस के लिए इमटैक की प्रौद्योगिकी 'शाशुन कैमिकल्स एवं ड्रग्स लिमिटेड 'चैने द्वारा 'क्लॉटबस्टर' (एलम्बिक) तथा 'लूपीफलो ' (लेूपिन) के ब्रांड नाम से वाणिज्यिक तौर पर भारतीय बाज़ार में उतारा गया । संस्थान द्वारा 24 नवम्बर, 2010 को संस्थान द्वारा थ्राम्बोलिटिकों की अगली पीढ़ी के विकास एवं वाणिज्यिकरण के अधिकार नॉस्ट्रम फार्मास्यूटिकलस इंक., यू. एस.ए को लाईसेस किए गए, 150 मिलियन डॉलर का यह ऐतिहासिक सौदा प्रगति मैदान, नई दिल्ली में 'सीएसआईआर टैक्नोफैस्ट 2010 'के दौरान हुआ ।

इस भव्य आयोजन में इमटैक ने भी सक्रिय तौर पर भाग लिया तथा अन्य प्रयोगशालाओं के साथ स्वास्थ्य के क्षेत्र में अपनी उपलब्धियों को प्रदर्शित किया तथा 'स्वास्थ्य' विषयक पैविलियन के लिए प्लैटिनम अवार्ड भी प्राप्त किया ।

विस्तार की संकल्पना पर आरम्भ किए गए अत्याधुनिक प्रोटीन विज्ञान केन्द्र तथा नया छात्रावास, जिनकी नींव इस अवधि में रखी गई थी, वे लगभग पूरे हो चुके है । इससे हमें प्रोटिओमिक्स तथा प्रोटीन इंजिनियरिंग के क्षेत्र में विशेषज्ञता अर्जित करने का अवसर मिलेगा तथा यह हमारे छात्रों की बढती हुई जरूरतों को भी पूरा करेगा । यह हमारा निरन्तर और प्रबुद्ध प्रयास रहेगा कि हम राष्ट्र के लिए उच्च योग्यता के वैज्ञानिक पीएचडी छात्र विकसित कर सकें । रिपोर्टाधीन अवधि में 40 छात्रों ने अपनी डॉक्टरल उपाधि प्राप्त की तथा 80 नए छात्रों को जवाहर लाल नेहरू विश्विद्यालय के साथ संयुक्त रूप से चलाए जा रहे पीएचडी कार्यक्रम में प्रवेश दिया गया ।

हमारे कुछेक वैज्ञानिकों को विज्ञान एवं प्रौद्योगिक मं उनके अभूतपूर्व योगदान के लिए पुरस्कार प्राप्त हुए । उल्लेखनीय उदाहरण हैं डॉ. जी. पी. एस. राघव, वैज्ञानिक ने वर्ष 2009 के लिए प्रतिष्ठित थॉम्सन र्‌यूटर्स रिसर्च एक्सीलेंस - इंडिया रिसर्च फ्रंट अवार्ड प्राप्त किया तथा डॉ प्रदीप चक्रवर्ती को भारतीय राष्ट्रीय विज्ञान अकादमी तथा भारतीय विज्ञान अकादमी का सदस्य चुना गया ।

मैं, विज्ञान एवं प्रौद्योगिकी मंत्रालय, परिषद् मुख्यालय तथा हमारी शोध व प्रबंधन परिषदों द्वारा दिए गए सहृदय समर्थन एवं सहयोग के लिए धन्यवाद देना चाहता हूँ । अन्य बाहरी विशेषज्ञों का भी आभार व्यक्त करना चाहता हूं जिन्होंने पूरी निष्ठा और समर्पण से हमें आगे बढ़ने के लिए प्रेरित किया ।

हमारे संकाय में सम्मिलित नए युवा सदस्यों का भी मैं गर्मजोशी से स्वागत करता हूँ तथा इमटैक को भविष्य का सर्वोत्कृष्ट शोध संस्थान बनाने के लिए आपसे से प्रत्येक के साथ काम करने को उत्सुक हूँ । हमारे संस्थान की इस रिपोर्ट को प्रस्तुत करने के लिए द्विवार्षिकी रिपोर्ट संकलन समिति के प्रयासों के लिए उनका आभार व्यक्त करता हूँ । अंत में, मै अपने सभी सहकर्मियों, सहयोगियों, छात्रों तथा इमटैक परिवार के अन्य सदस्यों को भी इस प्रतिवदेन के सफल समापन में उनके उदार प्रयासों तथा योगदान के लिए बधाई देता हूँ, तथा आने वाले वर्षो के लिए शुभकामनाएँ देता हूँ ।

सुखद पठन!

गिरीश साहनी
निर्देशक

From the Director's desk

It is a matter of great pleasure for me to present to you the 2009-2011 Biennium Report of the Institute of Microbial Technology, Chandigarh. This report highlights the scientific achievements during this period and also provides us a valuable opportunity to gauge our progress and to step up our R&D activities.

Established in 1984, this premier laboratory of India has acquired considerable recognition and repute in the field to microbial biotechnology. It today ranks amongst the top five CSIR laboratories not only in terms of average impact factor per paper, but also in terms of translating its basic research into useful products and processes for the society.

The year 2009, being IMTECH's Silver jubilee year, marked the onset of celebrations beginning January 24, 2009 with the organization of several commemorative events. This included the successful hosting of two International conferences: "Understanding and Managing Pathogenic Microbes" from 22-24 January, 2010 and "Computational Aspects of Working with Genomes" from 28-20 March, 2011 with participants from both India and abroad.

In recent years, IMTECH has embarked on several important projects of national importance. Some of these projects are highly inter-disciplinary in nature, requiring expertise drawn from multiple streams of activity within the institute to work together as a coherent team. The excellent work being undertaken by all the research groups in IMTECH is reflected with 191 publications being published in prestigious international journals and with 20 patents being filed and 16 being granted in India and abroad.

It is gratifying to record that this period was a momentous period on the translational science and business development front too. We registered milestone progress by licensing out technologies. On 10 July, 2009 IMTECH's Technology for Recombinant Streptokinase was commercially launched for Indian market by Shasun Chemicals & Drugs Ltd., Chennai under the brand names 'Klotbuster' (Alembic) and 'Lupiflo' (Lupin). The institute on 24 November, 2010 licensed the rights to develop and commercialize next generation thrombolytics to Nostrum Pharmaceuticals Inc., USA in a historic US 150 million dollar deal held during the 'CSIR Technofest 2010' at Pragati Maidan, New Delhi. During this mega event IMTECH also actively participated and showcased its R&D achievements in the field of healthcare along with other CSIR labs and won the Platinum Award for the 'Healthcare' theme pavilion.

Embarked on an envision of expansion, foundations laid during this period of the state-of-the-art 'Protein Science Centre' and a new student's hostel are nearing completion. This would provide us an opportunity to specialize in the field of Proteomics & Protein Engineering and also to cater to the growing needs of our students. It is our continuous and conscious endeavour to develop highly skilled scientific Ph.D. students for the nation. During the period under report, 40 Students obtained their doctoral degrees and 80 new students were admitted in our Ph.D. Program run jointly with Jawaharlal Nehru University, New Delhi.

Some of our Scientists received recognition for their excellence for their contribution to Science and Technology. Notable examples were Dr. G.P.S. Raghava bagging the coveted Thomson Reuters Research Excellence - India Research Front Award for the year 2009 and Dr. Pradip Chakraborti being elected as the Fellow of Indian National Science Academy and Indian Academy of Sciences.

I wish to acknowledge the wholehearted support and cooperation received from the Ministry of Science & Technology, CSIR headquarters and our Research and Management councils. I am also grateful to the external experts who provided us with a devoted and dedicated impetus to excel.

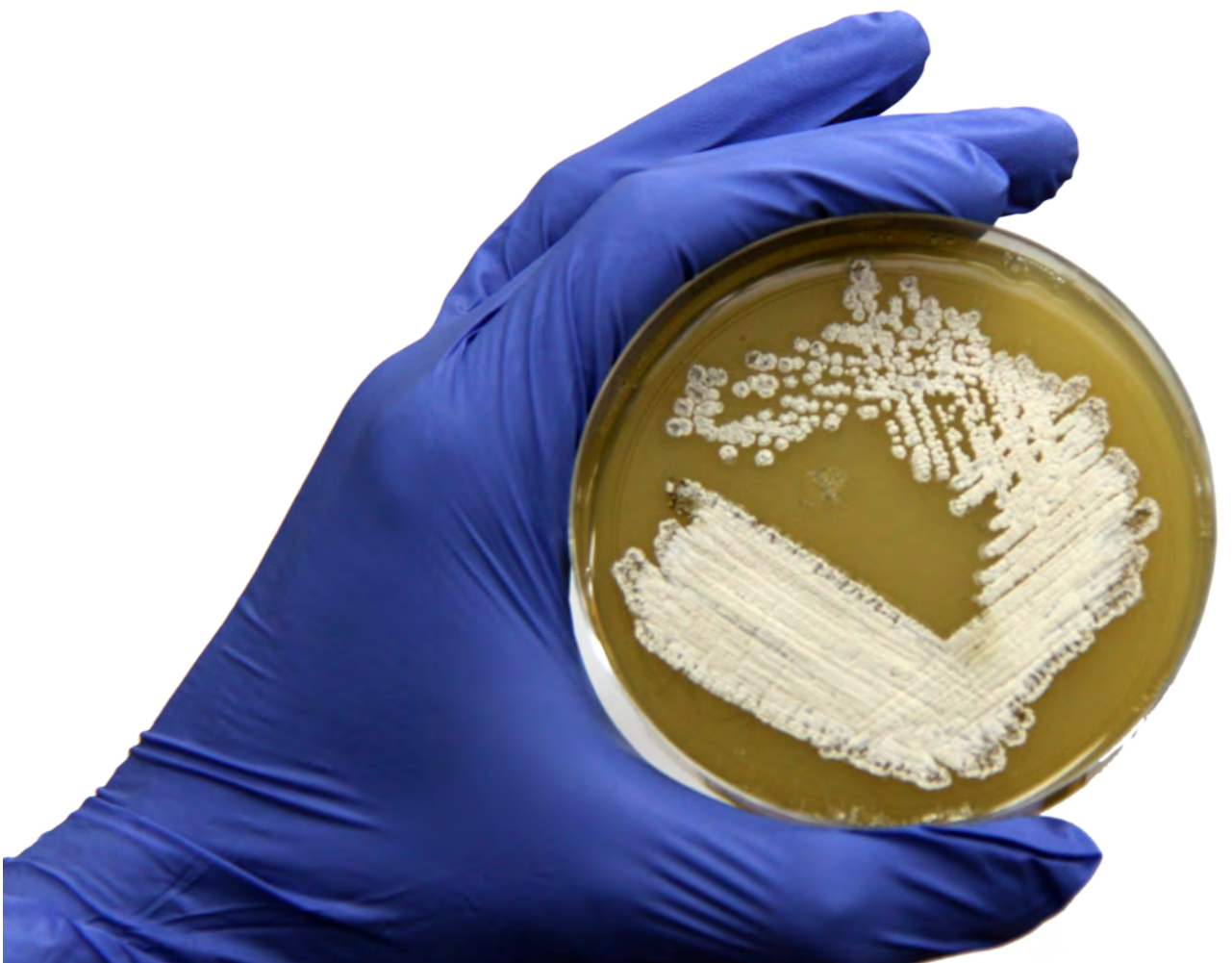
I also warmly welcome the young new additions to our faculty and also look forward to working with each and every one of you to make IMTECH the quintessential research institute of the future.

I would like to acknowledge the efforts of the Biennium Report compilation Committee for bringing out this report on our institute. Finally, I congratulate all my colleagues, associates, students and other member of the IMTECH family for their munificent efforts and contributions in the successful completion of this period, and I wish them the very best for the coming years.

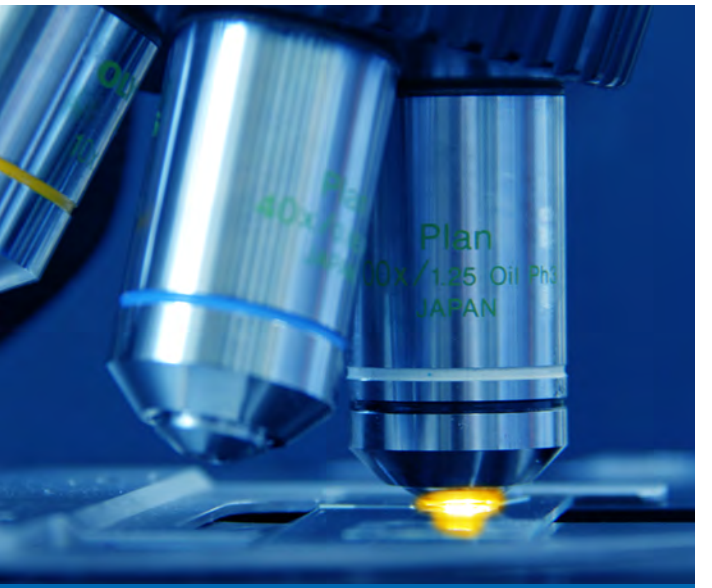
Girish Sahni

Director

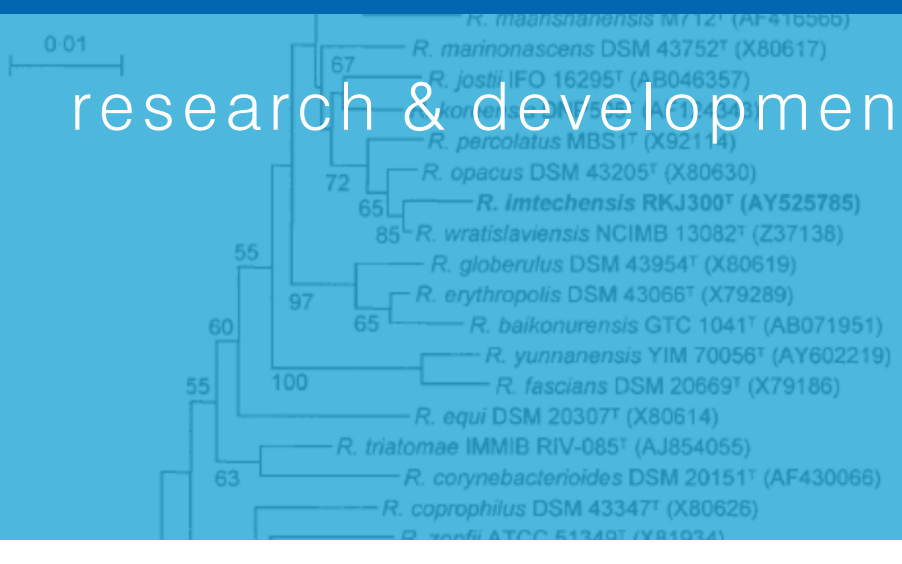
research & development programmes



microbial type culture collection (exploration of microbial diversity)



research & development programmes



Late R.K. Jain

1958 - 2011

Biodegradation of nitroaromatic compounds/ polynuclear aromatic hydrocarbons at the biochemical and molecular levels

Nitroaromatic compounds (NACs) are widely distributed in the environment because of their numerous applications. They are extensively used as herbicides, fungicides, insecticides, explosives and precursors for dyes and plasticizers. They are also released into the environment as hydrolytic products of some organophosphate insecticides or industrial wastes. Among NACs, nitrophenols are most extensively studied due to their acute toxicity. Most nitrophenols find their way into the environment during manufacturing and processing of these NACs. Some NACs degrading bacterial strains belonging to genera *Arthrobacter, Ralstonia, Burkholderia* and *Rhodococcus* were isolated, identified and characterized. The pathways for PNP and other NACs degradation strains have been elucidated using various biochemical/physiochemical techniques such as TLC, GC, GC-MS, HPLC, and enzyme assays. These novel pathways are also available at the University of Minnesota Biocatalysis/ Biodegradation Database. It was also established that the genes for the whole degradation pathways for p-nitrophenol and o-nitrobenzoate are encoded on large plasmids of app. 50-60 kilobase pairs in case of *Arthobacter protophormiae* and *Burkholderia cepacia* and some of the genes encoding the degradation pathways of these compounds have been cloned successfully. A fragment of 6.897 kb from the genomic DNA of *Ralstonia* sp. SJ98 has been cloned, sequenced, annotated and submitted to Gene Bank (Accession no. AY574278). Seven open reading frames were identified, out of which, six significantly matched with proteins of known function, *i.e*. 1,2,4-benzenetriol 1,2-dioxygenases, maleylacetate reductases, ferredoxin subunit of nitrite reductases and ring-hydroxylating dioxygenases, transcriptional regulator of GntR family, aldehyde dehydrogenase, and ATP binding ABC transporter.

The microcosm, pot, and field studies on bioremediation of P-nitro phenol (PNP) contaminated soil were also carried out in order to develop a bioremediation strategy for degradation of PNP by strain RKJ 100 since PNP is

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Rakesh K. Jain received his Ph.D. (1985) in microbiology from Monash University, Melbourne, Australia. He did post-doctoral research at University of Tennessee, Knoxville, TN, USA (1985-1987), and at the Tyndall Air Force Base, FL, USA (1992-1994) and had joined IMTECH in 1987.

the actual recalcitrant and toxic product of organophosphate degradation in soil. For this study, biodegradation of PNP in soil microcosms was used a model system to analyse the effect of various factors during the biodegradation. The efficiency of PNP degradation in soil by strain *Arthrobacter protopharmiae* was seen to be dependent on pH, temperature, initial PNP concentration and inoculums size. The results indicate that strain *Arthrobacter protopharmiae* has potential for use in *in-situ* bioremediation of PNP-contaminated sites. The pot and field study were carried out using the small- scale fields in which PNP-contaminated soil from agricultural field was bioaugmented with strain *Arthrobacter protopharmiae* under natural environmental conditions. PNP was totally depleted in 5 days. The fate of the released strain was also as monitored by plate counts, hybridization studies, and real-time polymerase chain reaction. Simultaneously, depletion of PNP was quantified in these samples to determine the effectiveness of the bioremediation strategy.

The *Ralstonia* sp., isolated by chemotactic enrichment technique as indicated above, showed that it is chemotactic to several nitroaromatic compounds, which are also utilized by this organism as sole source of carbon and energy. This was achieved by drop assay, swarm plate assay and capillary assay methods. Results obtained have indicated a correlation between chemotaxis and biodegradation of nitroaromatic compounds. The work carried out by the nominee on the above aspects is of significant value as far as both basic and applied aspects are concerned.

Soil probably harbors most of our planet's undiscovered biodiversity. Recent results from both culture dependent and culture independent molecular approaches indicate that soil microbial diversity is even higher than previously imagined. Studies were carried out to assess the biodiversity of site(s) contaminated by organophosphate pesticides in the nominee's lab *e.g.* parathion and methyl parathion using Amplified rDNA Restriction Analysis (ARDRA) and simultaneously studying changes in biodiversity of the site following bioremediation using Terminal Restriction Fragment Length Polymorphism (T-RFLP). Apart from the above studies he has also characterized several novel bacterial strains isolates from contaminated soils using polyphasic approaches based on chemotaxonomy, 16S rRNA sequencing, FAMES analysis, G+C content analysis and DNA-DNA hybridization.

One of the most recalcitrant and hazardous group of compounds are the PAHs which include naphthalene, anthracene, phenanthrene and PCBs. These compounds also constitute major fraction of crude oil. The potential carcinogenic effects of PAHs in the environment and to the human beings is of concern and, because of their gentoxicity, the US EPA has listed 16 PAHs as priority pollutants. In this study several organisms were isolated and characterized from Indian soil samples which have potential to be used for commercial scale particularly for the purpose of decontamination of oil polluted soil/ areas. Among others two bacteria namely *Pseudomonas putida* and *Arthrobacter sulphureus* isolated from Gujarat oil fields, were capable of utilising naphthalene and phenanthrene as sole source of carbon and energy. The whole pathways for the degradation of above compounds were established using several chemical and biochemical techniques. It was further shown that the genes for whole naphthalene degradation pathway are encoded on a large plasmid of app. 85 kilobase pairs present in *Pseudomonas putida* strain. This was confirmed on the basis of plasmid isolation, curing and conjugation studies. The genes for the whole degradation pathways were also cloned into *E. coli* using a broad host range plasmid vector pLAFR3 which were found to be located on a 25 kb *EcoRI* fragment. The stability studies on the cloned fragment in selective and nonselective condition were also studied. It was found that cloned 25 kb *EcoRI* fragment of recombinant plasmid (pRKJ3) carrying gene for naphthalene degradation is segregationally unstable in non-selective conditions but is stable in selective conditions. This indicates that the genetically manipulated organism(s) could have potential of degradation of polynuclear aromatic hydrocarbons if selective condition are provided during degradation / mineralization.

Publications:

• Arora, P. K., Kumar, M., Chauhan, A., Raghava, G. P. & Jain, R. K. (2009). OxDBase: a database of oxygenases involved in biodegradation. *BMC Res Notes* **2**, 67.

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Participants:

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Diversity, molecular phylogeny & exploitation of yeasts

We examine the diversity and molecular phylogeny of yeasts using sequence analysis of various genes of ribosomal RNA gene cluster, and some house-keeping genes. Our study has shown that flowers of wild plants harbor several interesting and new species. One such yeast is being used for developing a process for production of biopolymer pullulan in collaboration with a research group from IMTECH. Other interesting yeasts isolated from flowers have shown ability to produce biosurfactants, lipase and sugar alcohols such as xylitol and erythritol. Studies are in progress to isolate yeasts from guts of beetles and other insects to examine their role in utilization/fermentation of xylose and other 5 carbon sugars.

Studies on purine degradation pathway of yeasts: The ability to degrade purine compounds have been found in all kingdoms and can occur either aerobically or anaerobically, but by separate pathways. The various purine-degradative pathways are unique and differ from other metabolic pathways because they may serve quite different purposes, depending on the organism or tissue. In the aerobic pathway, the committed step in the degradation of purine bases is the oxidation of hypoxanthine and xanthine to uric acid, catalyzed by xanthine oxidase/ dehydrogenase. Some fungi and yeast species have the ability to grow compounds of the purine degradation pathway like adenosine, adenine, inosine, hypoxanthine, xanthosine, xanthine, guanosine, guanine, uric acid. In the well characterized fungal system of *Aspergillus nidulans*, hypoxanthine and xanthine are oxidized to uric acid and the latter is converted to ammonium through the conventional pathway involving urate oxidase, allantoinase, allantoicase, ureido-glycolase and urease. Recently it was reported that, in the fungi, in addition to xanthine dehydrogenase, a completely different enzyme xanA is able to catalyse the oxidation of xanthine to uric acid. In yeasts purine degradation has received far less attention. We have initiated work on understanding the mechanisms of purine transport and degradation in yeasts.

After preliminary screening of several yeasts for their ability to degrade uric acid, five potential strains were selected for further work. Using genome sequence data, primers were designed to amplify uricase gene from *Kluyveromyces lactis, Pichia stipitis, P. segobiensis, Yarrowia lipolytica, Lachancea thermotolerans.* After

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successfully cloning the uricase gene from *Kluyveromyces lactis*, optimal conditions to obtain soluble protein with good expression levels were standardized using various concentrations of IPTG and different incubation temperatures. As the protein has His-tag, the expressed protein was purified using Ni-NTA matrix. The purity checking of the eluted protein was examined on SDS PAGE.

Biochemical properties of the enzyme like optimum temperature, optimum pH, temperature stability, pH stability and kinetics were studied. The molecular weight as determined using MALDI was found to be 36 kda and the protein structure was analyzed by Circular Dichroism spectrum. It contained α helices – 5.1 %, β sheets – 56.7%, Turns – 1.4%, Random coils – 36.7 %. To study structural properties of *K. lactis* uricase, the purified protein was set for crystallization by PEG screening solutions. X-ray diffraction data was collected for this crystal at 2.6 Å resolution with help of Dr. S.Karthikeyan's group, and the enzyme was found to be a tetramer (Figure 1). Cloning and characterization of other enzymes involved in purine degradation pathway in *Kluyveromyces lactis* is in progress.

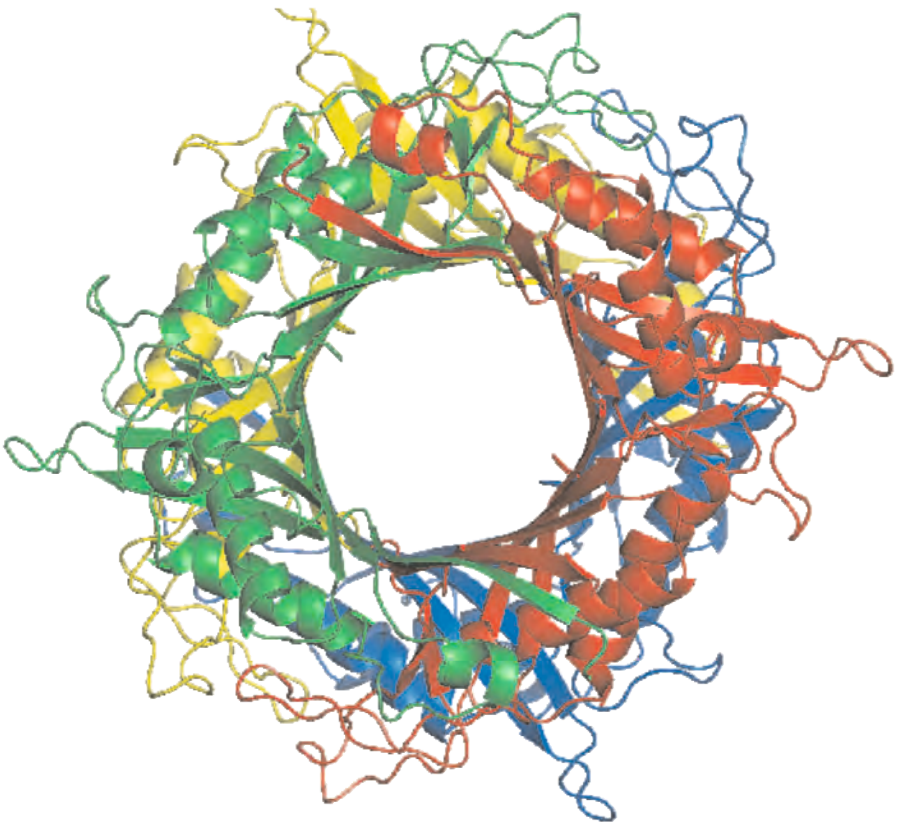

Figure 1: Crystal structure of uricase *Kluyveromyces lactis*

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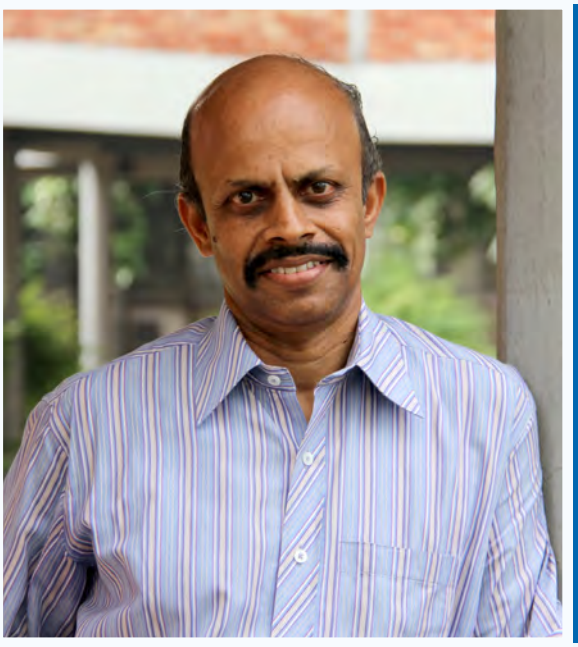

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Fungal biodiversity studies and culture collection related activities

I have been associated with the Microbial Type Culture Collection (MTCC) for more than twenty years. Pure, authentic and genetically stable microbial cultures are in demand all the time. Unfortunately culture maintenance in the best possible methods has been given low priority by the scientific managers. Commitment to such services are often neglected due to ill-manned collection centers which often suffer thro' inefficient staff and insufficient finances. It should be noted that the fungal organisms when introduced onto artificial media may be often indifferent in their phenotypic expression but their genetic make-up remain intact if preserved judiciously. The reputation of collection centers mainly rest on prompt supply of authentic cultures and reliable identification reports.

My assignments include preservation, maintenance, supply and identification of fungal cultures .With the top-quality service as the main objective, our section is working towards maintaining the highest standards with regard to supply, authentic identifications, processing of cultures or any related activities as expected from reputed culture collection centers. Regular collection trips are undertaken to different forest areas to bring as many fungi as possible to culture form. We periodically procure the type cultures from different culture collection centers to enable the identification assignments to be as authentic as possible. The 'Fungi' section holds the largest number of cultures and type cultures and the cultures are supplied in freeze-dried forms (wherever possible) as well as on agar slants. We always make sure that the cultures supplied from our centre and those received/ procured from outside sources undergo strict quality control tests before they reach their respective destinations.

Apart from the routine biodiversity studies, we are also working towards the revisions and monographic studies of a few fungal genera. In this direction we have started procuring the type cultures of all the species described under them. Such systematic studies have not been carried out nowadays and a great number of such

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monographic studies are outdated and badly need revisions based on new findings and culture collection centers, as in the past, have a definite role to perform.

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Exploration and exploitation of bacterial diversity

For the past one decade our group has been studying the bacterial diversity of different ecological niches of India and described several novel bacterial taxa (*Agrococcus carbonis, Microbacterium assamensis, Kocuria assamensis, Yaniella fodinae, Pontibacter rhizosphera, Cohnella ferri, Planococcus plakortidis* and *Actinoalloteichus spitiensis* which produces immunosuppresor molecule). Besides, few novel bacteria like *Bacillus horneckiae* sp. nov., isolated from a clean room where the Phoenix spacecraft was assembled; *Bacillus canaveralius* sp. nov., isolated from a spacecraft assembly facility; *Tetrasphaera remsis* sp. nov., isolated from the Regenerative Enclosed Life Support Module Simulator (REMS) air system. Currently our group is working on exploration and exploitation of unseen prokaryotic diversity using polyphasic approach. We propose to extend it to next generation sequencing and comparative genomics. Our group has also initiated work on applied enzymology, focusing on cholesterol oxidase, NRPS, and PKS through culturable and metagenomic approach.

Detection and expression of biosynthetic genes in marine *actinobacteria*: Most microbial organic molecules are secondary metabolites which consist of diverse chemical structures and range of biological activities. *Actinobacteria* are the prolific producers of these metabolites. Numerous novel secondary metabolites have been isolated from marine *actinobacteria*, although exploitation of this group is still at its infancy. A growing number of novel, bioactive secondary metabolites produced by marine invertebrates as well as marine bacteria appear to be structurally biosynthesized by polyketide synthases (PKS) and/or non-ribosomal peptide synthetases (NRPS). These enzymes are organized in a modular fashion and utilize specific domains to sequentially catalyze the condensation of simple carboxylic acids for PKS systems or amino acid building blocks for NRPS systems into a growing chain. Polyketides are classified as aromatic, polyenes and polyethers including macrolides. The PKSs are responsible for the synthesis of several pharmacologically important bacterial polyketides such as antibiotics (*i.e.* erythromycin, tetracycline), antitumor, immunosuppressive and cholesterol-lowering agents. Non-ribosomally produced peptides exhibit a remarkable spectrum of biological and pharmacological activities such as the antibiotics vancomycin and penicillin, immunosuppressive agent

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cyclosporine and the antitumor compound bleomycin. Thus, given the promising potential of marine *actinobacteria*, we will be employing different strategies to fully exploit the capacity of marine *actinobacteria* to produce bioactive secondary metabolites through detection and expression of biosynthetic genes specially PKS and NRPS.

Cholesterol consists of a sterol group (a combination steroid and alcohol). It is present in the cell membranes of all body tissues, and transported in the blood plasma of all animals. Cholesterol plays a central role in many biochemical processes, but is well known for its association with cardiovascular disease. Cholesterol Oxidase (EC 1.1.3.6) or 3-β-hydroxysterol is the enzyme which involved in cholesterol degradation pathway by microorganisms. Cholesterol oxidases are produced by many microorganisms from the different environments. It includes the member of *Actinobacteria* genera like *Arthrobacter Brevibacterium, Cellulomonas, Corynebacterium, Nocardia, Rhodococcus*, *Streptomyces Streptoverticillium*. Cholesterol oxidase is an enzyme of great commercial value. It is used by industries as well as in clinical chemistry. This is the second most frequently used enzyme after glucose oxidase in clinical chemistry. Due to its increasing applications recently much attention has been directed to screening and isolation of microorganisms which can produce cholesterol oxidase extra-cellularly. Our lab is focusing on screening of new cholesterol oxidase enzyme from various unexploited habitats, particularly marine habitats and also its purification, characterization and structural analysis.

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Participants:

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Studies on fungal biology

We are currently working on multi-gene phylogenetics and DNA-based identification of the members of economically and ecologically important fungal genus *Colletotrichum*. These fungi are mainly known and studied for their involvement in plant diseases such as anthracnose spots, blights and post-harvest rots of aerial plant parts, especially of commercially important fruits, vegetables, and ornamentals. Identification of *Colletotrichum* species, however, is complicated mainly due to the absence of a rigorous phylogenetic classification system. This study aims to improve our understanding of taxonomic diversity of *Colletotrichum* in India by multi-gene sequence-data analysis. Maximum likelihood analyses of an ITS dataset of 205 *Colletotrichum* isolates of Indian origin and selected reference sequences (mainly the type sequences) from NCBI-GenBank have revealed the presence of many cryptic species in this genus. Our recent studies based on translocation elongation factor gene sequence-data have revealed the existence of more than 15 cryptic species within *C. gloeosporioides* species complex. This suggests that there is an urgent need to develop a multi-locus DNA barcoding or a polyphasic approach in identification and delimitation of *Colletotrichum* species - especially those implicated in plant diseases.

We are also working on molecular phylogenetics of hypocrealean insect-associated fungi based on sequence-analysis of protein-coding genes. Insects and fungi (*i.e.* entomogenous fungi) are shown to have intimate interactions in various habitats. Entomogenous fungi have a great potential as a biological control agent to control insect populations. These fungi are polyphyletic and taxonomically diverse. Considering their economic and ecological importance, there is a need to resolve species boundaries of entomogenous fungi, especially of entomopathogenic fungi and pursue further research to reveal the genetic make-up of these fungi. One of the entomopathogenic fungal cultures, *Hirsutella thompsonii* MTCC 3556 has been selected to study the structure-function relationships of fungal chitinases.

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Publications:

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Studies on microbial diversity of aerobic and anaerobic ecosystems and their biotechnological applications

Prokaryotes are the dominant form of life on earth that survive under any conditions of the biosphere. They are considered to be the ancestors to all modern life and their evolution established the central plan for the living cell. We are using an integrated approach to understand the nature of free-living prokaryotes by studying microbial diversity and their ecology. This information provides an insight into the systematics of these prokaryotes and their biotechnological exploitation. The biotechnological applications extensively studied in our laboratory include bioactive production and bioremediation. Microorganisms existing in complex environments often require capability to produce antimicrobial compounds as a competitive advantage to producing strains for their establishment in these ecosystems. It is estimated that between 30-99% of the total bacteria and archaea produce at least one bacteriocin. We have explored few ecosystems to obtain bacteriocin producing strains. Among them, soil samples obtained from subsurface environments were processed for isolation of bacteriocin producing bacteria using dilution plate technique. From different samples few isolates (including both aerobic and anaerobic bacteria) exhibiting antimicrobial activity were obtained. These bacteriocin producing isolates were identified and the strains as well as bacteriocins exhibited differences with available bacteriocins were further characterized in detail.

Characterization of bacteriocins produced by diverse microorganisms: The 16S rRNA gene sequence and BLAST analysis of one of the strain designated as GI-9 had high similarity with species of genus *Brevibacillus* and exhibited 98.4% similarity with *B. laterosporus*. The bacteriocin produced by strain GI-9 was purified from supernatant using hydrophobic interaction chromatography and reverse-phase HPLC. While Electrospray TOF (ESI-TOF) studies determine the precise molecular mass of the peptide to be of 9892.15 Da, N-terminal sequencing of the peptide reveals low similarity with reported bacteriocins. The thermo stable bacteriocin with

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pH stability between pH 2.0-10.0 was significantly resistant to proteases and displays broad-spectrum yet specific antibacterial activity without any growth-inhibitory effect on yeast or fungi. Blast analysis of N-terminal sequence indicated that the peptide is a novel bacteriocin. Another strain IE-3 is found to be an obligate anaerobe and its 16S rRNA gene sequence BLAST analysis affiliated it to the genus *Pediococcus*. The thermostable bacteriocin produced by strain IE-3 inhibited growth of *Listeria monocytogens* and did not show any activity against other gram positive, gram negative or yeast test strains.

Studies on bioremediation: Screening of bacteria, both aerobic and anaerobic, for chloronitrobenzoates bioremediation has been done through PCP and other chloronitrobenzoates enrichment of sediment samples obtained from polluted environments and industrial effluent. Among the isolates, few strains exhibited high ability to grow on PCP as sole carbon source at concentrations of up to 1.5 mM and could utilize different chloronitrobenzoates. These isolates are identified as species belonging to *Enterobacteriaceae*, *Bacillus*, *Burkholderia* and *Pseudomonas* using 16S rRNA gene sequencing and biochemical characterization.

Biochemical characterization of pentachlorophenol (PCP) degradation: PCP degradation studies were carried out using strain PD3 to identify the degradation intermediates. The HPLC analyses of sample collected at 0 h time point showed presence of only PCP peak. However, sample collected at 20 h time point showed presence of three metabolites with retention time of 7.5, 5.0 and 3.2 min and these peaks were closely matching to those of observed with the standards of 2, 3, 5-trichlorophenol, 3, 5-dichlorophenol and 3-chlorophenol. However, there were no peaks observed with samples collected at same interval from un-inoculated controls. Further, the results obtained from HPLC were confirmed by GC-MS with m/z values and the mass spectra showed similar to those metabolites. The strain PD3, being a facultative anaerobe, it was also checked for the degradation of PCP compound under aerobic condition. Results of aerobic degradation experiments indicated that it could not degrade PCP in aerobic conditions, but could utilize TCP, DCP and mono chlorophenol as sole carbon source under aerobic conditions. In support to this, liberation of $^{14}CO_2$ from ^{14}C-labeled chlorophenols, used as substrate, confirmed that the strain PD3 is capable of mineralizing TCP, DCP, and mono chlorophenols under aerobic conditions. The preference to degrade lower chlorinated phenols under aerobic conditions indicates the absence of enzyme PCP-4 monooxygenase in strain PD3, essentially required for PCP degradation under aerobic conditions yielding hydroquinones as intermediates. In conclusion, anaerobic cultures of bacterial strains capable of degrading PCP into lower chlorinated phenols are very useful in remediation of contaminated soils, especially environments like sludge or sediments where anoxic conditions prevail. Therefore, strain PD3 in the present study, could be an excellent model for *in-situ* remediation of PCP contaminated environments.

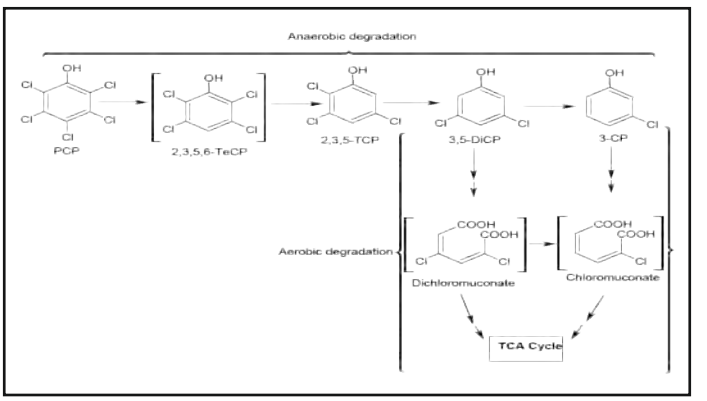

Figure 1: Proposed pathway for PCP degradation under anaerobic and aerobic conditions by *Escherichia* sp. strain PD3. 2, 3, 5, 6-TeCP (2, 3, 5, 6-tetrachlorohydrophenol), 2, 3, 5-TCP (2, 3, 5-trichlorophenol), 3, 5-DCP (3, 5-dichlorophenol), 3-CP (3-chlorophenol), dichloromuconate and chloromuconate. Metabolites shown in the brackets were not detected in this experiment.

Industry-sponsored projects (entitled "Studies on soil analysis for RCGM approval"): The soil analyses studies for transgenic brinjal and mustard has been carried out for RCGM approval. These soil analyses studies were performed for biosafety regulatory approval.

- "Microbial comparative studies on soil ecosystem of Bt and Non-Bt brinjal crops" sponsored by Bejo Sheetal Pvt. Ltd.

- "Assessment of impact of transgenic *Brassica juncea* (mustard) on soil microflora" sponsored by Delhi University.

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Phototrophic bacterial diversity of marine ecosystems of India based on culture dependent studies

Phototrophic bacteria are those which use light as energy source for growth, more specifically the conversion of light energy into chemical energy in the form of ATP. Prokaryotes that can convert light energy into chemical energy include the photosynthetic "*Cyanobacteria*", the purple and green bacteria. The "*Cyanobacteria*" conduct oxygenic photosynthesis; the purple and green bacteria conduct anoxygenic photosynthesis.

Oxygenic phototrophic bacteria: "*Cyanobacteria*" (also known as blue-green algae) is a phylum of bacteria that obtain their energy through photosynthesis. The name "*Cyanobacteria*" comes from the color of the bacteria (Greek Kyanos = blue). The ability of "*Cyanobacteria*" to perform oxygenic photosynthesis is thought to have converted the early reducing atmosphere into oxidizing one, which dramatically changed the composition of life forms on Earth by stimulating biodiversity.

Distribution of Cyanobacteria: "*Cyanobacteria*" are distributed in all possible biotypes of the world. They have several attributes and strategies, which enable them to colonize and survive under extreme habitats. The factors that affect the distribution of "*Cyanobacteria*" include pH, soil moisture, mineral nutrients and combined nitrogen. "*Cyanobacteria*" can be found in almost every environment, from oceans to fresh water to bare rock to soil. "*Cyanobacteria*" proved themselves successful in occupying freshwater, brackish, marine and hypersaline environments. Marine forms employ both halophily and halotolerance as survival strategies. The open ocean, the largest and ecologically most stable marine environment is the home of picophytoplaktonic unicells, classified within genera, *Synechocystis*, *Synechococcus* and *Prochlorococcus*. These tiny microorganisms contribute significantly to the primary production of lakes, oceans and lagoon water. Bloom forming nitrogen-fixing filamentous "*Cyanobacteria*", taxonomically clustering around the genera *Kathagnymene* and *Trichodesmium* are common in all tropical oceans.

Bloom forming planktonic heterocystous "*Cyanobacteria*" , on the other hand, seem to be restricted to eutrophic,

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enclosed epicontinental seas and phosphorous-overloaded lakes.

Anoxygenic phototrophic bacteria: Anoxygenic phototrophic bacteria (APB) are a group of phototrophs performing photosynthesis without the evolution of oxygen. The photosynthetic apparatus in these bacteria differs from that in oxygenic phototrophic bacteria, algae and green plants in that they lack photosystem II and the electron donor is not water. They require an electron donor more reduced than water such as H_2, reduced sulfur compounds (sulfide, thiosulfate, tetrathionate *etc*.) or simple organic compounds or sometimes-ferrous iron. The photosynthesis is mediated by photosynthetic pigments comprising bacteriochlorophylls and carotenoids. The phototrophic bacteria contain a great variety of carotenoid pigments. They function as accessory light harvesters and also serve to protect the photosynthetic apparatus against photo-oxidation. Some of the carotenoids are characteristic of families, genera and even species of phototrophic bacteria, and together with the chlorophylls they are largely responsible for the various colors of organisms within the group. Thus, some purple bacteria may be brown, or even green. Likewise, green bacteria may be brown or even orange, as in the case of chloroflexus growing in bright light.

The APB have the ability to fix CO_2 as well as utilize organic carbon compounds. Autotrophy is the preferred growth mode in purple and green sulfur bacteria while heterotrophy is favored by purple non-sulfur, green non-sulfur and heliobacteria. The purple non-sulfur bacteria are the most versatile in growth modes being able to grow under photoheterotrophy, photoautotrophy, chemo auto and heterotrophy and by fermentative metabolism. They also utilize a wide variety of organic compounds *viz*., organic acids (aliphatic and aromatic), amino acids, alcohols and simple sugars [rarely complex sugars] which serve different functions depending upon the growth mode. Under phototrophic conditions they are the primary source of electron donor and/or carbon while under chemotrophic conditions they are the sources of carbon/e^- donor as well as energy.

Distribution of Anoxygenic phototrophic bacteria: Anoxygenic phototrophic bacteria are widely distributed in aquatic and a few terrestrial environments where light of sufficient quality and quantity is available and anaerobic conditions prevail from where they can be enriched and isolated. Their ideal ecological niches include habitats such as lakes, waste lagoons, intertidal zones, sediments of ditches, ponds and moist soils. They are found in diverse aquatic habitats such as fresh water, hyper saline, alkaline and acid water to hot springs, Arctic and Antarctica. The pH, temperature, salinity, light intensity and concentration of sulfide and oxygen are important for the occurrence of different groups of APB in their habitat where they play an important role in the carbon, nitrogen, hydrogen, and sulfur cycles.

Marine Ecosystems: Marine ecosystems are a part of the largest aquatic system on the planet, covering over 70% of the Earth's surface. The habitats that make up this vast system range from the productive near shore regions to the barren ocean floor. Some examples of important marine ecosystems are: Oceans, estuaries, salt marshes, mangrove forests and coastal areas like Lagoons.

India, being a tropical country abounds in biodiversity. In terms of marine environment, India has a coastline of about 8000 km, an exclusive economic zone of 2.02 million sq. km adjoining the continental regions and the offshore islands and a wide range of coastal ecosystems such as estuaries, lagoons, mangroves, backwaters, salt marshes, rocky coasts, sandy stretches and coral reefs, which are characterized by unique biotic and abiotic properties and processes.

The primary goal of the project is to explore the diversity of phototrophic bacteria of marine habitats of India. Polyphasic taxonomic analysis of the isolates obtained and validation in the International code of bacterial nomenclature. For the past one year, the group is working on the diversity aspects of phototrophic bacteria and their screening for carotenoids, and biopolymers. Highlights of the work carried out by the group are as follows

Sampling: Samples were collected from mangrove forests of Bitherkanika (Orissa) and Namkhana (West Bengal) and salterns, seashore water of Kakinada (Andhra Pradesh).

Isolation of Phototrophic bacteria: The samples were inoculated in respective media for different phototrophic bacteria (Figure 1 & Figure 2). Positive enrichments were purified by repeated streaking on agar slants of respective media.

The isolates were identified using 16S rRNA gene sequence analysis (Table 1, Figure 3).

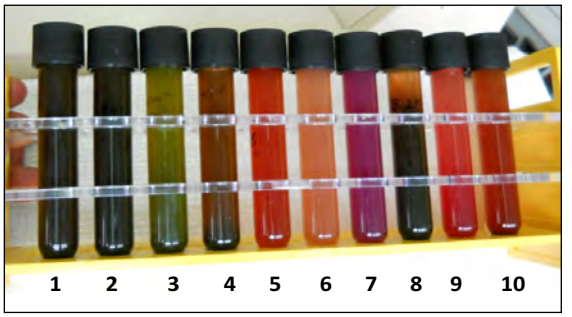

Figure 1: Anoxygenic phototrophic bacteria (representing both green and purple bacteria).

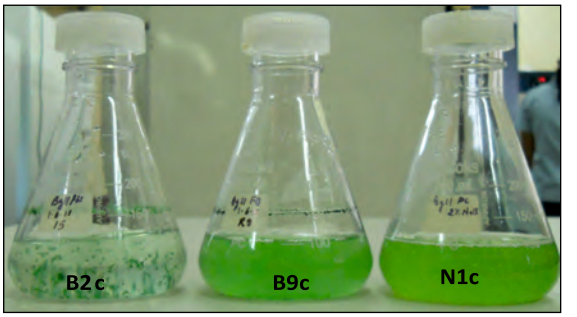

Figure 2: Cyanobacteria - isolated by our group.

Table 1. Identity of the isolates based on 16S rRNA gene sequence analysis.

GSB, green sulfur bacteria

S. No.	Sample	Nearest neighbor	Absorption spectra of acetone extracts [wave length (nm)]
1	B2-p	*Rhodoplanes pokkaliisoli* (99.2%)	578, 504, 474, 450, 419
2	B56-ps	*Marichromatium indicum* (98.5%).	577, 501, 473, 446
3	B6-p	*Rhodovulum imhoffii* (99.2%)	576, 481
4	N1-ps	*Marichromatium indicum* (99.2%).	577, 502, 473, 445
5	N2-ps	*Marichromatium gracile* DSM 203(T) (99.8%)	578, 501, 473, 448, 359
6	N16-ps	*Marichromatium gracile* DSM 203(T) (97.7%)	577, 500, 473
7	N22-ps	*Thiorhodococcus bheemlicus* (98.6%)	577, 501, 473
8	N24-p	*Bradyrhizobium japonicum* LMG 6138(T) (98.8%)	578, 481
9	N3-aa	*Erythrobacter gaetbuli* SW-161(T) (99.3%)	480, 452
10	B9-aa	*Porphyrobacter dokdonensis* DSW-74(T) (98.6%)	461

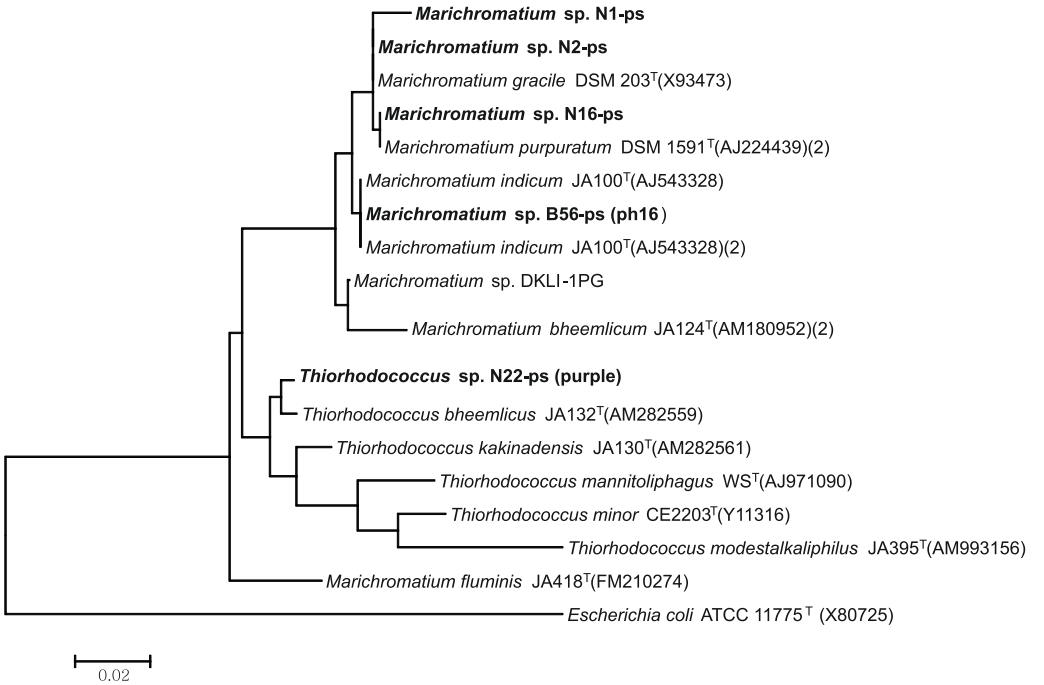

Figure 3: Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of the isolates within the family *Chromaticaeae*. Bar, 2 nucleotide substitutions per 100 nucleotides

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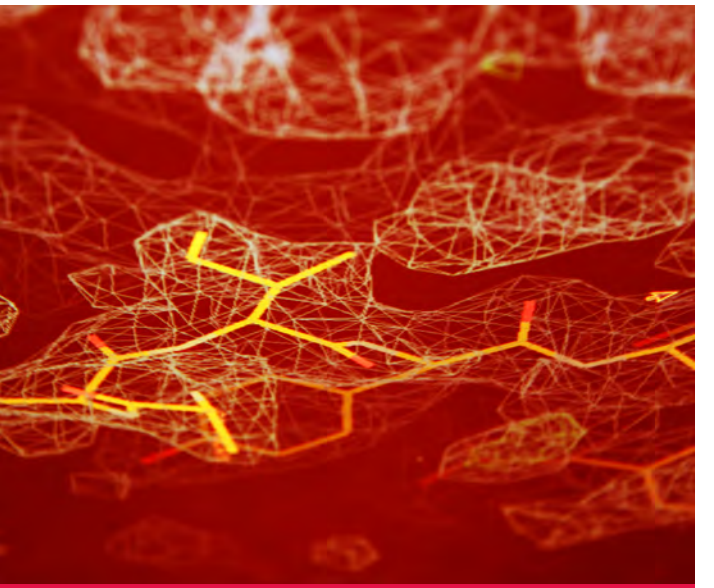
protein science and engineering



research & development programmes

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Fundamental and applied studies on (A) the mechanism of human plasminogen activation by the bacterial co-factor protein, streptokinase, and (B) microbial Community studies on soil metagenomes

Search for new exo-sites in Streptokinase that mediate enzyme-substrate interactions: Interest in our laboratory has long focussed on elucidating the structure-function inter-relationships that confer the remarkable substrate specificity to the streptokinase-plasmin activator complex for the single scissile peptide bond of human plasminogen. The establishment of such structure-function correlations in this system is of central importance to the design of more effective clot dissolving proteins.

Recently, we identified a new exosite of streptokinase that is involved in substrate HPG recognition and catalytic turnover, that is located in the beta-domain of this three-domain protein (Aneja et al., 2009; *J Biol. Chem.* 284, 32642–32650). In order to identify possible new exo-sites/substrate interacting sites in the gamma (γ) domain of streptokinase, mutants were generated by error-prone random mutagenesis of the gamma domain and its adjoining region in the beta domain, followed by functional screening specifically for substrate plasminogen activation. By this procedure, single-site mutants derived from various multi-point 'mutation-clusters' identified the importance of discrete residues in the gamma domain that are important for substrate processing. Among the various residues, Aspartate at position 328 was identified to be critical for substrate HPG activation, as evidenced through extensive mutagenesis of its side-chain, namely D328R, D328H, D328N, and D328A. Other mutants found to be important in substrate plasminogen activation were, namely R319H, N339S, K334A, K334E and L335Q. When examined for their 1:1 interaction with human plasmin, these streptokinase mutants were found to retain the native-like high affinity for plasmin, and also generate amidolytic activity with partner plasminogen in a manner similar to wild-type streptokinase. Moreover, cofactor activities of the mutants, pre-complexed with plasmin, against microplasminogen as the substrate, as well as *in silico* modeling studies

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(Figure 1) suggested that the region 315-340 of the γ domain interacts with the serine protease domain of the macromolecular substrate. Overall, our results clearly identify the presence of a new substrate-specific exosite in

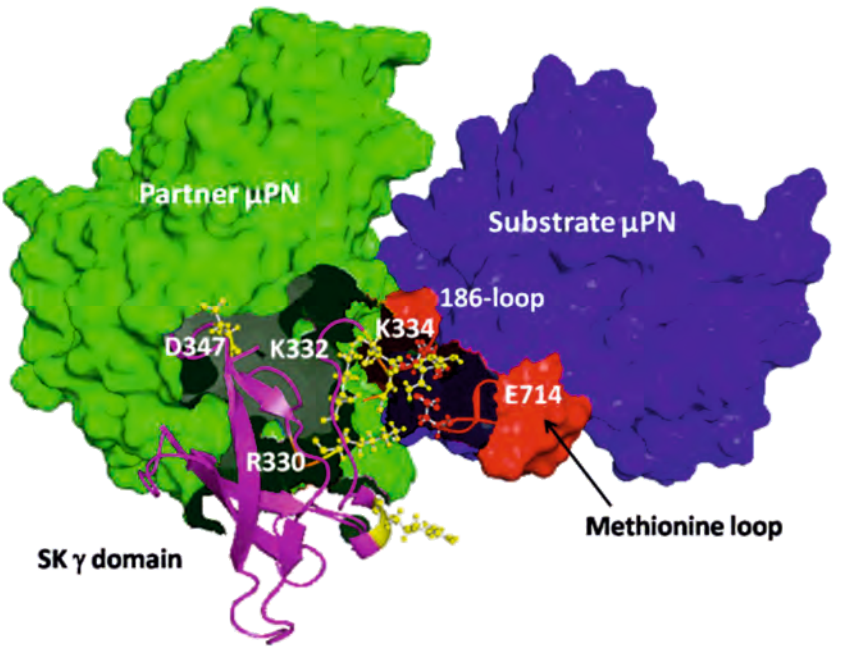

Figure1. Putative ternary interaction model of partner and substrate µPG interaction/s with the SK domain highlighting the newly discovered exosite in the domain. The ternary interaction model of µPN.SK.µPN complex generated by ZDOCK. Close-up of interactions of SK domain (*pink*), catalytically important residues (*yellow; ball and stick forms*) can be observed to be interacting with substrate µPN (*blue*) particularly residues of methionine loop and 186-loop *(red; ball and stick),* and the region of SK domain visualized to be interacting with partner µPN (shown in *green*); (reproduced from Yadav et al., 2011., *J Biol Chem* 286:6458-69).

the γ domain of streptokinase. The discovery of several such exo-sites in the streptokinase-plasmin complex provides novel mechanistic insights whereby these are used to transform the relatively non-specific active site of plasmin into a highly specific one, that exhibits almost exclusive fidelity for the single, scissile peptide bond in its macromolecular substrate, plasminogen. A more general translation of this concept, if made possible, through protein engineering approaches, for specifically targeting intrinsic human proteases towards infectious agents, such as viruses, offers tantalizing possibilities for bio-medical applications of the future.

Comparative Metagenomic Profiling of Soil samples isolated from Assam's Kaziranga Wild life sanctuary using Next-generation sequencing technologies: The soil metagenome is an important genetic resource for discovery of altogether new genes, enzymes and natural products which have long gone unnoticed due to lack of the knowledge of the right culturing conditions. We report here the results of the whole metagenome sequence information of the Indian sub-Himalayan belt, namely Assam's Kaziranga Wild life sanctuary, which is recognised to be a world biodiversity hot-spot. The data, which represents the phylogenetic as well as functional metabolic diversity profile present within the microbial community of the soil, was then also compared with those obtained from other important sites reported earlier viz. Sargasso sea, Acid Mine drainage biofilm, and Luquillo experimental forest soil. Overall, the sequence information of the soil metagenome of Kaziranga provided invaluable and, in many ways, unique bio-resource information. The comparative analysis of different metagenomes highlighted the much higher level of genetic complexity in the Kaziranga eco-system as compared to sources like Sargasso Sea and acid mine drainage biofilms (which were much more simple in terms of biodiversity), but was qualitatively similar to more complex metagenomes from soils such as Luquillo experimental Forest soil, which reinforces the uniqueness of each metagenome in terms of the underlying

adaptation dynamics and the biogeochemical condition of each environment.

In addition to the gene-profiling, we have isolated several new genes encoding cellulases and other carbohydrate processing enzymes of biotechnological interest from soil metagenomic libraries.

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Patents:

• Shekhar Kumar, Neeraj Kumar Maheshwari and Girish Sahni. Mutants of streptokinase and their covalently modified forms. Indian Patent Application No. 1845/DEL/2010 (31.3.2008); PCT/IN2009/000212 (31.3.2009).

• Neeraj Maheshwari and Girish Sahni. Protein Fusion constructs possessing thrombolytic and anticoagulant properties. Indian Patent Application No. 1845/DEL/2010 (5.8.10); PCT/IB2011/001825 (5.8.2011).

Participants:

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Studies on structure-function & molecular mechanism of haemoglobin function(s) in *M. tuberculosis*

Mycobacterium tuberculosis carries unusual single and two domain hemoglobins having novel structural features. Expression profile and ligand properties of these oxygen binding proteins are quite distinct from each other suggesting that they may be catering different physiological functions. The present study aims to understand the molecular mechanism of hemoglobin functions in *M. tuberculosis* and evaluate their role in intracellular survival and pathogenicity. Previous studies in our laboratory demonstrated that HbN carries a potent oxygen dependent nitric oxide dioxygenase activity and is capable of supporting growth of a recombinant *E. coli* under gaseous nitric oxide and nitrosative stress (Pathania *et. al.*, Mol. Microbiology, 2002; Lama *et. al.*, FEBS Lett., 2006) and HbO associates with the respiratory membranes and stimulate its respiratory activity that is dependent on cytochrome *o* (Pathania *et. al.*, J. Biol. Chem., 2002). Expression of HbN and HbO in an intracellular pathogen *Salmonella enterica* Typhimurium conferred distinct growth advantage within the microphages (Pawaria *et.al.*, Microb. Pathogen. 2007) and a substantial level of HbN and HbO is produced by *M. tuberculosis* during *in vivo* infection (Pawaria *et. al.*, Appl. Environ. Microbiol. 2008).

HbN of *M. tuberculosis* carries two unusual structural features; a 12 residue long highly charged N-terminal motif (Pre-A) and a tunnel system within the protein matrix having short and long tunnels. Recently, we have shown that the NO-scavenging ability of HbN is regulated by its N-terminal Pre-A region (Lama *et al.*, J. Biol. Chem, 2009) that modulates the protein dynamics in a unique way and facilitates ligand migration towards the active site.

In continuation of previous study, we further extended our attempt to explore the role of these structures in modulating the NO-scavenging properties of HbN of *M. tuberculosis*.

• **Role of Pre-A motif : Site-directed and deletion mutagenesis of Pre-A region:** Deletion of Pre-A region from the N-terminus of HbN resulted in attenuation of its NO-dioxygenase activity. Molecular modeling studies on Pre

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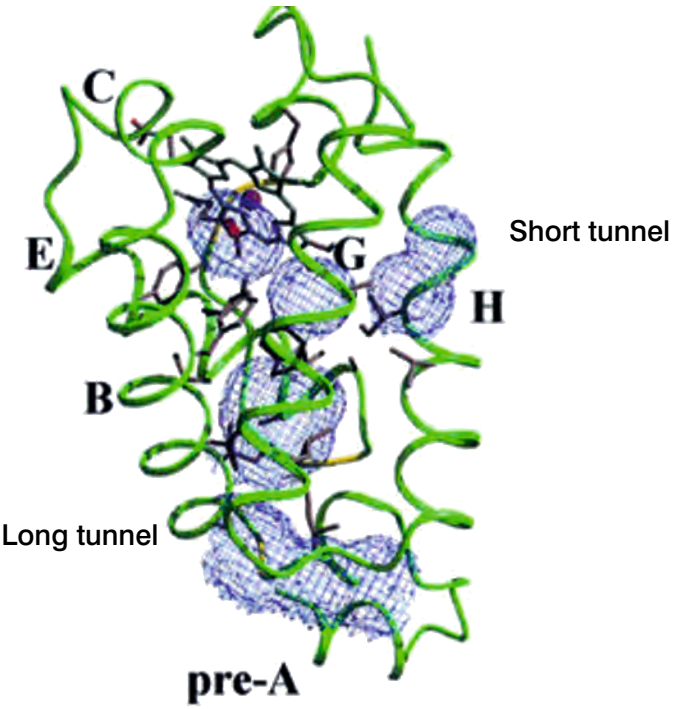

Figure 1: Structure of truncated hemoglobin, HbN of *Mycobacterium tuberculosis* showing existence of long and short tunnels within the protein matrix.

A deleted mutants indicated distinct changes in dynamics of protein backbone and blockage of long protein tunnel gate due to altered position of Phe62 residue. To understand the molecular basis of this effect, site directed mutants of Pre-A region, where positively charged residues were individually or in combination were mutated into alanine. There are five positively charged residues within the 12 residue N-terminal motif of HbN. These positively charged residues were replaced with alanine through site-specific mutagenesis and mutant protein was expressed and characterized. Replacement of charged residues within the Pre-A region resulted drastic reduction in the ability of HbN to resist toxicity of acidified nitrite, NO and nitrosative stress. We further mutated individual charged residues of Pre-A region and each mutant was characterized with respect to NOD activity. These studies indicated that Arg6, Arg7 and Lys9 are crucial for the protein function but Arg10 can be replaced without having any significant effect on protein function.

• **Role of long protein-tunnel gate on protein function: site directed mutagenesis of Ph62 of HbN:** HbN carries a branched tunnel within the protein matrix. It has been speculated that long and short tunnels of protein are involved in controlling the entry of oxygen and NO respectively. within the active site. To investigate the role of this tunnel in ligand access and NO-detoxification function of HbN, Phe62 residue that reside at the gate of long tunnel, was replaced with Ala, Trp, Tyr or Ile. These mutants were cloned and expressed and mutant proteins were purified. Oxygen and CO binding studies on these mutants indicated that these mutants are able to bind oxygen very similar to wild type HbN, however, a drastic change in their NO binding properties were observed indicating Ph62 at the long tunnel gate is modulating the NO entry and access to the active site. NO uptake and pattern of NO oxidation by oxygen bound protein indicated significant attenuation in their NO-dioxygenase activity and nitrate release.

Overall biochemical and physiological studies unraveled that Phe62 residue of long tunnel branch may be playing a vital role in acquisition of NO, thus, establishing the involvement of long tunnel branch of HbN in

modulating the NO-scavenging activity of HbN by controlling the access and diffusion of NO towards the active site.

Expression of HbN in *M. tuberculosis* alters lipid profile of cell wall and immune responses during intracellular infection: To gain insight into the functional role of HbN, we overexpressed HbN in *M. smegmatis* and *M. tuberculosis* and observed its effect on cell physiology and immunological responses during macrophage infection. Overexpression of HbN resulted in altered cell morphology and lipid profile of cell membranes. Such effects were not observed when HbN was expressed in *M. smegmatis* suggesting that the HbN mediated changes in cellular metabolism are specific to *M. tuberculosis*. Lipid profile of cells where level of fatty acids show significant changes with presence of a few new lipids. These interesting observations indicated the involvement of HbN in modulating lipid biosynthesis. However, the mechanism by which HbN is conferring these effects is not clear at present.

Since, overexpression of HbN resulted in altered cellular morphology and lipid components of *M. tuberculosis*, we compared the infectivity and immunological responses of wild type and HbN expressing cells of *M. tuberculosis* during intracellular infection into THP-1 macrophages and mouse peritoneal cell lines. When equal number of control and HbN expressing cells of *M. tuberculosis* were allowed to infect these cell lines, HbN expressing cells displayed higher infectivity as compared to control cells as determined by CFU (colony forming units) count. HbN expressing cells of *M. tuberculosis* H37 Ra were also found to enhance the level of IL-10 and cytokines during macrophage infection.

Overall, the present studies on mycobacterial hemoglobins, have unveiled novel mechanism of NO-dioxygenation by HbN of *M. tuberculosis*. Furthermore, alterations in lipid profile and immune reponses of HbN overexpressing cells of *M. tuberculosis* during intracellular infection have provided new insights into the involvement of hemoglobins in modulating the lipid biosynthesis virulence of tubercle bacillus. Attempts are underway to explore these novel aspects of mycobacterial hemoglobins and their relevance during intracellular infection.

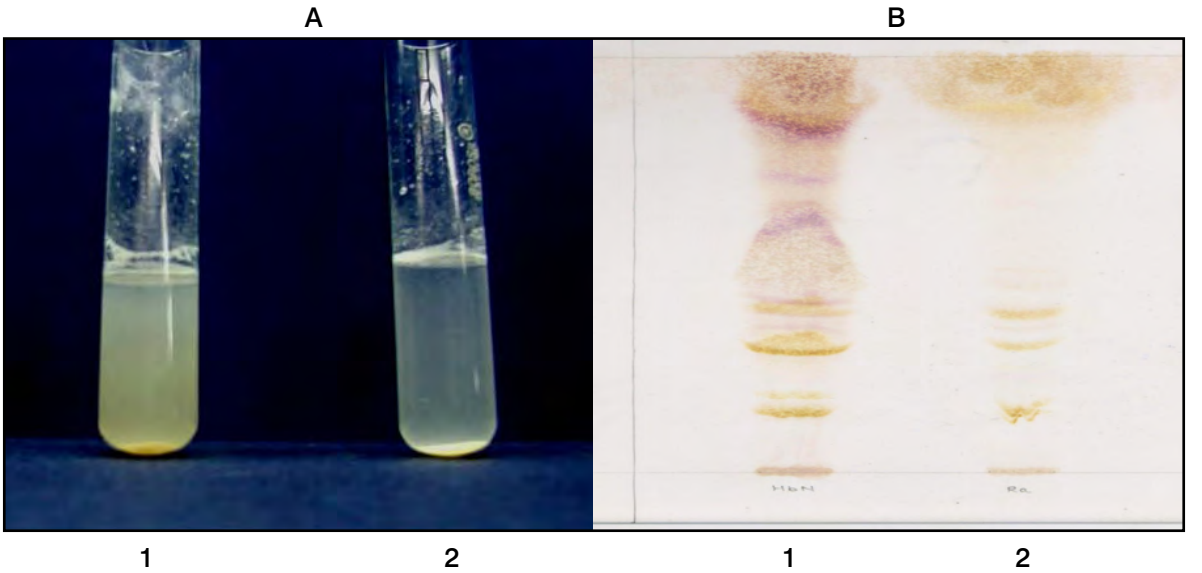

Figure 2 : Growth (A) and lipid profile (B) of cell membrane of HbN overexpressing (1) and the wild type (2) cells of *Mycobacterium tuberculosis* H37Ra.

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• Anand, A., Duk, B. T., Singh, S., Akbas, M. Y., Webster, D. A., Stark, B. C. & Dikshit, K. L. (2010). Redox-mediated interactions of VHb (Vitreoscilla haemoglobin) with OxyR: novel regulation of VHb biosynthesis under oxidative stress. *Biochem J* **426**, 271-80.

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• Stark, B. C., Dikshit, K. L. & Pagilla, K. R. (2011). Recent advances in understanding the structure, function, and biotechnological usefulness of the hemoglobin from the bacterium *Vitreoscilla*. *Biotechnol Lett*. Epub ahead of print, PMID 21603987.

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Conformational analysis of bio-active peptides and model-peptides of biological relevance

In recent years, the establishment of structure-property relationship of peptide *mimics* containing two- or three methylene units, by inserting readily available non-proteinogenic unsubstituted aliphatic β- or γ-amino acid segment, continue to attract considerable attention. Primarily, besides our group, Gellman and co-workers have exploited unsubstituted β- or γ-amino acids to design and construct well-defined unusual folded-unfolded structural features, stabilized by intramolecular hydrogen-bonds. It is widely known that methylene units usually adopt energetically preferred an all-*trans (*or a fully-extended or an all-*anti*) conformation. As a part of our investigation aimed at assessing the conformational features that can be accommodated across unsubstituted β- or γ-amino acid residues, we recently investigated single crystal X-ray structure of a model peptide: Boc-Pro-γ-Abu-OH (**1**) particularly, lacking the C-terminus amide group. Interestingly, the peptide **1** favoured an overall tightly folded backbone topology. In conjunction with an unusual type *a* disposition of the urethane moiety, the three methylene units with four degree of conformational freedom: ϕ, θ_1, θ_2 and ψ, facilitated the formation of a weak non-conventional C_i-H$\cdots$O$_i$ type intramolecular hydrogen-bond, encompassing a six-membered ring motif, as depicted in Figure 1. *(N. Kumar et al. 2010).*

Concurrently, we have been investigating the preferred structural features of an immunomodulating tetrapeptide, rigin (H-Gly-Gln-Pro-Arg-OH). Of note, the phagocytosis index of rigin is found to be the same as that of another structurally related and extensively investigated immunomodulating tetrapeptide, tuftsin (H-Thr-Lys-Pro-Arg-OH). In order to explore conformational characteristics of rigin, a combined use of high-temperature unrestrained MD simulations in implicit water and one- and two-dimensional ^{1}H NMR spectroscopic techniques have been employed. The distribution of backbone torsion angles revealed the predominance of *trans* conformers across the Xaa-Pro peptide-bond. The results of MD simulations indicated that of the five predicted families **A -E**, the predominant families: family **A** (92 structures), family **C** (63 structures) and family **D**

R. Kishore did Post M.Sc. Diploma in Molecular Biophysics and Ph.D. (1987) in Molecular Biophysics from the Indian Institute of Science, Bangalore before joining IMTECH in 1994.

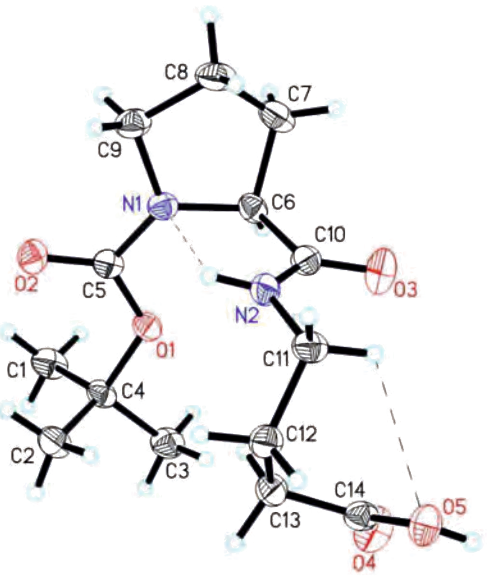

Figure 1: The crystal molecular structure of Boc-Pro-γ Abu-OH showing the folded topology across the γ -Abu moiety.

(31 structures), could be complemented by extensive 1D- and 2D ^{1}H NMR parameters acquired in aqueous phosphate buffered saline (PBS, pH 7.4) solution. Although, a significant variation in side-chains orientations have been observed. A survey of specific inter- and intraresidue NOEs substantiated the predominance of an unusual type VII β-turn structure, defined by two torsion angles *i.e.*, $\psi_{Gln} \sim 155^{o}$ and $\phi_{Pro} \sim -65^{o}$ across the Gln-Pro segment. The proposed semi-folded kinked topology precluded formation of any intramolecular hydrogen-bond or electrostatic interaction, as depicted in Figure 2.

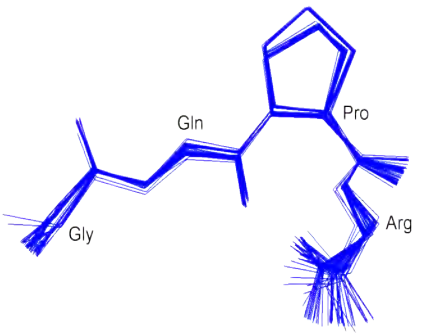

Figure 2: An unusual type VII β-turn like secondary structure *i.e.*, $\psi_{Gln} \sim 155^{o}$ and $\Phi_{Pro} \sim -65^{o}$, of rigin, not stabilized by any intramolecular interaction.

We also examined the chiroptical properties of rigin in solvents of varied polarities *i.e.*, a polar aqueous PBS and a hydrophobic 2,2,2-trifluoroethanol (TFE), to assess its overall structural stability. Far-UV CD spectral characteristics of rigin in aqueous PBS solution and in structure promoting organic solvent TFE revealed strong solvent dependence. In PBS solution, the presence of a strong negative shoulder at ~ 198 nm and an extremely weak negative shoulder at ~ 234 nm could be ascribed to a small population with ordered and/or semi-folded topology. However, in TFE, the CD spectrum showed a prominent negative maximum at ~ 221 nm and positive ellipticity centered at ~ 194 nm, indicative of high proportion of ordered structures. We propose that the plausible structural attributes *i.e.,* type VII β-turn structure, may be exploited for design and rigidification of the bioactive conformation of this immunomodulator towards improved immunopharmacological properties.

Publications:

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• Kumar, N. & Kishore, R. (2010). Determination of an unusual secondary structural element in the immunostimulating tetrapeptide rigin in aqueous environments: insights via MD simulations, (1)H NMR and CD spectroscopic studies. *J Pept Sci* **16**, 456-64.

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Engineering the folding, aggregation, stability and functions of globular proteins

Engineering of protein function: Our group has invented and demonstrated the efficacy of a novel protein engineering method for the grafting of functional protein surfaces amongst structurally-homologous proteins. A key insight resulting from this work is the understanding that conditions causing enzyme inactivation need not be correlated to those effecting enzyme unfolding, with the resulting application being that novel enzymes can be built that independently dissect these two characteristics away from each other and re-assort them in ways that nature, and evolution, do not employ.

Understanding kinetic structural stability of hyperthermophile proteins: Our group has discovered and experimentally established the novel idea that the high kinetic stability of conformationally stable proteins owes to a reduction in the overall cooperativity of the global unfolding process, which is achieved through increased structural autonomy of individual sub-structures (consisting of supersecondary structural elements). The group has demonstrated this increased sub-structural autonomy through protein truncation experiments; established that a key factor is enhanced surface salt bridge interactions, and demonstrated that destruction of selected salt bridges can dramatically reduce kinetic stability and even facilitate mesophile protein-like behavior (e.g., cold denaturation) in hyperthermophile proteins.

Role of molecular recognition in protein aggregation: Our group has discovered and demonstrated that protein aggregates can consist of chains containing significant levels of native-like structure, explaining why certain aggregates arise from homo-molecular recognition, while others tend to be hetero-molecular, and also why amyloid formation is linked to protein structural stability, in addition to demonstrating approaches for developing three novel types of reagents for the blocking of protein aggregation. Further, the group has also developed a novel understanding of the mechanism of amyloid formation, and demonstrated evidence of a negative correlation between protein structural stability and the tendency to form amyloids. It is noteworthy that

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Purnananda Guptasarma received his Ph.D. (1994) in Molecular Biophysics and Biochemistry from the Centre for Cellular and Molecular Biology, Hyderabad. He did post-doctoral research at the Dept. of Biochemistry, University of Cambridge, U.K., before joining IMTECH in 1996.

the group's work over the last several years, together with the collaborating group of Dr. M. Luthra-Guptasarma in PGIMER, Chandigarh, has led to several novel insights into how aggregates might cause disease. These are summarized in Figure 1.

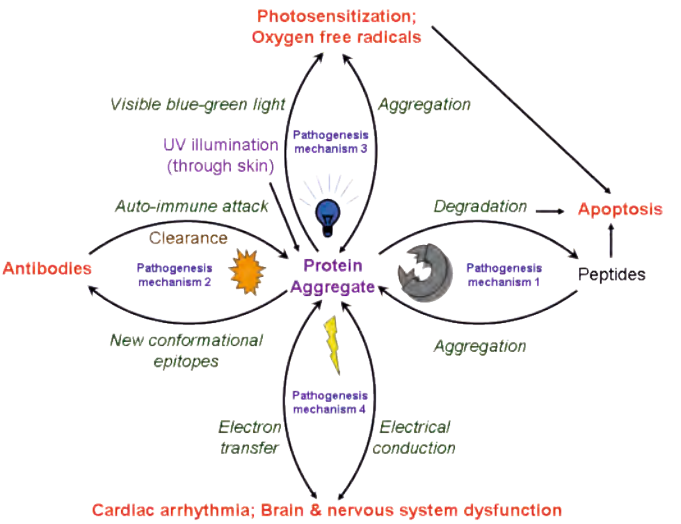

Figure 1.

Protein folding and the sequence-structure relationship: Our group has explored several novel aspects of the sequence-structure relationship in proteins through protein engineering-based approaches querying the effects of altering sidechain identity without altering sidechain nature, OR altering polypeptide backbone direction, OR shuffling substructures around within symmetric protein structures. The group has shown, using a model protein (chymotrypsin inhibitor 2) that global replacement of residues with other residues of like nature, but altered identity, does not necessarily alter chain folding tendencies, indicating that residue nature is more important at early stages of folding, with residue identity potentially becoming important only during later stages of residue packing, affecting stability. The group has also shown how an excised beta-finger can be used to form a novel all-beta protein. These studies have shed light on aspects of protein folding that have not been hitherto explored.

Novel analytical methods and tools: Our group has discovered and characterized a novel blue fluorescence in proteins derived from hydrogen-bonded peptide bond orbitals, and shown that this fluorescence is sensitive to protein secondary structure, like far-UV circular dichroism. This discovery has now been confirmed by independent studies from a group in Europe. Our group has also shown that the phenomenon responsible for the novel fluorescence facilitates the use of proteins as conducting electronic materials. Further, in terms of the development of novel analytical methods, the group has invented and demonstrated a novel method for experimentally discovering and destroying secondary structures in mRNA that inhibit translation by ribosomes (thus affecting protein expression), through a novel DNA sequencing-based techniques.

Industrial enzymes and therapeutic proteins : We have cloned and expressed various thermostable enzymes of potential industrial value (proteases, cellulases, amylases etc) from thermophile genomes through heterologous overexpression in bacteria. We have also worked in protein engineering of therapeutic proteins (interleukin-2 and interferon-gamma) to sidestep known problem-causing characteristics in these proteins, e.g., aggregation or facile degradation.

More details about our group's work may be found at http://www.guptasarmalab.in

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• Chandrayan, S. K. & Guptasarma, P. (2009). Attenuation of ionic interactions profoundly lowers the kinetic thermal stability of *Pyrococcus furiosus* triosephosphate isomerase. *Biochim Biophys Acta* **1794**, 905-12.

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• Guptasarma, P., Kapoor, D., Singh, B. & Karthikeyan, S. (2010). A functional comparison of the TET aminopeptidases of *P-furiosus* and *B-subtilis* with a protein-engineered variant recombining the former's structure with the latter's active site. *Enzyme Microb Technol* **46**, 1-8.

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Riboflavin biosynthesis pathway: Potential anti-bacterial drug target

Multiple-drug resistance in pathogenic microorganism is at a steadily increasing rate and poses significant threat both in hospitals and more recently in the community. This increasing incidence of antibiotic resistance urged the scientists worldwide to look for discovery and development of new antibacterial drugs. As the current drugs in the market attack only handful of targets, the development of drugs that can act on new targets is urgently needed. Among the known strategies for designing new antibiotics, the enzymes involved in the cofactor/coenzyme biosynthesis offers several advantages for drug development as most of the microorganisms are strictly dependent on endogenous biosynthesis of cofactors. While, genomic studies have suggested that the riboflavin/FAD biosynthesis pathway is essential for many bacteria, this pathway is absent in humans. Therefore, the enzymes involved in riboflavin/FAD biosynthesis pathway are considered as potential drug targets. In the development of drug against a target, the crystal structure plays a vital role by revealing the position of active site at atomic level of the target molecule and the key interactions with the substrates and products. This key information is important for rational drug design and help to identify the lead compounds more quickly. Moreover, the determined three-dimensional structure can be used for virtual screening of the chemical library of compounds that can be a potential antagonist specific for the enzyme. As a first step, our group is focusing on the crystal structure determination of enzymes involved in riboflavin biosynthesis pathway from pathogenic bacteria to develop it as potential drug target.

Structural characterization of ribB from *Salmonella typhimurium*: 3,4-dihydroxy-2-butanone 4-phosphate synthase (DHBPS) encoded by ribB gene is one of the first enzymes in riboflavin biosynthesis pathway and catalyzes the conversion of ribulose-5-phosphate (Ru5P) to 3,4-dihydroxy-2-butanone 4-phosphate and formate. We cloned, expressed and purified the *Salmonella* DHBPS enzyme to its homogeneity. The recombinant DHBPS enzyme requires magnesium ion for its activity and catalyzes the formation of 3,4-dihydroxy-2-butanone 4-phosphate from ribulose-5-phosphate at a rate of 199 nmol min^{-1} mg^{-1} with K_m value of

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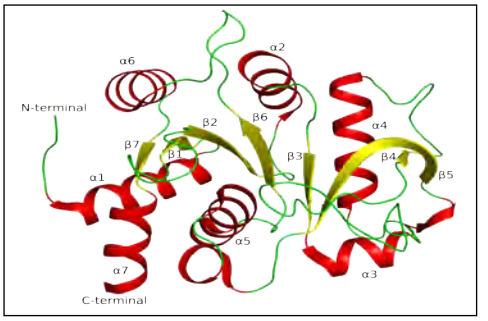
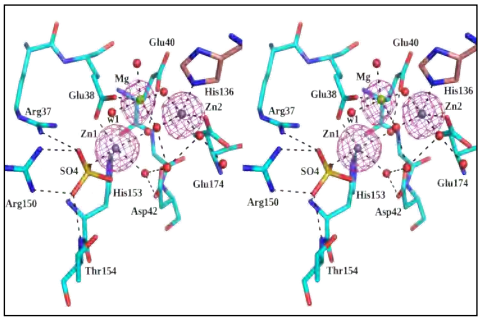
S. Karthikeyan obtained his Ph.D. (1999) from All India Institute of Medical Sciences, New Delhi. He did his post-doctoral research at Harvard Medical School and at UTSW-Medical Center, Texas, USA. He was at IIT, Roorkee as an Assistant Professor before joining IMTECH in 2005.

116 μM at 37^{0}C. Further, we have determined the crystal structures of *Salmonella* DHBPS in complex with sulphate, ribulose-5-phosphate and sulphate-zinc ion at a resolution of 2.80, 2.52 and 1.86 Å respectively. Analysis of these crystal structures reveals that the acidic loop (residues 34-39) responsible for the acid-base catalysis is disordered in the absence of substrate or metal ion at the active site. Upon binding either substrate or sulphate and metal ions, the acidic loop becomes stabilized, adopts a closed conformation and interacts with the substrate. Our structure for the first time reveals that binding of substrate Ru5P alone is sufficient for the stabilization of the acidic active site loop into a closed conformation. In addition, the Glu38 residue from the acidic active site loop undergoes a conformational change upon Ru5P binding, which helps in positioning the second metal ion that stabilizes the Ru5P and the reaction intermediates.

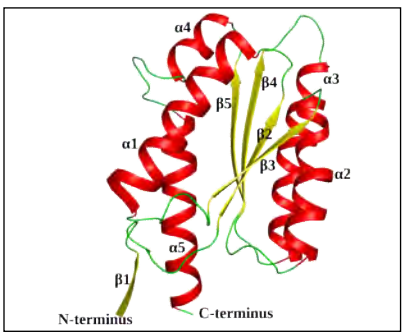


Cartoon representation of sDHBPS with secondary structure elements labeled. The α-helices are shown in red, β-strands are shown in yellow and loops are shown in green.

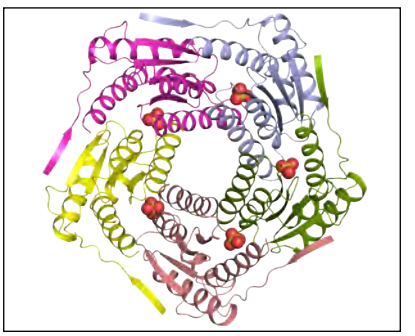


Stereoview of the active site of sDHBPS bound with sulphate, magnesium (green) and zinc (purple) ions. The difference Fourier electron density map covering the Zn^{2+} ions are contoured at 5.0 σ level.

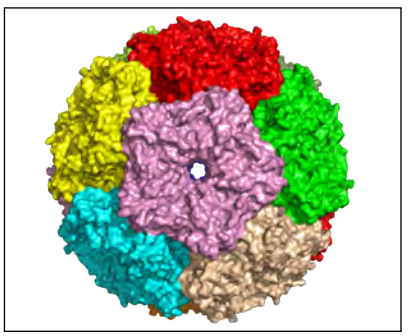
Structural characterization of Lumazine synthase from *Salmonella typhimurium*: Lumazine synthase, an enzyme involved in the penultimate step of riboflavin biosynthesis, catalyzes the formation of 6,7-dimethyl-8-ribityllumazine from 3,4-dihydroxy-2-butanone 4-phosphate and 5-amino-6-ribitylamino-2,4-(1*H*,3*H*)-pyrimidinedione. We have cloned, over-expressed and purified the lumazine synthase from *Salmonella typhimurium* (sLS) and crystallized it in three forms, each with different crystal packing. We have determined the crystal structure of sLS in monoclinic $P2_1$ space group with 60 subunits per asymmetric unit, packed as an icosahedron, at 3.57 Å resolution. Interestingly, the sLS has a N-terminal proline residue (Pro11) which was previously suggested to disrupt the icosohedral assembly formation.



Cartoon representation of the subunit fold of sLS.

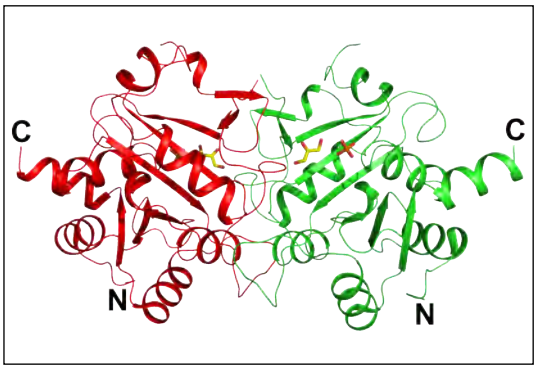


Pentamer assembly of sLS where each subunit is represented in different colour.

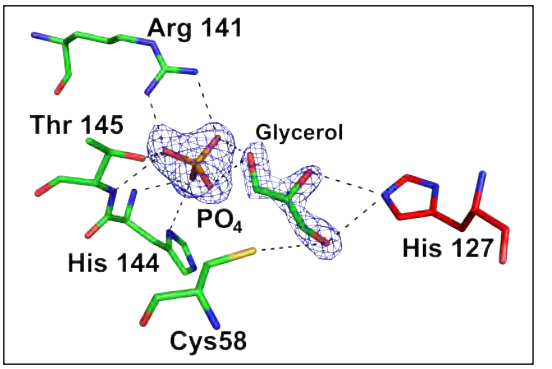


Surface representation of icosahedral assembly of sLS showing each pentamer in different colour.

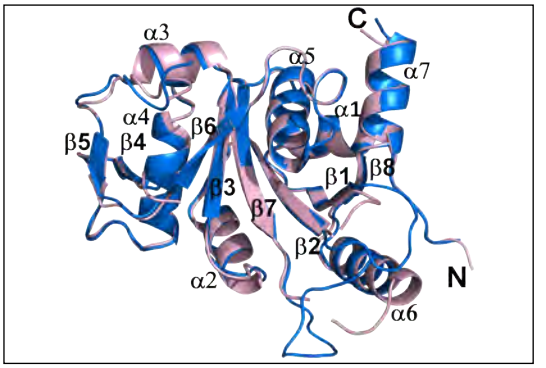
Structural basis for pH dependent monomer-dimer transition of ribB from *M. tuberculosis*: In *Mycobacterium tuberculosis* (*Mtb*), the *ribA2* gene (Rv1415) encodes for the bi-functional enzyme with DHBPS and GTPCH-II domains at N- and C-termini, respectively. We have determined three crystal structures of *Mtb*-DHBPS domain in complex with phosphate and glycerol at pH 6.0, with sulphate at pH 4.0 and with zinc and sulphate at pH 4.0 at 1.8 Å, 2.06 Å and 2.06 Å resolution respectively. The hydrodynamic volume and enzyme activity studies revealed that the *Mtb*-DHBPS domain forms a functional homo-dimer between the pH 6.09.0, however, at pH 5.0 and below, it forms a stable inactive monomer in solution. Furthermore, the functional activity of *Mtb*-DHBPS and its dimeric state could be restored by increasing the pH between 6.0 to 9.0. The comparison of crystal structures determined at different pH revealed that the overall three-dimensional structure of *Mtb*-DHBPS monomer remains the same. However, the length of the α6-helix at pH 6.0 has increased in pH 4.0 achieving a higher structural stability at pH 4.0. Taken together our experiments strongly suggest that the *Mtb*-DHBPS domain can transit between inactive monomer to active dimer depending upon its pH values, both in solution as well in crystal structure.



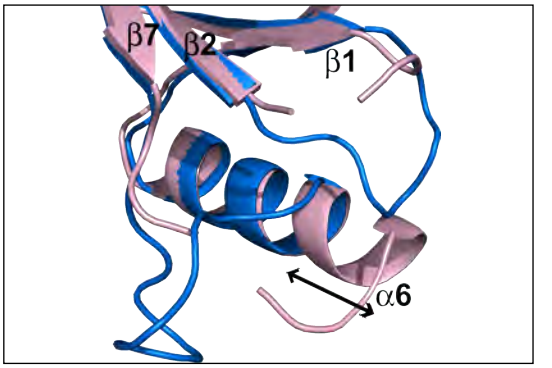
Cartoon representation of functional homo-dimer of *Mtb*-DHBPS domain.



The final $2F_o$-F_c electron density map covering glycerol and phosphate molecule is contoured at 1.0 σ level. Residues marked in red are from the neighboring subunit.



Superposition of *Mtb*-DHBPS monomers from pH 6.0 (blue) and pH 4.0 (pink).



The close view of conformational change of loops connecting β7 and α6 and β1 and β2.

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• Kapoor, D., Singh, B., Subramanian, K. & Guptasarma, P. (2009). Creation of a new eye lens crystallin (Gambeta) through structure-guided mutagenic grafting of the surface of betaB2 crystallin onto the hydrophobic core of gammaB crystallin. *Febs Journal* **276**, 3341-53.

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• Das, A. K., Pathak, A., Sinha, A., Datt, M., Singh, B., Karthikeyan, S. & Sarkar, D. (2010). A single-amino-acid substitution in the C terminus of PhoP determines DNA-binding specificity of the virulence-associated response regulator from *Mycobacterium tuberculosis*. *J Mol Biol* **398**, 647-56.

• Kumar, P., Singh, M., Gautam, R. & Karthikeyan, S. (2010). Potential anti-bacterial drug target: structural characterization of 3,4-dihydroxy-2-butanone-4-phosphate synthase from *Salmonella typhimurium* LT2. *Proteins* **78**, 3292-303.

• Kumar, P., Singh, M. & Karthikeyan, S. (2011). Crystal structure analysis of icosahedral lumazine synthase from *Salmonella typhimurium*, an antibacterial drug target. *Acta Crystallogr D Biol Crystallogr* **67**, 131-9.

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Development and characterization of Probiotics and Designer Probiotics

Probiotics are defined as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" WHO conference 2001. Every human has certain amounts (10^{8}- 10^{11}) of useful bacteria in the gut, intestine, and colon known as domestic microbes. These bacterial population differs from human to human in their diversity and density. It is well understood that lesser the domestic bacteria brighter the chances for frequent illness *e.g.,* bacterial infections, intestinal bowel disease, diarrhea, *etc*. Our objective lies in reducing the disease burden in those humans by developing human consumable probiotic formulations by using lactobacillus strains. In order to achieve our goal we isolate, identify and characterize certain microbes for their efficacy as probiotics.

Recent developments in our lab has lead to the isolation, identification and characterization of novel bifidobacterial strains with high probiotic efficiency. During the process of their characterization we succeeded in identifying and developing a purification system for novel peptide which functions as an antagonistic against gram positive and gram negative microbes such as *Listeria monocytogenes, streptococcus, staphylococcus, clostridium, Vibrio Cholerae etc*. Presently we are unraveling its sequence, structure *etc*.

We also deal with development of recombinant probiotics, here our main objective lies in utilization of bifidobacterium for delivery of certain antigens for therapy. The field of bifidobacterium for delivery is in infantile stage therefore we are in the process of developing, cloning and expression plasmids which will be later utilized for cloning, expression of antigens such as human tumor necrosis factor-α (hTNF). Although, TNF has the ability to regress the tumors, it cannot be used for cancer treatment because of its severe side effects *eg*. Ischemia and un-wanted signal transduction pathway initiation. Therefore, the best way of delivery is only through lactobacillus. Hence, our main objective lies in development of recombinant probiotics for therapy.

In our future plans we intend to develop probiotics and recombinant probiotics for therapy. Recent study in our

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lab lead to find novel bacteriocins from some of our novel bacterial isolates. Our main objective lies in understanding bifidobacterial bacteriocins their origin, structure and mechanism. After which we would like to develop designer bacteriocins having improved functions.

Unique features of the bacteriocin purified are:

- Mol.wt above 5kDa and approx 9kDa.
- Wide spectrum antimicrobial activity.
- Free thiols are essential for activity.
- Active pH 2 to pH 12 and after autoclaving also.

Publications:

- Rajagopal, K. & Bhalla, N. (2010). Simple gene synthesis method bypassing overlap extension. *Int J Gen Eng Biotechnol* **1**, 131-139.
- Ranjan, R. K. & Rajagopal, K. (2010). Efficient ligation and cloning of DNA fragments with 2-bp overhangs. *Anal Biochem* **402**, 91-2.

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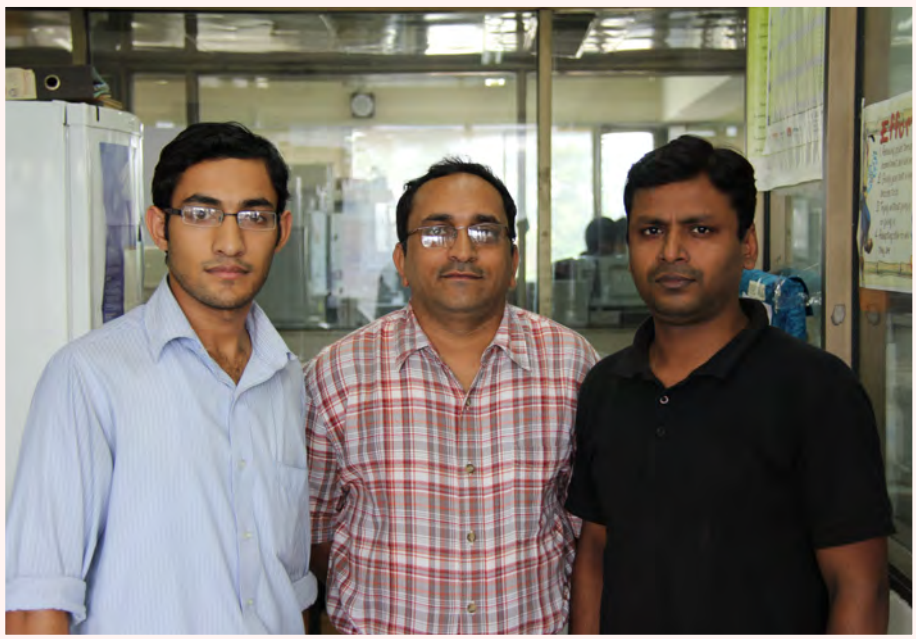

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Understanding the evolutionary mechanisms that shape our protein universe

Proteins are the molecular machines that enable various cellular processes. All life forms sequenced to date share a significant portion of their protein repertoire. Proteins are composed of one or more domains - elementary units that can independently function and evolve. The current estimate is that several billion different proteins may exist in the myriad life forms found on Earth. This enormous diversity has most likely evolved from a small number (possibly a few thousand) of ancestral protein domains. Over the course of several billion years, these domains have diverged to perform a wide variety of functions. My research interests focus on identifying and categorizing the evolutionary relationships between protein domains, and understanding the prevalent mechanisms by which proteins evolve.

Recent advances in genome sequencing and in structural genomics have contributed substantially to our perception and knowledge of the size and nature of the protein universe. These efforts open uncharted regions of the protein universe for exploration and offer a means to uncover new insights into the evolution and diversity of protein structure and function. Our research interests are in methodology development to find similarities between proteins and to understand how protein sequences and structures evolve over time. In addition, one of the long term goals of our group is to develop a comprehensive framework for annotation and structural classification of all proteins.

Sequence-Structure-Function Relationships of Zinc Finger Proteins: Zinc fingers are small protein domains, typically 20-50 amino acids long, in which the zinc ion plays a structural role contributing to the overall stability of the domain. A diverse set of functions including protein-DNA, protein-RNA, protein-protein and protein-small-molecule interactions are mediated by zinc finger domains. A comprehensive structural classification of zinc fingers has been initiated in order to understand and appreciate the relationships between these functionally versatile domains. The protein database is scanned regularly to identify novel members of this interesting group

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Srikrishna Subramanian received his Ph.D. (2001) in Molecular Biophysics from the Indian Institute of Science, Bangalore. He did post-doctoral research at the University of Texas Southwestern Medical Center at Dallas and was Assistant Professor (Research) at the Sanford | Burnham Medical Research Institute, La Jolla, California, USA before joining IMTECH in 2010.

of proteins and such proteins are further studied by *in silico* sequence and structure similarity methods to place them in the correct evolutionary context and derive functional hypothesis for these proteins.

Recently, we identified that the bridge-region of the Ku superfamily is an atypical zinc ribbon domain. In this study, we showed by sequence and structural analysis that the bridge region of the dsDNA repair protein Ku is in fact a segment-swapped zinc ribbon domain that in most organisms has lost its ability to chelate metal. Ku is a crucial component of the non-homologous end-joining repair of double-stranded breaks in DNA and is involved in a variety of repair, recombination and end protection processes, including the maintenance of genomic stability. Our findings allow for a better understanding of how this protein senses double-stranded breaks in DNA.

Sequence - Structure - Function relationships among members of the Ferredoxin-like fold: The ferredoxin-like fold is one of the most populated folds for which three-dimensional structural information is available. We have initially focused on the classification of an interesting superfamily of the ferredoxin-like fold termed the alpha+beta barrel group. The proteins of this group either homo-dimerize or contain two evolutionarily-related domains of the ferredoxin-like fold that are arranged in a back-to-back manner to form a barrel. Several proteins structures of this group exist many of which have no known function. We have focused on identifying all proteins belonging to this superfamily. Remote sequence similarity methods as well as structure similarity tools have been used to identify novel members of this group. Detailed analysis to shed light on the evolution of these proteins and their mutual relationships are being carried out.

Publications:

• Krishna, S. S. & Aravind, L. (2010). The bridge-region of the Ku superfamily is an atypical zinc ribbon domain. *Journal of Structural Biology* **172**, 294-299.

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Molecular mechanism of signal transduction and gene regulation by *M. tuberculosis* PhoP-PhoR system

Bacterial adaptation usually takes the form of transcriptional regulation of genes, products of which specifically help the bacterium cope with the given microenvironment. One of the systems bacteria most frequently use to sense chemical or physical changes in the environment is the two-component regulatory systems. Such a system typically consists of a histidine protein kinase that functions as an environmental sensor and a response regulator that mediates the transcriptional processes. The sensor kinase is usually autophosphorylated in response to environmental signals and subsequently transfers the phosphate to the regulator. In general, the response regulator functions as phosphorylation-activated switches that often mediate a cellular response through transcriptional regulation.

A number of investigations show that inactivation of *phoP* of *M. tuberculosis phoP-phoR* system leads to significant growth attenuation. Biochemical results reveal that PhoP regulates biosynthesis of sulfolipids, diacyltrehaloses and polyacyltrehaloses and absence of these complex lipid molecules in the *phoP* mutant is a major reason for its attenuated growth in a mouse model. While two independent studies show that a point mutation in PhoP contributes to avirulence of *M. tuberculosis* H37Ra and also accounts for the absence of polyketide-derived acyltrehaloses in H37Ra, more recently PhoP has been implicated in the ESAT-6 secretion and specific T-cell recognition during virulence regulation of the bacilli. Thus, growing evidences strongly suggest that PhoP is a key regulator in *M. tuberculosis*. However, molecular mechanism of how the regulator functions remains largely unknown.

As a part of our ongoing effort on understanding mechanism of PhoP function, we have identified PhoP-regulated genetic determinants in the regulatory region of the *phoP* gene. We further investigated the origin(s) of binding specificity in protein complexes, and probed the determinants of complex formation using biochemical assays. Our results show that two molecules of *M. tuberculosis* PhoP protein recognizes a 23-bp sequence of

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Dibyendu Sarkar received his Ph.D. (1998) in Biochemistry and Molecular Biology from the Department of Biophysics, Molecular Biology and Genetics, University of Calcutta. He did his post-doctoral research in the MCB Department, Brown University, USA before joining IMTECH in 2002.

the *phoP* upstream region comprising a direct repeat motif to promote transcription regulation. Our data are consistent with a model in which PhoP binds to DNA in a head-to-head orientation to form a symmetric dimer via N-terminal receiver domain of the protein. This is in broad agreement with the most recently-solved crystal structure of the full-length PhoP.

To explore structural effect of protein phosphorylation and to examine effect of phosphorylation on DNA binding and/or transcription regulation, we are interested in the molecular mechanism responsible for transmission of the conformational change associated with phosphorylation of PhoP that is expected to influence DNA binding by the C-terminal domain. To better understand the functions of the N-terminal domain and inter-domain interaction(s) in effector domain regulation, we sought to investigate domain structure of PhoP. Our results identify an 11-residue long inter-domain linker that tethers two functionally-independent domains of PhoP together and regulates inter-domain interactions. While the newly-identified linker region is not required for either domain functions of PhoP, most strikingly, it plays an essential role for phosphorylation-dependent DNA binding to target promoter, previously suggested to be regulated by PhoP (Figure 1). These observations invite speculation on additional level(s) of complexity in the regulation of PhoP-controlled transcription processes. Together, our results suggest that although the DNA binding energy and specificity of regulator-promoter interactions is contributed primarily (but not entirely) by the C-domain, linker region of the protein likely allows the regulator to adopt a different phosphorylation-dependent conformation enabling it to discriminate target promoters while it regulates a vast array of genes to either activate or repress transcription (Figure 1).

In summary, our primary objective is to continue to explore and probe protein-protein and protein-nucleic acid interaction(s) that constitute the signaling network of *M. tuberclosis* PhoP-PhoR system, which is of key importance to understand and unravel the molecular mechanism of virulence regulation of the pathogenic organism.

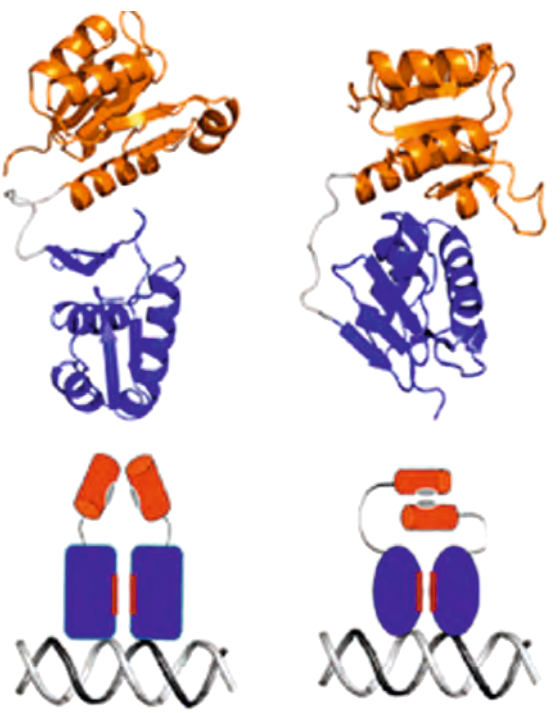

Figure 1: Two different conformations of the *Mycobacterium tuberculosis* PhoP with dual mode of DNA binding by the linker-independent and the linker length-dependent form of the protein.

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Understanding nuclear receptors and engineering therapeutic proteins

Host-Pathogen Interaction (*Mycobacterium tuberculosis*, *Mtb*): *Mtb* is the etiologic agent of tuberculosis in humans and is responsible for more morbidity than any other bacterial disease (Global Tuberculosis Control, *WHO report 2010*). The resurrection of tuberculosis globally, despite the usage of Bacille Calmette-Guerin (BCG) vaccine and the introduction of Directly Observed Therapy (DOT) in many high-burden regions, has led to escalation in the efforts to understand the cellular mechanisms of *Mtb*–macrophage interactions. Recent studies have shown evidence that *Mtb* hijacks host cellular factors for its survival. Interactions of *Mtb* lipids with macrophage membrane receptors for sensing and phagocytosis of *Mtb* have also been reported. We are investigating host–pathogen interactions, particularly those between host lipid-sensing nuclear receptors (LSNRs) and *Mtb*-macrophage lipids, which contribute to *Mtb* pathogenesis or clearance (Figure 1). We have a "look beyond NF–κB approach", an overrated pharmacological molecule in macrophages, to other nuclear receptor that have recently been reported to find expression in immune cells. Also a "look beyond *Mtb* lipid repertoire conventional functional understanding" and address their ability to modulate host lipid sensing nuclear receptors.

Autoimmune Disorder and Immune Cell Plasticity: Various effector CD4+ T cells – designated T helper type 1 (Th1), T helper type 2 (Th2), interleukin (IL)-17-producing T helper cells (Th17), IL-9-producing T helper cells (Th9) and regulatory T cells (Treg) – are characterized by their distinct patterns of cytokine production. They are also characterized by signature expression of nuclear receptors such as Th1:Tbet, Th2:GATA3, Th17:RORγ, and Treg:FoxP3 (Figure 2). Several other nuclear receptors, some of which are lipid-sensing, find expression in these various subsets, without clearly described functions. Although our major focus is to explore the ever-increasing complexity of the differentiation and plasticity among T helper cells at the level of molecular pharmacology, we also plan to investigate the role of these nuclear receptors during Th17 expansion (*e.g.*, Th17 with IL-23R surface receptor expression, as well as IL-17 and IL-23 cytokine expression). We are also assessing their ability to

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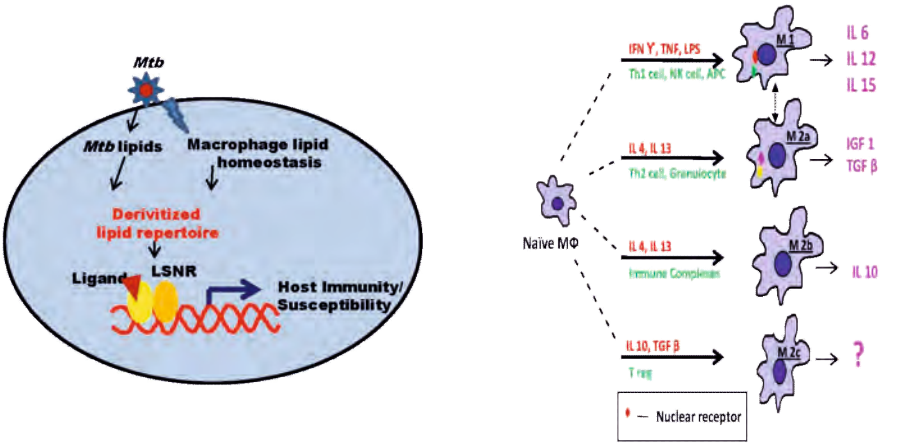

Figure 1: Regulation of macrophage lipid sensing nuclear receptors by *Mtb* - Mϕ lipids and associated Mϕ plasticity.

modulate the signature surface receptors and cytokines of the Th17 phenotype, specifically the surface expression of IL-6R and IL-21R receptors, and expression of cytokines IL-17, and IL-21. We also plan to investigate their role in TGFβ/TGFβ+IL-6 induced plasticity among Treg/Th17, for understanding and combating autoimmune diseases. We are also investigating how environmental lipids can modulate lipid-sensing nuclear receptor in dendritic cells (DCs) maturation and function and their priming (IL-6) of T-Helper cells so as to modulate these diseases. The information thus gathered can become the basis for therapeutic pharmacological modulation of diseases. We are working on mouse autoimmune models of collagen-induced arthritis (CIA) and myelin oligodendrocyte glycoprotein-induced encephalomyelitis (MOG-EAE) and also clinical samples to study differential expression and function of transcription factors in plasticity, polarization and activation of T cell, B cell, DCs and macrophage.

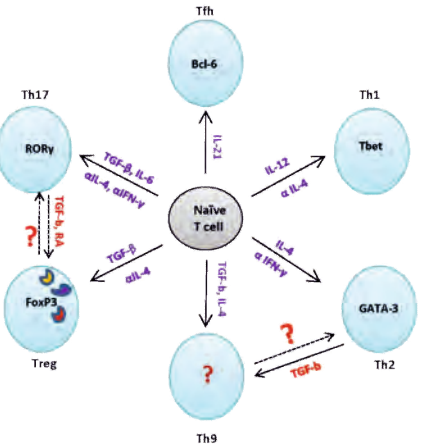

Figure 2: Nuclear receptor and T-cell plasticity.

Proteomics: We are deciphering novel post translational modification (PTM) to, and complex regulation of, orphan/nuclear receptors. Also proteome-vide screening for all-trans retinoic acid, vitamin B6 and acrolein conjugation. Look beyond conventional understanding of Vitamin A's physiological form: retinoic acid (atRA) which is known as a ligand. atRA initially was identified to be conjugated with some proteins and was subsequently identified as a ligand. As an inherent bias towards the significant observation of it being a ligand, its other non-genomic role in signaling and as a PTM have largely been ignored. Our studies strongly suggest 'Retinoylation' as a novel PTM. Look beyond conventional understanding of vitamin B6 as a cofactor of a large number of essential enzymes in the human body. There are physiologies of vitamin B6 that cannot be explained by its role just being a cofactor. Besides, vitamin B6 has been known to be associated with non-enzymatic complexes. We hypothesize that vitamin B6 function has potential role in physiology as a PTM event 'Pyridoxylation' beyond our current understanding of it being a simple co-enzyme.

SMART and Switchable Therapeutic Proteins: Protein engineering is the process of developing useful or valuable proteins by rational design and directed evolution. The protein engineering market is forecasted to rise to $118 billion by 2012, with majorly a recombinant therapeutic market of $53 billion. These recombinant therapeutics include but are not limited to hormones, growth factors, interleukins, interferons, proteases,

subunit vaccine, humanized antibodies-derivatives etc. With most of these products already in the market and because of issues related to them, there is an emergent need of next generation recombinant therapeutics which are more smart, controlled and effective. We are working on Selective Mutagenesis and Rational Transplantation (SMART) of therapeutic and membrane traversing peptides from stem bromelain, a phytotherapeutical protein. Switchable recombinant erythropoietin is being attempted for expression in insect and mammalian cells. Protein Nucleic Acid (PNA) mimetics for selective antigene/antisense therapy towards infectious disease is also being attempted.

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Participants:

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Biology of Macromolecular assemblies: Architectural and regulatory features

My lab is interested in synthesizing the knowledge of assembly, structure, and regulatory mechanisms of macromolecular assemblies that form biological circuits. We use the knowledge of biology, chemistry, and physics to understand the regulatory strategies of macromolecular assemblies and define their physiological role in the cell. Our goals will be achieved using a variety of techniques including molecular biology, cell biology, X-ray crystallography, analytical ultracentrifugation, isothermal titration calorimetry (ITC), fluorescence & CD spectroscopy, mass spectrometry, surface plasma resonance, light scattering, steady state & pre-steady state kinetic approaches, and computational methods.

Assembly, structure, and regulatory strategies of multi-enzyme complexes: We study the regulatory protein-protein & protein-ligand interactions of cysteine synthase, a multi-enzyme complex from pathogenic bacteria. Cysteine synthase is a molecular sensor which senses levels of metabolites and regulates the activities of its component enzymes by dissociation and association of components. Our goal is to understand the evolutionary and physiological significances of this molecular sensor.

Currently, we are investigating the regulatory mechanisms of cysteine synthase complex from *Salmonella typhimurium*. Cysteine biosynthesis in microbes is the last step in sulphur assimilation and occurs in two steps. In the first step, serine acetyltransferase (SAT) transfers acetate from acetyl-coA to L-serine generating O-acetylserine (OAS). In the second step, O-acetylserine sulfhydrylase (OASS) catalyzes the insertion of sulphide into O-acetylserine in a β-replacement reaction to generate cysteine and acetate. The intracellular cysteine levels are controlled through three types of regulatory mechanisms, i) Feedback inhibition of SAT by cysteine, ii) Transcriptional level regulation in which OAS regulate the expression of genes encoding components of sulphur metabolism and iii) An important biochemical regulation involves physical association of SAT and OASS to form a multi-enzyme complex called cysteine synthase (CS) that act as molecular sensor of metabolites. The third

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regulatory mechanism is the subject of our research program. Our recent study discovered multiple binding modes for OASS (Banerjee et al, 2011).

Assembly & Regulation of Transcriptional Regulatory Complexes (TRC's): Bacterial transcriptional regulators play a vital role in virulence, pathogenicity, symbiosis, and survival. Assembly of functional transcriptional regulatory complexes (TRC) requires a highly coordinated sequential set of events that dictate temporal association of various components (transcription factors, RNA polymerase, *etc.*) of TRC. A conditional assembly of TRC depends on many regulatory steps preceding the assembly. Our studies are aimed at understanding the regulatory principles of assembly of functional TRC which dictates the frequency of transcription of genes. We hope to provide a physical, mechanical and physiological basis of regulation at transcriptional level.

We aim to address the role of transcriptional factors (TFs) involved in Mg^{2+}signaling (PhoP), sulfur assimilation (CysB), and drug resistance (TetR). Our recent study has revealed a "rate-limiting" step in the formation of active-dimeric TF-DNA complex and proposed a novel role for phoshporylation mediated gene regulation. Based on our results, we propose that phosphorylation of PhoP overcomes the "rate-limiting" dimerization step by stimulating the cooperative binding of two monomers on the adjacent sites.

Understanding the molecular origin and signatures of promiscuity of enzymes: Although specificity has been thought of corner stone of catalysis/molecular interactions, recent observations that multiple functions/reactions associated with single molecular entity indicate that promiscuity in molecular recognition is more common than generally appreciated. Some advantages of promiscuity could be, high tolerance of organism to changing environments, multitasking by enzymes/proteins to compensate for the loss of genes. Two types of structural architectures that could serve as platforms for promiscuity; multiple binding sites within the protein as one gorup and single site with ability to interact with multiple ligands as another. Our lab is interested in studying mechanism of promiscuity of the latter which represents differential ligand binding to single site.

Metallo peptidases (MPs) are prevalent in nature and are associated with multiple medical disorders including cancer, malaria, and diabetes. Many of MPs show promiscuity in peptide binding, although specific in catalysis, are good candidates for studying principles of promiscuity. We aim to conduct interdisciplinary protein/peptide research of both experimental and computational nature to develop new concepts for promiscuity and use the knowledge gained to succeed in designing a MPs inhibitor. Our recent observation that non-substrate peptides could bind to MPs active site with high affinity encourages us to design and develop a novel peptide inhibitor (Kaur *et al.* 2011). We recently determined the crystal structure of a metallo peptidase from yeast. At 2.4 Å. We use protein engineering and biochemical approaches to map structural and molecular features of promiscuity.

Host-Pathogen Interactions: The central theme of our research is to study host-pathogen relationship at the molecular level and identify key macromolecular targets for designing therapeutic agents. Many intracellular pathogens including *Mycobacterium tuberculosis (Mtb), Salmonella typhimurium,* and *Yersinia pestis* share the ability to survive within macrophages. Macrophages provide hostile, highly oxidizing, and nutrient limiting environment. The adaptability and successful survival of these bacteria to a very unfriendly intracellular environment is achieved through a net work of highly specific macromolecular interactions that co-ordinate the recruitment of essential nutrients/minerals for the survival of bacteria, removal of harmful metabolites, and manipulate the physiology of the host in favor of pathogens. However, such specific interactions between macromolecules impose many constraints on the survival as well as evolution of these pathogens. Often, constraints are translated into structural, thermodynamic, and kinetic features of molecular interactions. We aim

to exploit these constraints for therapeutic purposes. Our current research interest is focused on studying molecular interactions of two families of proteins; Coronin-1 and 14-3-3 proteins.

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Understanding structure-function relationship of proteins with bio-medical relevance

Towards our long-term goal of designing minimal or bonsai version of proteins with bio-medical relevance, we are involved in understanding solution structure-function relationship of some proteinogenic systems. To gain insight into the global shape profile, we analyze small angle x-ray scattering data and supplement the information by *in-silico* modelling and secondary structure information by circular dichroism and infrared spectroscopy. Some interesting findings are:

How tetravalent antibody CD4-IgG2 neutralizes HIV-1 much faster than any other broadly neutralizing antibody?: CD4-IgG2 contains four copies of first two domains of human CD4 grafted in place of variable domains of the heavy and light chains of human IgG2. Unlike soluble CD4 and chimeric proteins having CD4 grafts, CD4-IgG2 potently neutralizes a broad spectrum of HIV-1 isolates. SAXS data analysis and constraint-based modeling of structure showed that in unliganded state, this antibody acquires a symmetric shape slightly longer in dimension than another IgG2 antibody (NC-1) and is characterized by a radius of gyration (R_G) and maximum linear dimension (D_{max}) of 60.4 and 215 Å, respectively. Estimated values of scattering intensity at zero angles (I_0) supported that upon adding one, two, three and four moles of full-length HIV-1 gp120 to one mole of CD4-IgG2, predominantly di-, tri-, tetra- and pentameric complexes were formed in respective mixtures. Interestingly, comparison of the average structures restored within shape constraints of the acquired SAXS datasets revealed that though the two engineered Fab arms adopt a symmetric shape relative to central Fc portion in the unliganded antibody, their antigen binding behavior is differential. Against the expected notion, we observed that the first two gp120 molecules unambiguously bind to the same arm of CD4-IgG2 instead of one gp120 binding to each Fab arm (Figure 1).

Structural evidence on pH-activation of plasma gelsolin: Gelsolin is a key actin cytoskeleton modulating protein primarily regulated by calcium and phosphoinositides. In addition, low pH has also been suggested to

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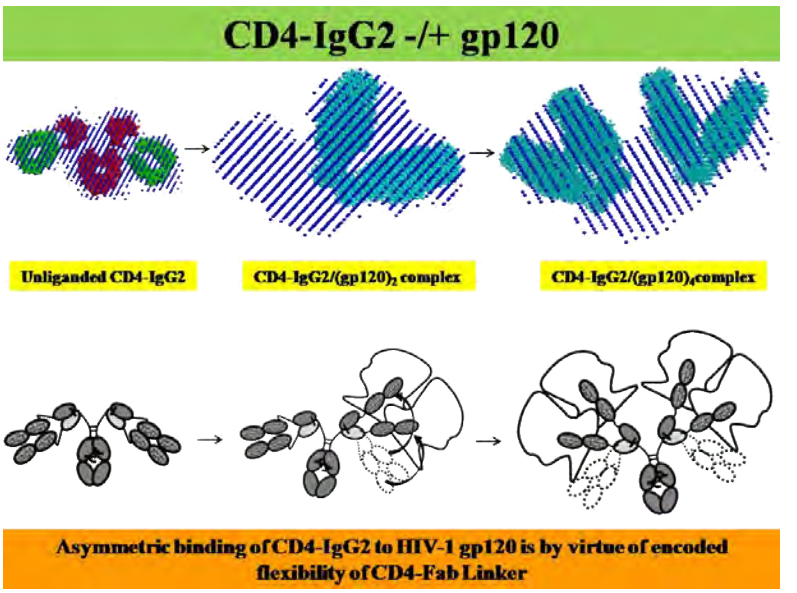

Figure 1: SAXS based insignt into the global shape of tetravalent antibody -/+ HIV-1 gp120.

activate gelsolin in absence of Ca^{2+} ions, although no structural insight on this pathway is available except for a reported decrement in its diffusion coefficient at low pH. Analysis of the small angle X-ray scattering data collected over same pH range indicated that the radius of gyration and maximum linear dimension of gelsolin molecules increased from 30.3 to 34.1 Å and from 100 to 125 Å, respectively. Models generated for each dataset indicated that similar to Ca^{2+} induced process, low pH also promotes unwinding of this six-domain protein but only partially. It appeared that pH is able to induce extension of G1 domain from rest of the five domains while the Ca^{2+}-sensitive latch between G2 and G6 domains remains closed. Our results provide insight into how under physiological conditions, a drop in pH can fully activate F-actin severing shape of gelsolin with micromolar levels of Ca^{2+} available (Figure 2).

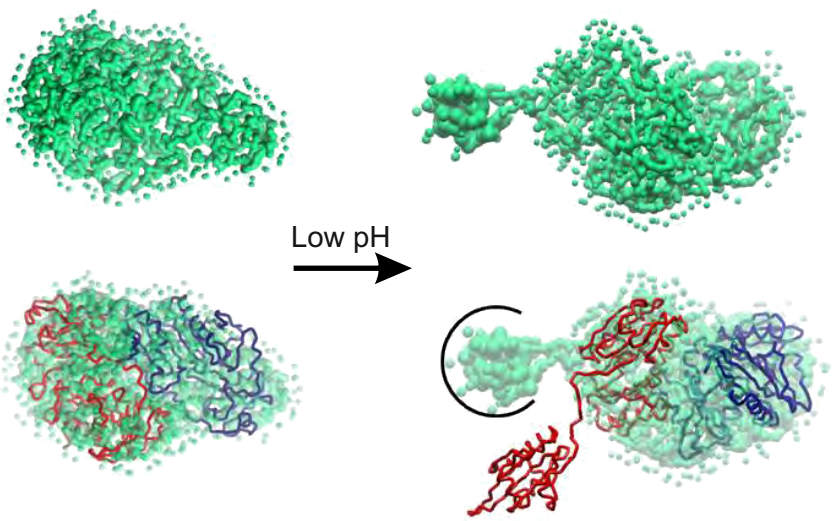

Figure 2: SAXS based insight into opening of G1 domain of plasma gelsolin in low pH.

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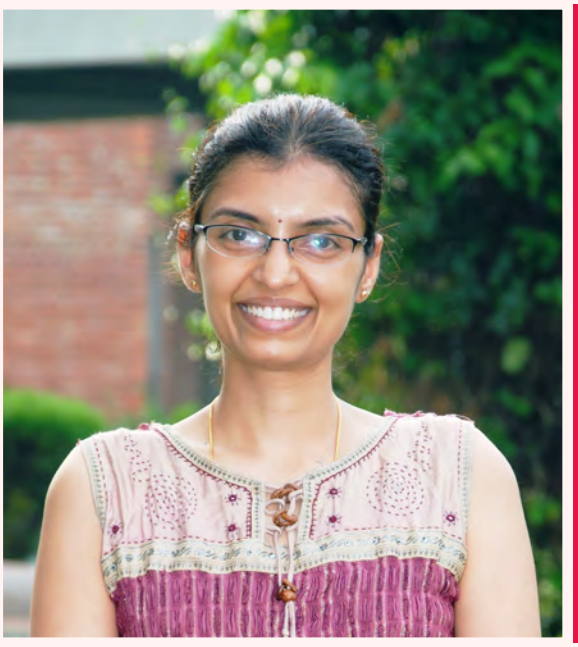

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Lectin-glycoconjugate interactions in complex biological phenomena

The broad research interests of our lab are in the interface of glycobiology and molecular and cell biology with emphasis on elucidating the molecular and cellular events involving glycoconjugate-lectin interactions in various complex biological phenomena such as inflammation, innate immunity and host-commensal interactions. The objective is to understand the physiological roles of lectin-glycan interactions in the cellular milieu and explore the possibility of translating the knowledge gained towards the design of novel therapeutic agents. Our goals will be achieved using a variety of molecular biology tools together with novel, improvised or existing chemical biology and glycobiology tools to detect, visualize and track glycoconjugates.

Understanding the role of ligand binding by the human intelectins in inflammation: Intelectins are GPI-anchored or secreted glycoprotein lectins that are expressed in the endothelial cells of the intestine, lung and several other tissues, with multiple molecular functions that include recognition of galactofuranose residues present on pathogenic microbes, lactoferrin binding and adipocytokine function. Intelectins are associated with roles in innate immune response (particularly in nematode infections) and inflammation (in asthma, Crohn's disease and other disorders). In particular, intelectins in the mucus cells of the airway epithelium are upregulated following allergen exposure, and associated with the expression of chemokines implicated in inflammatory mediator release by eosinophils and basophils, and hence are potential therapeutic targets for allergic airway inflammation. The thrust of our ongoing research is to characterize the effect of ligand binding and trafficking of intelectin-ligand complexes vis-à-vis their role in inflammation with the ultimate aim of designing high affinity ligands that can manipulate intelectin function, and thereby mitigate inflammation.

Exploring the functions of F-type domain containing proteins in bacteria: The F-type lectins or fucolectins are glycan-binding proteins with a characteristic sequence motif and are distributed in a range of organisms from bacteria to vertebrates. While a pathogen-recognition role has been implicated for several F-type lectins in invertebrates and vertebrates, the varied architectural contexts and spatio-temporal expression of the F-type

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lectins suggest that the sugar-binding ability may be harnessed for accomplishing a variety of functions. In bacteria, the F-type domain is found in a variety of gene architectures in a small number of diverse bacterial species. While sugar binding has been demonstrated for the virulence factor and F-type lectin, SP2159 from *Streptococcus pneumoniae*, little is known about the majority of the proteins containing F-type domains in bacteria. The focus of our current research is to characterize F-type domain containing proteins in bacteria with the objective of understanding how the F-type domain has evolved to recognize different oligosaccharide specificities and participate in different biological functions as part of different architectural contexts.

Host-commensal interactions mediated by glycan-lectin interactions: The intestinal epithelium is heavily glycosylated with a variable and highly regio-specific pattern of glycosylation. Despite the rapid turnover of epithelial cells and the brisk rate of peristalsis, some microbial species are able to entrench themselves in a given intestinal niche, suggesting that symbionts inhabit the polysaccharide rich mucus gel layer overlying the gut epithelium through stable associations with host glycoconjugates and constitute a biofilm-like community. Their retention in such a matrix benefits the host by promoting functions served by the microbiota, including digestion of luminal contents and fortification of the host defense system. Our research focus will be to identify microbial glycan binding proteins that interact with host glycoconjugates and enable stable microflora associations in the gut.

Effect of host glycogenes on the gut microbial community: Microbial ecosystems are guided by a complex interplay of environmental and genetic factors. This is beautifully illustrated by the highly diversified community of microorganisms that resides in our distal gut with densities approaching 10^{12} microbes per milliliter of luminal contents, providing a variety of benefits such as the regulation of energy balance and the maturation and regulation of immune function. The gut microbial community is known to demonstrate significant inter-individual variability and it is well known that the host dictates many of the conditions under which members of the human microbiome competes. This is in fact evident from the observation that notwithstanding some temporal variability, persistent changes in the gut microbiome are difficult to achieve even by rigorous antibiotic regimens. Our research will be aimed at understanding how the intestinal glycobiome may influence microbiome composition.

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Broad investigative studies on the occurrence of glycosylation in bacterial proteins/proteome across the domain Eubacteria

In the year 2010, our lab has initiated studies towards exploring glycosylation in bacteria, a newly identified post translational modification of proteins in prokaryotes. The usual seat of glycosylation of proteins in eukaryote is lumen of endoplasmic reticulum (ER) and golgi body, the two structures that are absent in prokaryotes. Therefore, for years together it was believed that glycosylation is an exclusively eukaryotic phenomenon until year 1974, when the first true bacterial glycoprotein named S layer protein was identified in archaebacteria, *Halobacterium salinarum.* Although, now it is widely accepted that different bacteria does express glycoproteins and harbor genes responsible for glycoprotein synthesis in their genomes, yet understanding the glycome (the totality of glycan structures), and its subset glycoproteome (a sum total of the proteins containing a covalently attached glycans) is far more complex and challenging than the genome or proteome of a cell. Our lab's primary interest is in investigating the spread, horizons and versatility of protein glycosylation in bacteria across the domain eubacteria, eventually to discern the role of bacterial glycoproteins in the general cell biology as well as in deciphering its importance in increasingly more evident interaction between host defenses and bacterial virulence factors. A glimpse of various roles attributed to bacterial glycoproteins is depicted in Figure 1.

In order to lay a foundation for this new line of research at IMTECH, we have developed first exclusive database of prokaryotic glycoproteins (PROGLYCPROT): A compilation of experimentally characterized glycoprotein of Prokaryotes (Figure 2). The ongoing efforts towards detecting glycosylation in selected species of phylum *Actinomycetes*, indeed is indicative of the fact that glycosylation could be a well conserved phenomenon in the bacteria.

Apart from this, we have a research project funded by DBT where in we are trying to study *Mycobacterium tuberculosis* (*M. tuberculosis*) homologs of conserved hypothetical proteins forming basic survivasome of the pathogen.

Alka Rao received her Ph.D. (2006) from International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi. She was Assistant Professor at Dept. of Bio & Nanotechnology at Guru Jambheshwar University for Science & Technology, Hisar before joining IMTECH in 2009.

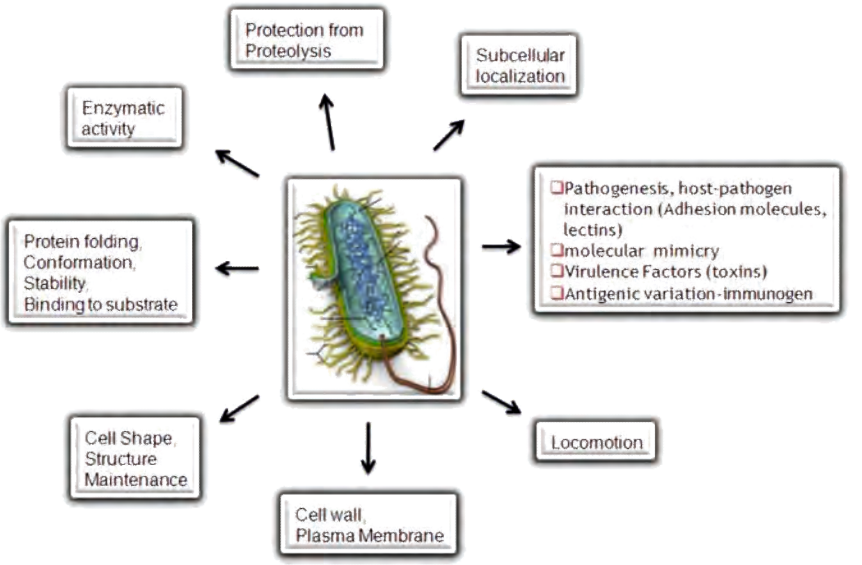

Figure 1: Various structural/ functional and virulence associated roles attributed to bacterial glycoproteins.

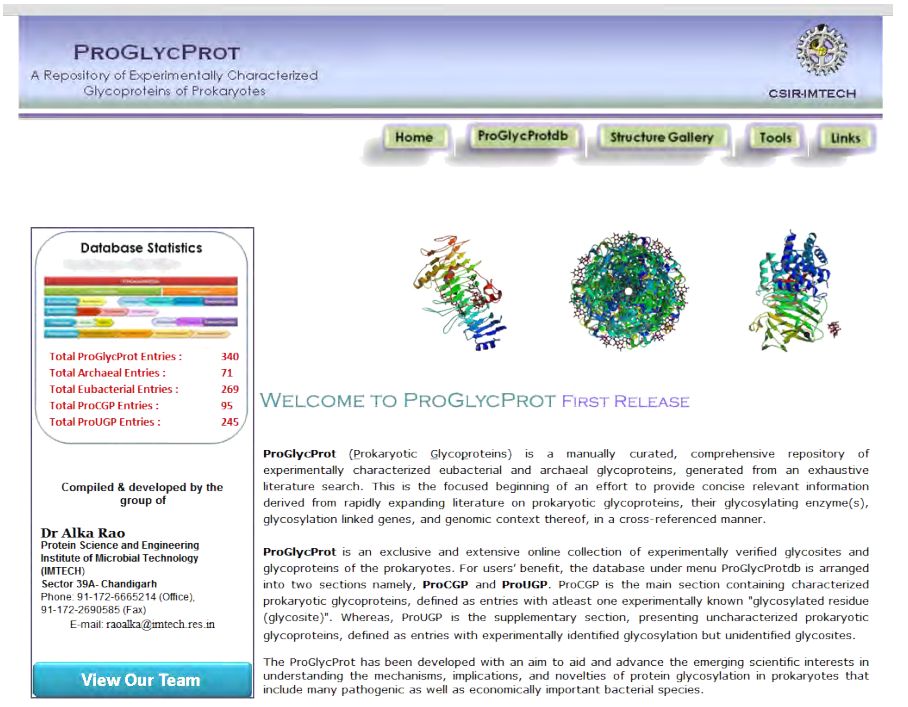

Figure 2: PRO GPDB: A database of experimentally characterized glycoproteins.

M. tuberculosis, an obligate pathogen with a mid size genome has a large complement approximately 35% of its total annotated protein coding ORFs found as essential. Further within these essential genes a notable fraction codes for (approximately 25%) hypothetical proteins with annotations like hypothetical/ conserved hypothetical, probable and putative *etc*. Our study is focused on delineating the fundamental/metabolic pathway/s associated with GCP-ALR cluster of genes (predicted glycoprotease-alanine racemase operon) in *M.*

tuberculosis. Some of the member ORF's have already been shown important for survival in macrophage and pathogenesis of *M. tuberculosis* by mutagenesis approach. The information gained may turn out directly useful in identifying the eubacteria specific members of GCP-ALR cluster as novel drug targets. At the same time this study may help understanding the projected involvement of GCP homolog in an essential tRNA modification in the context of *M. tuberculosis*. Experiments are under progress.

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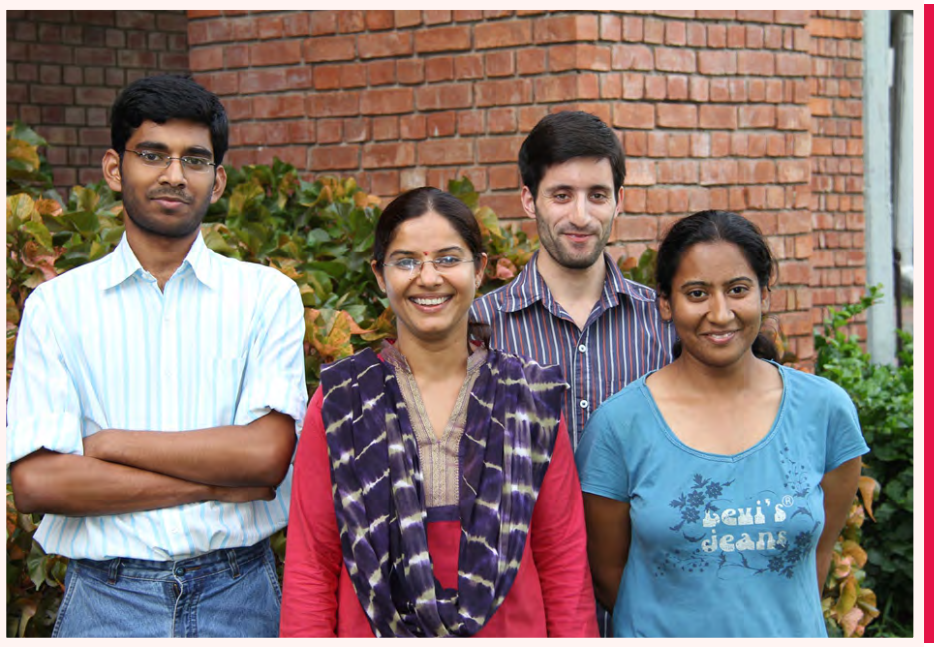

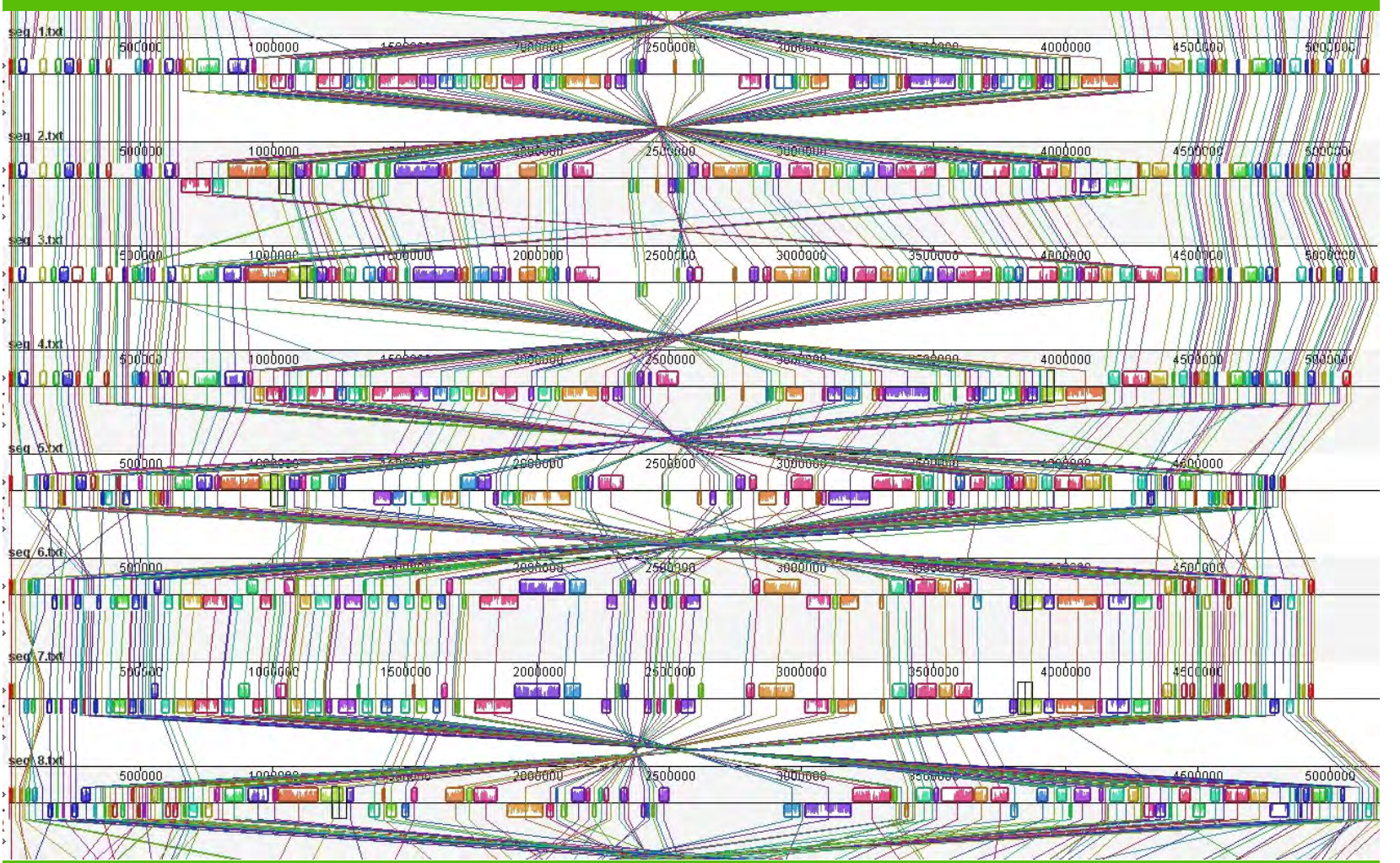
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molecular and microbial genetics

research & development programmes

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Studies on enzyme(s) involved in mycobacterial N-terminal methionine excision pathway

The N-terminal methionine excision (NME) in both prokaryotes and eukaryotes is an essential co-translational proteolytic process responsible for the diversity of amino-termini of proteins. In prokaryotes and eukaryotic organelles since the initiator methionine is formylated, NME requires the action of a metalloprotease, peptide deformylase (PDF), which removes the N-formyl group present on all nascent polypeptides synthesized. This is followed by specific removal of terminal methionine by another enzyme, methionine amino peptidase, in all organisms if the penultimate residue is non-bulky and uncharged. It is an irreversible reaction, which occurs soon after N-terminal residues of the nascent polypeptide chain emerge from the ribosome exit tunnel before the commencement of protein folding. Since involvement of these enzymes is mandatory during nascent protein synthesis in different microbes, their importance has long been realized. In this context, we concentrated on structure-function analyses of these enzymes, particularly from *Mycobacterium tuberculosis,* to assess their potential as drug targets. We previously reported the characterization of PDF from *M. tuberculosis* (Saxena and Chakraborti, *Biochem. Biophys. Res. Commun.*, 332, 418-425, 2005; Saxena and Chakraborti, *J. Bacteriol*, 187, 8216-8220, 2005; Saxena et al. *J Biol Chem,* 283: 23754-23764, 2008) and presently focused on MetAP1.

We cloned genes encoding two such metalloproteases (*Mt*MetAP1a and *Mt*MetAP1c) present in *Mycobacterium tuberculosis* and expressed them as histidine-tagged proteins in *Escherichia coli*. Though the presence of two MetAP1s was known with the availability of the *M. tuberculosis* genome sequence, both of them (*Mt*MetAP1a and *Mt*MetAP1c) are active has recently been shown by us and others. In fact, it is still not known whether they are merely complementing each other for their functionality and therefore redundant within the genome or their presence is because of the specific needs of the bacterium. However, the initial step of structure-activity relationship with these two proteins has not been addressed properly. In this context, to carry out systematic study of these two enzymes from *M. tuberculosis*, here we have attempted to analyze MetAP1s to elucidate subtle differences in their characteristics.

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Our results indicated that *Mt*MetAP1c had strikingly high enzyme turnover rate with the same substrate (Met-Ala-Ser) compared to *Mt*MetAP1a (~350-fold more), although they had different substrate preferences (Table 1).

Kinetic parameters of *Mt*MetAPs

System	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (mM^{-1} s^{-1})	No. of experiments
***Mt*MetAP1a** (Met-Gly-Met-Met)	2.85±0.69	9.63±1.42	3.47±0.7	3
***Mt*MetAP1a** (Met-Ala-Ser)	3.3±0.35	11.17±0.97	3.32±0.18	4
***Mt*MetAP1c** (Met-Ala-Ser)	1.36±0.25	1516±174	1156±317	4

Table 1.

The outcome of our CD as well as activity assays with these mycobacterial enzymes illustrate that *Mt*MetAP1a can sustain high temperature (up to 50°C) compared to *Mt*MetAP1c. In fact, T_m value calculated based on CD spectra for *Mt*MetAP1a was 53.6°C as opposed to 42.7°C for *Mt*MetAP1c. When the structure of *Mt*MetAP1a was modeled based on available structure of *Mt*MetAP1c and compared, we observed that the active site residues in both the enzymes are essentially same but their arrangement in space is different. Furthermore, these amino acids are crucial because any alteration in them (for both the enzymes) yielded an inactive protein.

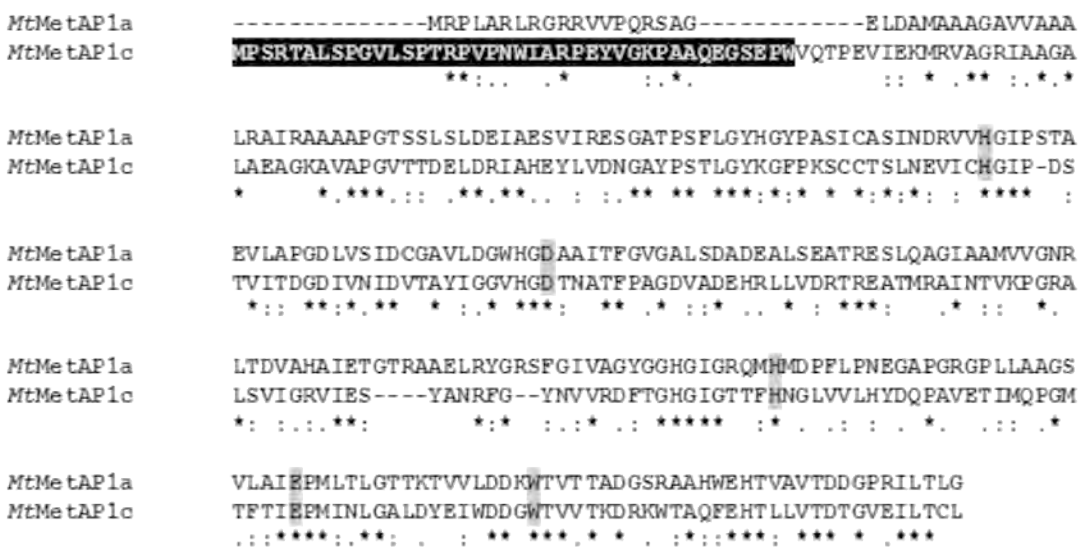

Figure 1: Sequence alignment of the two mycobacterial methionine aminopeptidases using Clustal X program. Gaps in the sequences were introduced for optimum alignment. Asterisk and dots denote identical and similar amino acids, respectively. Residues highlighted with black represent the 40 amino acid long N-terminal extension present in *Mt*MetAP1c and those with gray are the mutated amino acids in the two *Mt*MetAPs.

Sequence analysis revealed that the remarkable feature of *Mt*MetAP1c compared to *Mt*MetAP1a, is the presence of 40 amino acid long N-terminal extension (Figure 1). It has been suggested that this extension may be involved in the interaction of *Mt*MetAP1c with the ribosome. This led us to explore the role of the N-terminal

extension present in *Mt*MetAP1c towards the activity of the enzyme. We constructed a series of deletion mutants removing nine (Δ2-10), fourteen (Δ2-15), nineteen (Δ2-20), twenty-nine (Δ2-30) and thirty-nine (Δ2-40) amino acids from the N-terminal end of the *Mt*MetAP1c. Enzyme assays with these constructs using even 10 fold excess of proteins compared to the wild-type revealed that there was a considerable loss in activity in Δ2-10 or Δ2-15 variants and no activity at all in mutants, like Δ2-20 or Δ2-30 or Δ2-40. Intriguingly, following resolving on SDS-PAGE, analysis of the mutant proteins in Coomassie Brilliant Blue stained gels reflected that purification profile of Δ2-10 or Δ2-15 mutants was similar to that of the wild-type but variants like Δ2-20, Δ2-30 and Δ2-40 exhibited loss in purity. Hence, it seems logical to postulate that the amino acid residues between 15 and 20 of *Mt*MetAP1c are crucial for its activity. In fact, evaluation of structural changes through far-UV and near-UV CD spectra revealed that up to 15 residues from the N-terminal extension of *Mt*MetAP1c are dispensable for the folding of the protein but required for its activity.

To identify the critical residue(s) for the activity of the protein, we analyzed the sequences of MetAPs from different Gram positive bacteria with N-terminal extension and found the presence of conserved Val-18 and Pro-19 in between amino acid residues 15 and 20 (Figure 2). This observation prompted us to create point mutations at 18th and 19th position of *Mt*MetAP1c (one at a time or together with type-to-type/drastic substitutions) and examine their effect on the activity of the enzyme. While V18A and P19A were partially active, V18AP19A did not

Figure 2: Sequence alignment of MetAP1c from different gram positive bacteria. M tb-*Mycobacterium tuberculosis* (P0A5J2); M bovis-*Mycobacterium bovis* (P0A5J3); M avium-*Mycobacterium avium* (A0QJ09); M marinum-*Mycobacterium marinum* (B2HJQ5); M paratuberculosis-*Mycobacterium paratuberculosis* (Q73VS7); M leprae-*Mycobacterium leprae* (Q9CBU7); S coelicolor-*Streptomyces coelicolor* (Q9RKR2); S scabies-*Streptomyces scabies* (C9ZHA6); R erythropolis-*Rhodococcus erythropolis* (C0ZY62); N farcinica-*Nocardia farcinica* (Q5YSA3); G bronchialis-*Gordonia bronchialis* (D0LBG0); C diphtheriae-*Corynebacterium diphtheriae* (Q6NGL5); C glutamicum-*Corynebacterium glutamicum* (Q6M437); B mcbrellneri-*Brevibacterium mcbrellneri* (D4YNZ0); L xyli-*Leifsonia xyli subsp. xyli* (Q6AFH6). Accession numbers are indicated in parantheses.

show any activity. On the other hand, P19G exhibited partial activity but neither V18G nor V18GP19G were active. Further, to assess if these mutations resulted in any structural alterations, far-UV CD studies were carried out. Interestingly, the mutation caused alteration in the secondary structure of the protein. Thus, our results argue the importance of these two residues (Val-18 and Pro-19) exclusively and mutually towards the enzymatic activity of *Mt*MetAP1c. It appears very likely that the structural alterations that occurred due to mutation at Val-18 and/or Pro-19 of *Mt*MetAP1c affected the active site conformation of the protein. We, therefore, carried out molecular dynamic simulation studies with these proteins in collaboration with Dr. Kumaran's group. Our results (both 5 ns and 50 ns) indicated that the conformational changes generated at the site of mutation (V18GP19G and V18G) propagated via connecting loops and helices to the enzyme active site resulting in the alteration in the

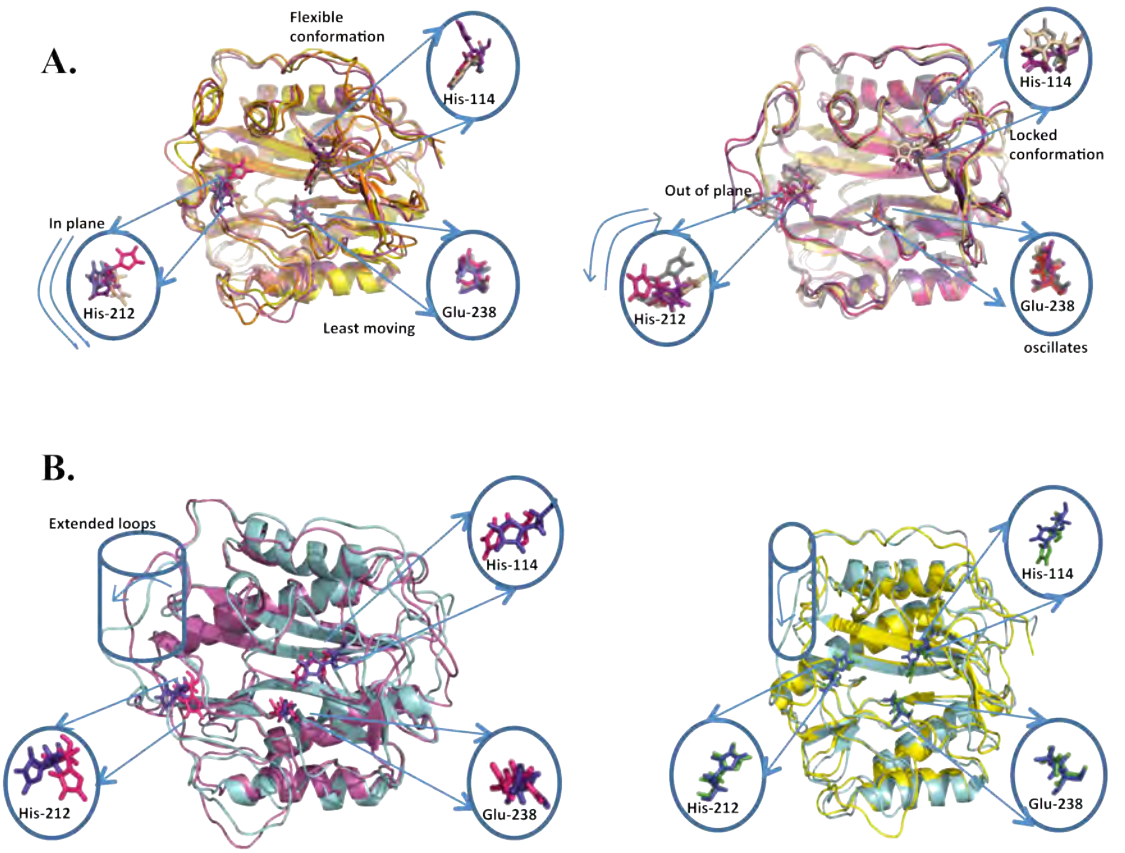

Figure 3: MD simulations. (A) The snapshots superimposed for wild-type (left) and V18GP19G (right) for 15 ns run. (*Inset*), depicts the highlighted regions of snapshots at 5ns, 7.5ns, 10ns, 15ns. (B) Snapshots of single mutant (left, V18G, blue-gray) superimposed with double mutant (pink). Both overlap each other to maximum probability compared to the wild-type protein. The loop proximal to the site of mutation is out of plane of the active site. MD simulations snapshots of single mutant (right, P19A, yellow ribbon blue sticks) superimposed with wild-type (blue-gray). Both overlap each other to maximum probability compared to the double mutant. The loop proximal to the site of mutation shows configurations similar to wild-type in 3-D space *i.e.* in plane to the active site.

movement/positioning of residues like His-114, His-212 and Glu-238 (Figure 3), which are critical for the enzyme activity as revealed in our biochemical studies. These variations in the V18GP19G or V18G mutant proteins presumably made the active site environment unfavorable for any activity. Thus, these results illustrate that residue(s) without being in the active site of an enzyme is capable of modulating its activity, which does not seem to be unusual.

Our findings unequivocally emphasize that N-terminal extension of *Mt*MetAP1c contributes towards the functionality of the enzyme by regulating active site residues through "action-at-a-distance" mechanism and this is presumably its unique function in *Mt*MetAP1c, which we are reporting for the first time. Since Val-18 and Pro-19 are conserved residues throughout the Gram positive bacterial MetAP1s with N-terminal extension, it remains to be seen whether they are universal in contributing towards the functionality of the enzyme.

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Studies on the 'DUG complex' involved in the alternative pathway of glutathione degradation in yeasts

We had previously described the discovery of a novel and alternative pathway for glutathione degradation that appeared to be fungal specific. This pathway, that we named the DUG pathway, involved three new proteins, encoded by the DUG1, DUG2 and DUG3 genes.

The DUG1 encoded an M20 metallopeptidase that we demonstrated was a cys-gly specific dipeptidase. We also showed that the human homologue, CNDP2 was a cys-gly peptidase, thereby defining a new family of Cys-gly peptidases that are likely to be the predominant Cys-gly peptidase in living cells.

We have also focussed on the Dug2p and Dug3 proteins. The Dug2p contains a WD40 repeat domain at the N-terminal region and a metallopeptidase domain at the C-termiinal region. Mutational analysis revealed that the Dug2p metallopeptidase domain is not a functional peptidase, suggesting that the Dug2p is a scaffolding protein. The Dug3p protein was also subjected to mutational analysis and revealed that it had a catalytically active Glutamine Amidotransferase Type II domain. The Dug2p and Dug3p proteins were individually purified and while the Dug2p could homodimerize with itself, the Dug3p protein was a monomer in solution. The Dug2p also strongly interacted with Dug3p suggesting that the Dug2p-Dug3p formed a strong dimeric heterodimeric complex. Efforts to purify the complex are ongoing. We were also able to demonstrate glutathione degrading activity of the Dug2p-Dug3p complex.

In addition to the studies on the Dug pathway proteins in *S. cerevisiae* we initiated studies on the Glutathione utilization pathways in the pathogenic yeasts *Candida glabrata* and *Candida albicans*. By utilizing the *met15*Δ organic sulphur auxotrophy phenotype of these yeasts we could demonstrate that *C.albicans* but not *C. glabrata* could not utilize glutathione as a sulphur source. The inability to use glutathione by *C. glabrata* was found to be

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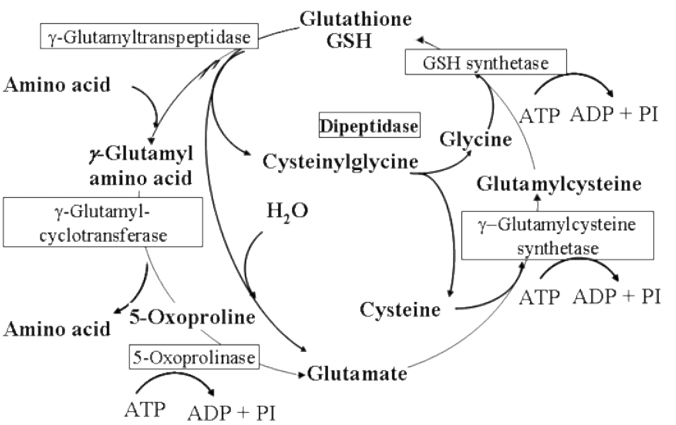

Figure 1: The gamma-glutamyl cycle.

due the absence of a glutathione transporter. To demonstrate the important of glutathione biosynthesis as opposed to glutathione transport in these yeasts *in vivo*, we carried out mice experiments and were able to demonstrate that glutathione biosynthesis was essential for the survival of both *C. albicans* and *C. glabrata in vivo*.

We have pursued our studies on the γ-glutamyl cycle in *S. cerevisiae* and were able to demonstrate that yeast contains a 5-oxoprolinase. Previously 5-oxoprolinase was thought to be absent in yeasts. We purified eukaryotic 5-oxoprolinase for the first time and were able to initiate structure function studies on this enzyme.

In addition to these studies, based on our understanding of glutathione metabolism and the γ-glutamyl cycle in yeast, we proposed a model to explain the key features relating to the pathophysiology of nephrotic cystinosis. Nephrotic cystinosis, an inherited disease caused by a defect in the lysosomal cystine transporter (CTNS), is characterized by renal proximal tubular dysfunction. ATP depletion appears to be a key event in the

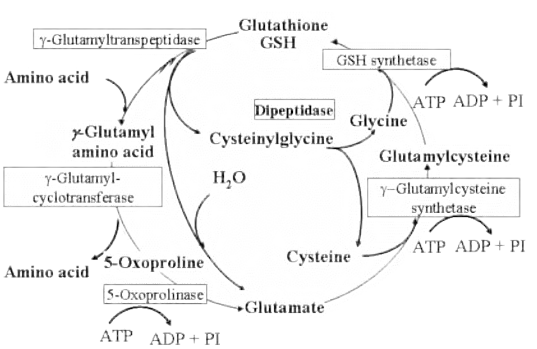

Figure 2: A model explaining the possible biochemical basis of the pathophysiology of Cystinosis.

pathophysiology of the disease, even though how ATP depletion occurs is still a puzzle. We have described a model that explains how a futile cycle that is generated between two ATP-utilizing enzymes of the γ-glutamyl cycle leads to ATP depletion.The enzyme γ-glutamyl-cysteine-synthetase (γ-GCS), under conditions of limiting cysteine levels in the cell, forms 5-oxoproline (instead of the normal substrate, γ-glutamyl cysteine) and the 5-oxoproline is converted into glutamate by the ATP-dependant enzyme, 5-oxoprolinase. Thus, in cysteine limiting conditions glutamate is cycled back into glutamate via 5-oxoproline at the cost of two ATP molecules without

production of glutathione and is the cause behind the decreased levels of glutathione synthesis, and also the ATP depletion observed in these cells. The model is also compatible with the differences seen in the human patients and the mouse model of cystinosis, where the renal failure is not observed.

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Biochemical and Genetic Interrelationships among the components of heterochromatin and RNAi machineries

During this period we have addressed some of the mechanistic aspects of establishment of heterochromatin in *S. pombe* and our findings warrant a revision of the currently held models as described below:

Cooperative interaction between Swi6 and Clr4 helps in establishment of the repressed epigenetic state at heterochromatin loci in *S. pombe*: According to the currently accepted model, the assembly of heterochromatin occurs by sequential actions of histone deacetylases to deacetylate histone H3 at Lys9 and 14 positions, followed by methylation of H3 at Lys9 by the histone methyltransferase Clr4, which creates a binding site for the heterochromatin protein HP1 to initiate heterochromatin. Recent results from our lab lead us to question the sequential model and support the concerted model wherein Clr4 is recruited by the tetramer of Swi6 to initiate H3-Lys9 methylation at the heterochromatin sites. A speculative model is shown in Figure 1.

Genetic interaction between RNAi pathway and heterochromatin proteins Swi6 and Clr2: It has been known for several years that RNAi pathway is required for initiating the heterochromatin mark of H3-Lys9 methylation. We have observed in our laboratory that *dcr1*Δ and *rdp1*Δ mutations cause an almost complete silencing defect at the mat3 locus, as monitored by the derepression of mat3-linked ade6 reporter, which is indicated by the pink phenotype of the colonies of the mutants as compared to the red color of the wt strain on adenine-limiting plates. Using the classical extragenic suppressor approach the heterochromatin genes encoding *clr2* and *swi6* were found to restore silencing at the *mat3*-linked *ade6* reporter in both *dcr1*Δ and *rdp1*Δ mutant strains (Figure 2). Thus, it is possible that Clr2 and Swi6 may interact with Dcr1 and Rdp1 proteins, thus providing a direct link between RNAi and heterochromatin machineries.

Earlier work from our lab had shown that the histone H3-Lys9 methyltransferase Clr4 has the ability to bind

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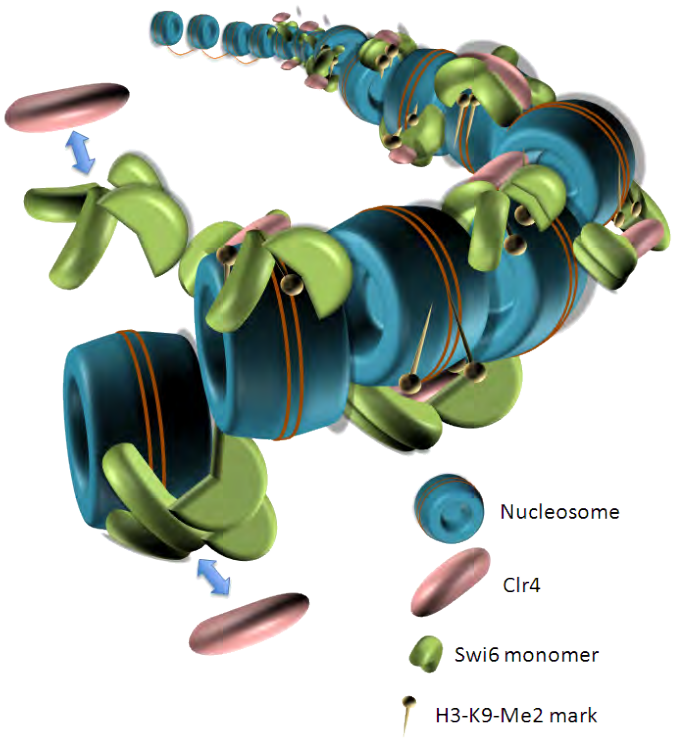
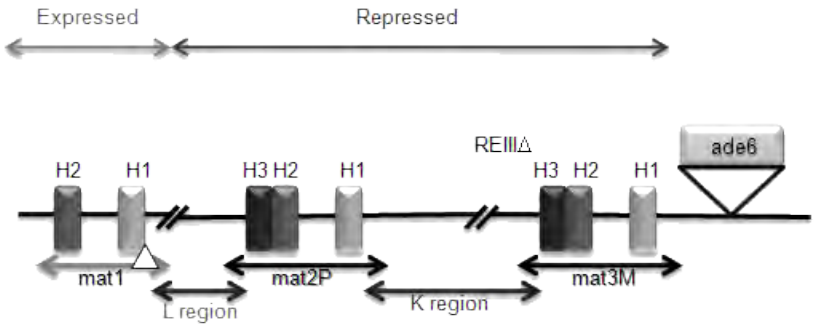


Figure 1: A speculative model visualizing a concerted mode of action of Swi6 and Clr4 in establishment and propagation of the heterochromatin in fission yeast.



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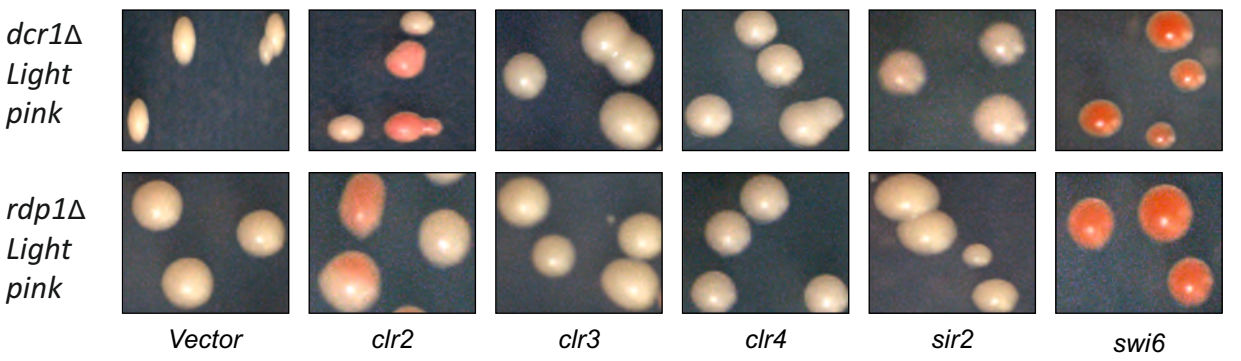

Figure 2: Suppression of the derepressed phenotype of *ade6* reporter at *mat3* locus in RNAi Mutants upon overexpression of *clr2* and *swi6* genes.

strongly with the siRNA sequences in hybrid with the DNA template *in vitro*. Further, the heterochromatin localization of Clr4 was reduced upon pretreatment of chromatin with RNaseH. To check the physiological relevance of these results we check the effect of expression of RNaseH on mating type silencing and centromere loci. Results indicate that RNaseH overexpression does cause derepression of *ade6* reporter inserted at the *otr1* locus (Figure 3) and also appears to affect the efficiency of mating type switching. Further experiments will check the effect of overexpression of RNaseH on localization of Clr4, Swi6 and other heterochromatin proteins.

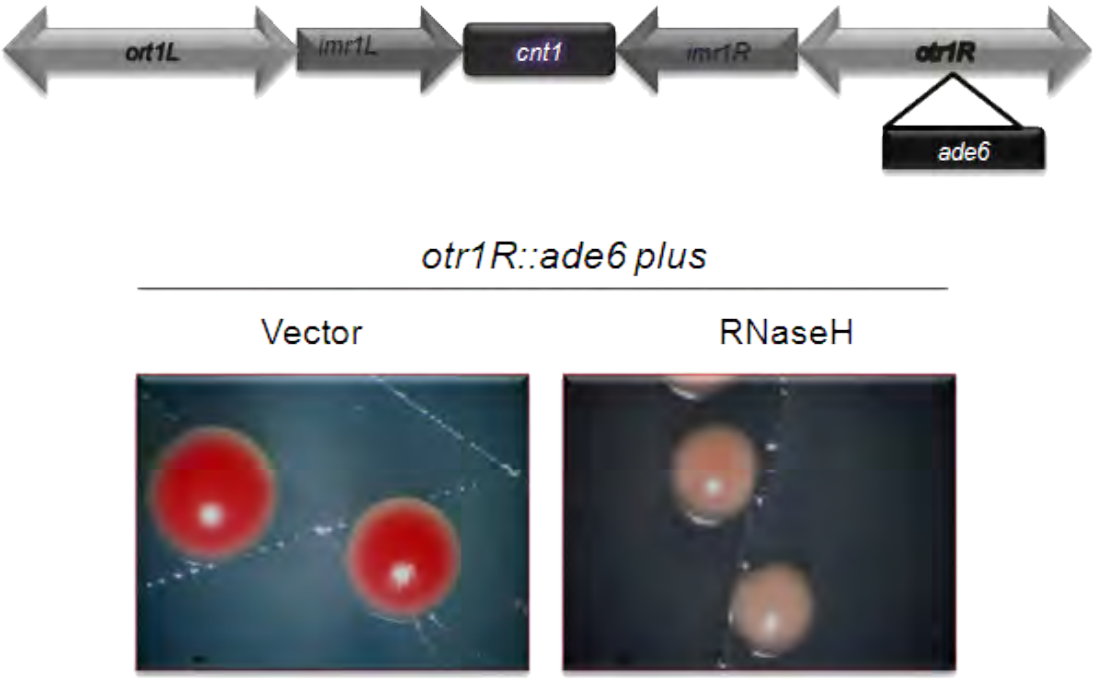

Figure 3: Derepession of *otr1R*-linked *ade6* reporter upon overexpression of RNaseH.

Ubiquitylation of Swi6 by the APC-E3 ligase: Earlier work had suggested that the silencing defect in the mutations in the subunits of the Anaphase Promoting Complex (APC) namely cut4 and cut9 was due to the reduced ubquitylation function fo the mutant APC complex. Our attempt to identify the target for ubiquitylation revealed that *in vivo* Swi6 is almost entirely in the monoubiquitylated form. Experiments are in progress to map the site of monoubiquitylation and its role in execution of Swi6 function.

Discovery of a hitherto strongest constitutive promoter in *S. pombe*: For the last several years, our lab has been engaged in developing new vectors based on strong promoters and strains with a view to develop *S. pombe* as a facile alternative expression system. Towards this goal we have recently isolated and characterized a super-strong constitutive promoter that yields an expression level of a few reporter as well as a therapeutic protein at levels approaching ~10% of total cellular protein without affecting cell viability. Work is in progress to optimize the conditions for maximizing expression levels and IPR protection.

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Studies on redox network of *Mycobacterium tuberculosis* H37Rv with special reference to WhiB proteins as novel redox system of *Mycobacterium Tuberculosis*

Mycobacterium tuberculosis (*Mtb*) has a remarkable ability to survive under hostile conditions it encounters during infection. Despite extensive research directed towards understanding the physiology of *Mtb* and its molecular pathogenesis, many fundamental questions about the mechanisms of survival during early infection and persistence remain poorly understood. Among several intriguing questions, are: (a) what are the bacterial determinants necessary for early infection, (b) how does the bacterium counteract or evade its host's defenses to survive the vigorous host immune response, (c) what regulates the transition from initial growth to persistence and back to active growth, (d) are the bacteria present in a non-replicating 'spore like' state or do they replicate at all during latency, and (e) how does the bacterium adapt to survive under the anaerobic and nutritionally altered environment within granuloma? Our objective is to provide answers to at least some of the questions if not all. To survive and establish successful infection, *Mtb* has acquired a strong network of genes to sense and respond to stress conditions; the properties of many of these are poorly understood. A family of genes, whiB, has received attention because of their involvement in cell division (*whiB*2), fatty acid metabolism and pathogenesis (*whiB*3), antibiotic *r*esistance (*whiB*7) and in sensing a variety of stress conditions. Seven genes, whiB1 / Rv3219, whiB2 / Rv3260c, whiB3 / Rv3416, whiB4 / Rv3681c, whiB5 / Rv0022c, whiB6 / Rv3862c and whiB7 / Rv3197A, were identified in *M. tuberculosis* as orthologs of the *whiB* gene of *Streptomyces coelicolor* A3(2), which has been shown to be involved in sporulation and septum formation. We have reported that WhiB proteins work as general protein disulfide reductases. Thus, our attention is focused to delineate function of WhiB proteins *in vitro*, study the protein -protein interaction network where WhiB proteins are involved and also to make attempt towards structure and functional analysis of these proteins. Mycobacterial WhiB proteins have 22-67% identity with WhiB

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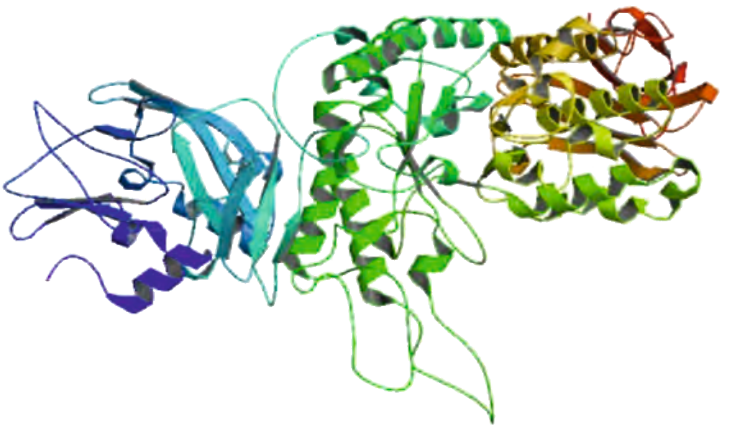

Figure 1: X-Ray Crystal Structure of *Mtb* GlgB.

protein of *S. coelicolor* A3(2). Sequence analysis of *Mtb* WhiB proteins shows the presence of four conserved cysteines arranged as 'C-X19-36-C-X-X-C-X5-7-C'. Notably, two cysteines are present in a conserved CXXC motif, except in WhiB5 / Rv0022c where it is CXXXC (CLRRC). Proteins with the CXXC motif have been implicated in diverse functions, for example, protein disulfide oxidoreductase activity, redox sensing and the coordination of metal cofactors. Though we have shown that except WhiB2 all other WhiB like protein of *Mtb* are protein disulfide reductases and are thioredxin like proteins but there reductases are yet to be identified. However, we have clearly shown that WhiB1 can indeed transfer its electron to GlgB/Rv1326c and reduce it, thus their interaction is redox dependent. The work has lot of significance because both *glgB* and *whiB*1 are essential genes of *Mtb*, therefore

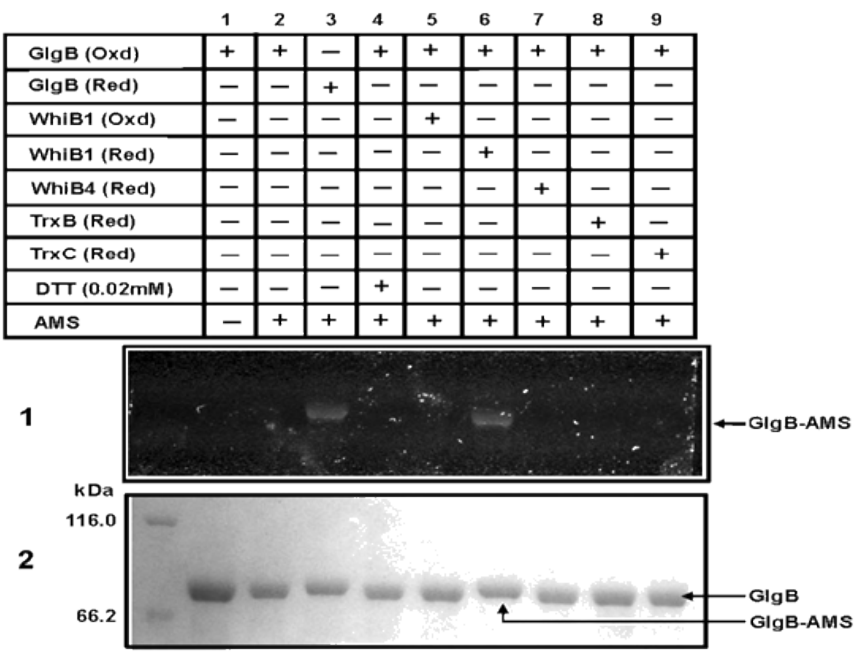

Figure 2: Reduction of GlgB's disulfide by reduced WhiB1. After interaction between WhiB1 and GlgB, the reduced cysteines of GlgB were trapped using AMS and the samples were resolved by 8% SDS-PAGE. Panels are: (1) resolved GlgB on gel, as visualized under UV light, the fluorescent band shows the binding of AMS to reduced cysteines of GlgB as labeled, (2) coomassie stained gel (after photographed under UV light) to show the presence of GlgB. The AMS labeled GlgB also showed mobility shift. Lanes are indicated on the top.

they are novel drug targets. Subsequently, we solved the crystal structure of full-length *Mtb* GlgB and have shown that though the crystal structure of *Mtb* GlgB and *E. coli* GlgB has lot of similarity, the inhibitors of *E. coli* GlgB does not inhibit the enzyme activity of *Mtb* GlgB.

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Molecular mechanisms of Amphotericin B resistance in *Candida*

Amphotericin B (AmB) is one of the frontline antifungals used in the treatment of invasive and life-threatening fungal infections. However, its toxicity, and emergence of AmB resistant clinical isolates limit its widespread use. The molecular basis of AmB resistance is poorly understood. Earlier, we have shown that farnesol, a quorum sensing molecule that accumulates in a cell-density dependent manner, increases AmB resistance of pathogenic yeasts *C. albicans* and *C. lusitaniae*. Farnesol has a similar effect on the yeast *S. cerevisiae* as well. Since this yeast is highly amenable to genetic and molecular analysis, we are using it as a model system in our studies. AmB resistance of *S. cerevisiae* mutants impaired in ergosterol biosynthesis, oxidative stress response or cell wall integrity pathways were found to be comparable to wild-type cells, indicating that the increased resistance is not mediated through these pathways. However, we have found that *PDR5,* involved in pleiotropic drug resistance, and its transcriptional regulators *PDR1* and *PDR3* are involved in modulating AmB resistance. Consistent with this, farnesol treatment increased the level of Pde5 protein, as seen by immunofluorescence. The role of *PDR5* in AmB resistance was not reported earlier, and thus we tried to understand the molecular basis for this phenotype. Results so far indicate that deletion of *PDR5* affects membrane lipid composition, in particular levels of various phospholipids. Perhaps these changes impact the ability of AmB to form pores in the membrane, thereby modulating the sensitivity of fungal cells to AmB. Consistent with the role of lipid composition, we have found that sphingolipids also modulate AmB resistance, since certain mutants impaired in sphingolipid biosynthesis are hypersensitive to AmB. Moreover, sphingolipids were also found to be critical for farnesol mediated increase in AmB resistance. In summary, AmB resistance as such, and farnesol mediated increase in AmB resistance, are mediated through *PDR* genes, as well as genes involved in sphingolipid biosynthesis. Thus, inhibitors modulating the function of the proteins encoded by these genes may potentiate AmB action. Use of such inhibitors as antifungals, in combination with AmB, can facilitate reduction in AmB dose, thereby minimizing AmB toxicity during therapy.

K. Ganesan obtained his Ph.D. (1985) in Life Sciences at Jawaharlal Nehru University, New Delhi. He continued his research at J.N.U. as a Research Associate, and later as a Research Scientist at the "Centre for Plant Molecular Biology", before joining IMTECH in 1993.

Besides the above, other ongoing work in my group includes metabolic engineering of yeast *S. cerevisiae* for fermentation of xylose, and studies towards a better understanding of acetic acid tolerance in yeast. Acetic acid, which is a byproduct of ethanolic fermentation, impairs ethanol production. We have identified a couple of yeast genes that modulate acetic acid tolerance; the overexpression of these genes in yeast may enhance acetic acid tolerance as well as ethanol production.

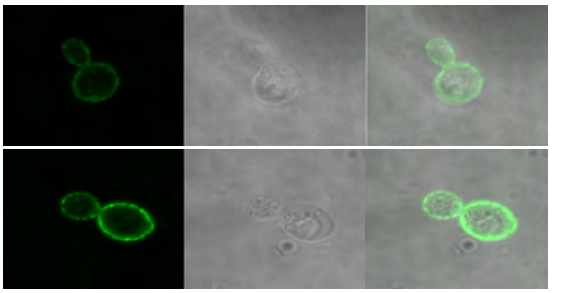

Figure 1: Farnesol induces the level of Pdr5p in yeast *Saccharomyces cerevisiae*. Yeast strain expressing HA-tagged Pdr5p was grown without (top panel) or with 50μM farnesol and analyzed by indirect immunofluorescence. The images shown are: Left panels – fluorescence; middle panels – bright-field; right panels – merged.

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Molecular genetics of halotolerance in yeast *Debaryomyces hansenii*

Debaryomyces hansenii is a biotechnologically important yeast which exhibits extreme halotolerance and osmotolerance. In the recent past it has become a model organism to understand halotolerance in yeast. For the past several years, the group is working to understand the molecular basis of the high halotolerance exhibited by this organism. Highlights of the work carried out by the group during the period under report are outlined below:

DhPpz1p, a PPP family of phosphatase plays important role in salt tolerance, cell growth and cell wall integrity pathway in *D. hansenii*: Phosphoprotein phosphatases (PPP) constitute an important family of phosphatases that regulate plethora of cellular processes in eukaryotes. Fungi, in general, contain several PPP family of phosphatase. Although corresponding orthologs for most of them could be found in higher eukaryotes, a few of them *e.g.* Ppz1p are quite unique and restricted to this kingdom of life only. Ppz1 orthologs regulate diverse physiological processes in fungi *e.g*. ion homeostasis, cell size, cell integrity *etc*. Although they are an important determinant of salt tolerance in fungi, their physiological role remained unexplored in any halotolerant species. In this context, the group undertook molecular and functional characterization of DhPPZ1 from *D. hansenii*. The group found that *DhPPZ1* have role in salt tolerance, cell wall integrity as well as in growth in *D. hansenii*. The group found that *dhppz1* mutant was highly resistant to toxic cations like lithium and Na^+/H^+ antiport appeared to have important role in this process. In case of *S. cerevisiae ppz1* mutant also exhibits higher salt tolerance however, through a mechanistically distinct process. The group has also identified a short, serine arginine rich sequence motif in DhPpz1p which is essential for its role in salt tolerance but not in other physiological processes. The group's work underscores a distinct role of DhPpz1p in *D. hansenii* and provides an example how organisms utilize the same molecular tool box differently to garner adaptive fitness for their respective ecological niches.

Thermotolerant xylitol dehydrogenase from *Pichia angusta*: Recent spur of interest to utilize renewable

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Alok Mondal joined IMTECH in 1987. He recieved his Ph.D. (1995) in Pharmacy from Jadavpur University, Kolkota.

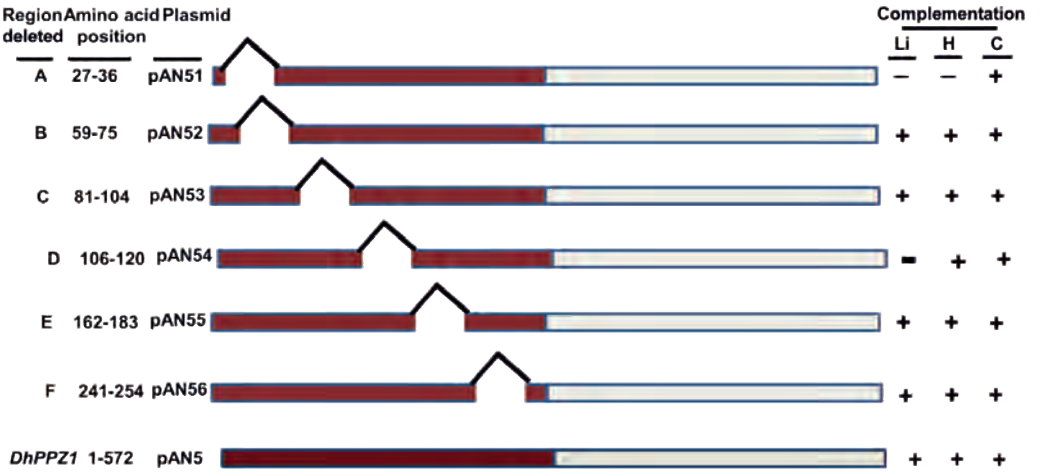

Figure 1: Functional analysis of the N- terminal non-catalytic domain of DhPpz1p.

resources like biomass for making biofuel has drawn considerable attention into xylose metabolizing microbes as they not only offer to be new platforms for these conversions but also are rich sources for novel enzymes which could be utilized to engineer organisms through synthetic biology. *Pichia angusta* is one of the limited numbers of yeast species that can grow at temperatures up to 50°C. It is capable of alcoholic fermentation of xylose at higher temperature a property that made it a very promising yeast species for ethanol production from lignocellulosic material. The group has cloned, expressed and purified two xylitol dehydrogenases (XDH) from *P. angusta*. XDH catalyzes second step in xylose assimilation pathway in yeast. Compared to XDHs from other species those have been characterized so far, PaXdh1p and PaXdh2p are thermotolerant enzymes a feature concomitant with the physiology of the host organism. They not only exhibited optimum activity at higher temperature, but also showed higher thermo stability and a higher thermal unfolding transition temperature. The group has also identified residues important for substrate binding and catalysis through modeling and mutagenesis approaches. These two enzymes are structurally distinct. They differ considerably in their affinity and catalytic efficiency to utilize xylitol as substrate. In this respect, PaXdh1p is much more efficient than the other and therefore could be more useful for biotechnological purpose. It is not clear at present what

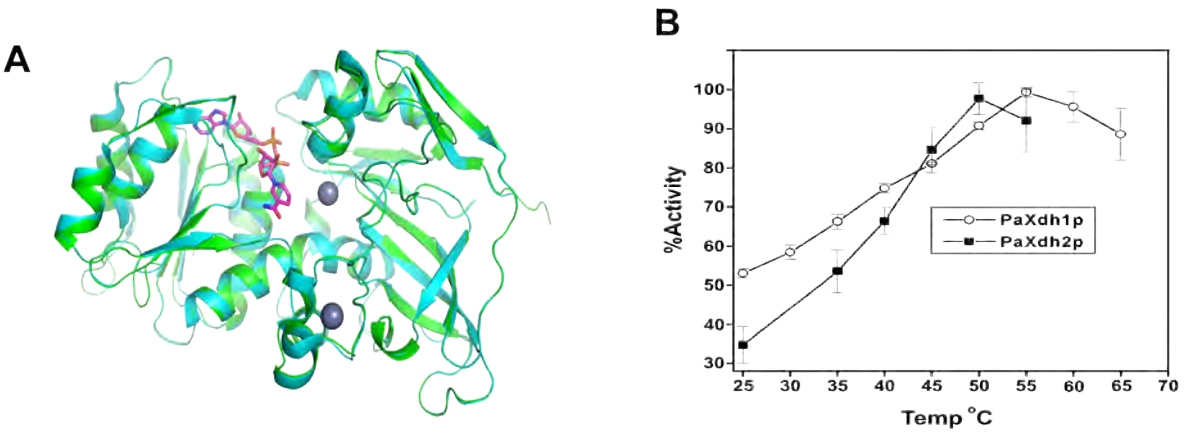

Figure 2: Modeled structure and temperature optima of XDH from *P. angusta*.

physiological benefits *P. angusta* could accrue by retaining these two redundant XDHs showing such disparate catalytic efficiency.

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Study of microbial influence on metalloid speciation in contaminated soil with special emphasis on Selenium using metagenomics

Selenium (Se), an essential element for various biological systems at low concentrations, is an extremely toxic metalloid. It was interesting to study how simple living, single-celled microbes survive and respond to elevated levels of selenium oxyions in their microenvironment. With this objective, three bacterial strains (*i.e.*, *Ochrobactrum* sp. S-5, *Bacillus licheniformis* JS-2 and *Bacillus cereus* CM100B) isolated from selenium rich soil were studied for their selenite reduction ability. Microscopic studies revealed the presence of spherical nanospheres of size ~200 nm as free deposits and also as aggregates adhering to bacterial cells. *Bacillus cereus* (strain CM100B), was of special interest as it rapidly reduced the toxic selenite oxyions to form extracellular red elemental Se^0 nanospheres which were highly stable due to the presence of high negative charge (-46.86 mV). This green route of biosynthesis of selenium nanospheres is a simple, economically viable and an eco-friendly process. It is also multi-metal tolerant indicating that this strain would have better adaptability and survival rate in the natural environments. To further gain insights into the metal-microbe interaction mechanism, FTIR study was performed which revealed the presence of numerous functional groups on bacterial cell surface involved in metal binding. The global gene expression analysis of bacterial stress response to elevated concentrations of Se and As oxyions was carried out to investigate the mechanism underlying bacterial tolerance to these toxic metalloids. The arsenite oxyions proved to be more toxic to the strain as indicated by the overall low cellular activity. It survives the arsenite toxicity by expelling out the arsenite ions with the aid of an efflux pump, ACR3. No specific selenite transporter was identified however, selenite reduction was observed to be associated mainly with the membrane fraction. It seems that the activation of various transcriptional networks cooperate to protect the cell from selenium toxicity. Thus, it can be concluded that multiple mechanism are involved in detoxification of selenite oxyions in this bacterium. Lastly, two novel species

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of bacteria, *Yaniella fodinae* ($G5^T$) and *Agrococcus carbonis* ($G4^T$) belonging to family *Yaniellaceae* and *Microbacteriaceae* were characterized by polyphasic approach. Bacterial strains were also isolated from soil samples from the nickel mine of Bhubaneswar, Orissa, and were studied for metal tolerance and transformation. Few bacteria were found to tolerate high concentration of NiSO4 (>10mM). These bacteria were identified as *Arthrobacter, Streptomyces*, *Bacillus*, and *Terrabacter* species. Apart from this, a toxicity study of bio-nano-selenium is being carried out on animal models. This bio-nano-selenium is produced by two strains isolated in our lab earlier viz., CM100B and JS2.

From the rhizosphere of *Parthenium hysterophorus* two different biosurfactant producing bacterial strains namely *Pseudomonas aeruginosa* A11 and *Bacillus subtilis* A21 were isolated. Strain A11 produced glycolipids type surface active agent which was characterized as Rhamnolipids by FTIR, NMR and LC-MS. Biosurfactant from strain A11 reduced the surface tension of water from 72 mN/m to 30 mN/m with CMC value of 80mg/L. Dirhamnolipids (L-rhamnosyl-beta-hydroxydecanoyl-beta-hydroxydecanoate) constituted major fraction of Rhamnolipids. Purified Rhamnolipids demonstrated antimicrobial activity against several bacteria. It caused lysis of susceptible bacteria within one hour of exposure to 2mg/ml solution. Coating of rhamnolipids on silicon tubes significantly reduced the adherence of several pathogenic bacteria. *Bacillus subtilis* A21 produced surface active lipopeptide while growing on unconventional and inexpensive source of energy-Ipomoea batatas extract. The Biosurfactant from A21 was characterized as Surfactin isoforms by TLC, FTIR, HPLC and MALDI-TOF. It reduced the surface tension of water from72 mN/m to 28 mN/m with CMC value of 33mg/L. Purified surfactin was able to remove metal like Cr, Cd, Co, and Fe from heavy metal contaminated soil.

Several strains have been screened for Lipases and we are working on the production and characterization of a couple of new lipases/esterases. During this period my group characterized three novel bacterial species from the rich biodiversity of India. Apart from this we are also working on the microbial degradation of PCBs, dyes and Petroleum fractions. Efforts are on in the direction of removal of S from oil. Another interesting area we are working on is the formation of Calcium Carbonate by microorganisms.

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Understanding the molecular basis of drug resistance by signalling proteins and secondary transporters in *Acientobacter baumannii*

Pathogenic bacteria have been evolving continuously and spreading resistance to diverse classes of antibiotics. As a result, our antibiotic arsenal becomes limited. Therefore, to overcome bacterial resistance, a deeper understanding of the many antibiotic resistance mechanisms is becoming increasingly essential for a broader audience of healthcare, and public health researchers. Signal transduction systems are woven within the fabric of bacterial cellular regulatory processes and are used to regulate the expression of genes involved in the virulence and antibiotic resistance responses of a large number of pathogens of major public health concern. The emergence of strains of pathogenic bacteria that are resistant to multiple antibiotics has driven the search for new targets and/or modes of action for antimicrobial agents. The presence of essential signal transduction systems in bacteria and the central role that these regulatory systems suggests that these systems may be novel targets for antimicrobial intervention. *Acinetobacter baumannii* is one of the most prevalent nosocomial multidrug pathogen with a high rate of mortality and morbidity worldwide including India. Previously, we had identified and characterized multidrug secondary transporters for the first time. Despite a worldwide increase in the incidence of *A. baumannii* infections, surprisingly, none of the signalling proteins have been characterized and its role in bacterial pathogenesis in general and its use as antibiotic target in particular has remained completely unexplored. Therefore, our current research interests focus on understanding the pivotal role of novel signalling proteins - protein kinases and secondary transporters-efflux pumps in multidrug resistance and host-pathogen interactions/pathogenesis. We use various molecular/protein engineering, genomic and proteomic approaches for our experiments. Implications of this research can be envisioned in terms of developing appropriate novel inhibitors to limit or prevent the spread of highly virulent multi-drug resistant clones from molecule to preventive action.

Govindan Rajamohan obtained his Ph.D. (2006) in Biotechnology from Institute of Microbial Technology, Chandigarh. He was a DAAD fellow (1999-2000) at Institute for Biochemistry-Gene Center, Ludwig Maximilian University, Munich, Germany and did post-doctoral research at The Ohio State University, USA (2007-2009) after joining IMTECH in 1994.

Novel bacterial signalling proteins from multidrug resistant *A. baumannii*: Signal transduction systems are intracellular information processing pathways that link environmental cues to adaptive responses in all living organisms. Reversible protein phosphorylation is a ubiquitous mechanism for the control of signal transduction networks that regulate diverse biological processes. Changes in protein phosphorylation affect the structure and activity of proteins regulating nearly all aspects of cell life including metabolic processes, DNA replication, gene expression, and the cell cycle. Enzymes called protein kinases (phosphorylation) and phosphatases (dephosphorylation) play a key role in this process. Protein kinases are classified into two families based on their similarities and enzymatic specifications; the histidine kinase superfamily (His Kinases) belonging to the two-component systems (TCS), which autophosphorylates a conserved histidine residue and the serine, threonine and tyrosine kinase superfamily (Ser/Thr and Tyr) that phosphorylates serine, threonine and tyrosine residues, respectively. Although the TCS represent the conventional prokaryotic mechanism for detection and response to environmental changes, however, the advent of genomics in recent years has provided interesting insights into the presence of multiple genes encoding one component system (OCS); Tyrosine or Ser/Thr kinases and phosphatases in prokaryotes. Coexistence of both His and Ser/Thr and Tyr kinases in several organisms as evident from genome sequence analysis may be significant in terms of functional differences between the two families of kinases. Recently, novel type of tyrosine kinase signalling mechanism is elucidated in bacteria which were initially considered to be intrinsic to eukaryotic hosts. Tyrosine phosphorylation is now recognized as a key regulatory device in bacteria and thus is considered an effective antibacterial target. Though there is plethora of information available regarding the physiological functions performed by bacterial tyrosine kinase (BY-kinase), however salient gaps exist in the field of tyrosine phosphorylation mediated bacterial signalling. Therefore, our aim is to decode the previously unprecedented/uncharacterized functions of signalling proteins (serine/threonine and tyrosine kinases) in drug resistance, bacterial pathogenesis and virulence using nosocomial pathogen *A. baumannii* as a model organism.

Molecular and functional characterization of novel bacterial protein kinase from multidrug resistant *A. baumannii*: Based on bioinformatic analyses using the previously described idiosyncratic bacterial tyrosine kinase and its cognate phosphatase in different bacteria have revealed the presence of similar kinase and phosphatase in multidrug resistant strain of *A. baumannii*. Bacterial tyrosine kinase and phosphatase among different *A. baumannii* strains exhibited more than 82% identity and 87% similarity. To our surprise, the number of two component system was noticeably less compare to other notorious pathogen such as *E.coli*, *Salmonella* spp. indicating that the sensing mechanism from other families (probably tyrosine kinase) might be playing vital role in responding to environmental cues. Therefore, *A. baumannii* consider be an ideal bacterial model to study the regulatory functions of bacterial tyrosine kinase and its phosphatase enzymes. Bacterial tyrosine kinaase consists of two main domains; an N-terminal domain bordered by two transmembrane helices which is located outside the cell and a C-terminal catalytic domain, following the second transmembrane helices, located in the cytoplasm. The intracellular C-terminal domain have common signature sequence for Walker A, A', B and a tyrosine cluster are the hallmark features of the BY-kinase family. In an attempt to functionally characterize the BY-kinase and phosphatase, both the genes were cloned and expressed in *E. coli.* The results demonstrated that both the genes are functionally active and its cognate phosphatase act as substrate for BY-kinase. Further, to delineate its role in native host, BY-kinase knock out was generated using standard procedures. Overall, efforts are underway to characterize both the genes in detail.

Characterization of a novel signalling protein with dual catalytic domain of protein kinases from multidrug resistant *A. baumannii*: In this study, we present the identification and first biochemical characterization of an unprecedented putative protein kinase (Ab-PK) from a multidrug resistant clinical isolate of *A. baumannii*. The Ab-PK gene encodes a 279 amino acid polypeptide (~33kDa protein) with dual overlapping catalytic domain

(spanning residues between 1-214 and 183-279) of protein kinases. The full length and respective deletion constructs with single catalytic domain was cloned from *A. baumannii* and expressed in *E.coli* as a fusion protein. *In vitro* kinase assays with full length protein exhibited autophosphorylation indicating that it is a functional kinase. Phosphorylation occurred in a time and concentration dependent manner with Mn^{+2} as a preferred cofactor within a pH range (7.0-8.0) at 37°C. Phosphoamino-acid analysis indicated that Ab-PK phosphorylates at serine and threonine residues. Transphosphorylation activity of purified Ab-PK kinase was demonstrated using the myelin basic protein as a surrogate substrate. Deletion constructs with a single catalytic domain was also found to be enzymatically active. Over expression of Ab-PK in a heterologous host *E.coli* displayed prominent cell division defects as observed by scanning electron microscopy. These results illustrate the currently undefined role of Ab-PK in cell division of *Acinetobacter*. Ab-PK is phylogenetically conserved within *Acinetobacter* genus which therefore indicates the operation of a novel protein phosphorylation mechanism during transduction of signals in *A. baumannii.* Overall, our findings provide insight into the functional role of the previously undescribed protein kinase in *Acinetobacter* for the first time.

Identification and functional characterization of novel secondary transporters (efflux pumps) from multidrug resistant *A. baumannii*: *A. baumannii* is a rapidly emerging nosocomial pathogen and causes severe life threatening infections. Alarmingly, this pathogen has raised concern in health care settings globally including India due to its intrinsic resistance to most clinically significant antibiotics. The emergence of multidrug resistant (MDR) /extreme drug resistant (XDR) bacteria has posed a serious problem worldwide. Active multidrug efflux processes, usually involving secondary transporters are now known to be important, especially in the intrinsic resistance of bacteria to antimicrobial agents. Analysis of published *A. baumannii* AYE genome sequence indicate the presence of efflux pumps from different family and more than 70% of them being the RND efflux pumps. More precisely, fifteen genes have been annotated as putative RND efflux pumps. It is worthy to state that till date only two such efflux pumps have been characterized, but the contribution of other pumps towards intrinsic drug resistance of this bacterium remains unclear.

Molecular mechanism of multidrug resistance in *A. baumannii*: Functional role of novel RND efflux transporter in cellular physiology: Our other area of research interest is to elucidate the molecular mechanisms and regulation of multidrug resistance with a prime focus on bacterial membrane efflux pumps. The objectives of the proposed study was to perform biochemical and genetic studies in order to understand the molecular architecture, function, and regulation of efflux pumps belonging to the resistance nodulation family in *A. baumannii.* The detailed knowledge about the efflux pumps is important for the development of MDR efflux-pump inhibitors, an important area of drug development. In this proposed study we investigated the contribution of previously uncharacterized efflux transporters in drug resistance of *A. baumannii*, using various approaches to unravel its essential role in conferring antibacterial resistance. The identified RND efflux operon has membrane fusion protein, transporter and outer membrane protein. Initially the transporter gene was targeted for making knock out, the gene was disrupted by incorporating the hygromycin cassette in the genome of native host. The generated mutant was further subjected to phenotypic analysis like antibiotic susceptibility testing and growth inactivation assays. The results of the assays suggested that it confers resistance to cell wall acting antibiotics. Growth inhibition and efflux assays were performed for both native and mutant strains in the presence and absence of selected antibiotics with two different efflux inhibitors. The results demonstrated the drastic reduction of efflux activity in the presence of inhibitors suggesting that mutated gene have a role in efflux activity of different antibiotics. In order to get an insight into regulation of the efflux pump, the promoter region of the RND type efflux pump was inspected. The promoter region was analyzed for presence of putative regulatory site using bioinformatic approach and analysis identified consensus sequence for the AraC and LysR type transcriptional regulator. The identified transcriptional regulators were cloned and expressed as fusion protein in

E.coli. Currently, work in progress to characterize both the regulators and its role in modulating the novel multidrug efflux transporter.

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Understanding the biology of *Vibrio cholerae* in the context of quorum sensing & model systems

Quorum sensing in *Vibrio cholera* - Lessons from natural variant: Studies on the quorum sensing signal network of *Vibrio cholerae* have produced a rich harvest of data where periodic appearance and performance of two regulatory proteins, namely LuxO and HapR determines the fate of a plethora of disparate cellular events. Of these, HapR has been given the status of a master regulator as it controls a wide range of diverse physiological activities, thus exerting a profound influence on the survival and pathogenic potential of this bacterium. Collectively, it represses biofilm development and the production of primary virulence factors while stimulates the production of HA/protease, promotes chitin induced competence, increases resistance to protozoan grazing, enhances the survival against oxidative stress and controls the expression of gene encoding Hcp. In a continuing effort, Zhu and coworkers have elegantly characterized additional novel direct targets of HapR and illustrated two distinct binding motifs (motif 1 and motif 2) in all target promoters. As it modulates a multitude of diverse cellular parameters, absence of a functional HapR affects the physiology of *Vibrio cholerae* to a great extent. Being a master regulatory protein of quorum-sensing circuit, a great deal of work has therefore been dedicated to understand the various structural and functional aspects of HapR. Although previous analysis has identified certain residues imparting to the DNA binding activity of HapR, the role of residues in the hinge region has not been evaluated in this context. While unraveling the necessary cause of protease negative phenotype of a non-O1, non-O139 strain of *Vibrio cholerae*, we discovered a variant HapR harboring a glycine to aspartate substitution in the hinge region. *In vitro* gel shift assay clearly suggested the inability of $HapR_{V2}$ to interact with various cognate promoters. Reinstatement of glycine at position 39 restores DNA binding ability of $HapR_{V2}$ ($HapR_{V2G}$), thereby rescuing the protease negative phenotype of this strain. The elution profile of $HapR_{V2}$ and $HapR_{V2G}$ proteins in size-exclusion chromatography and their circular dichroism spectra did not reflect any significant differences to explain functional discrepancies between the two proteins. To gain insight into the structure-function relationship of these two proteins, we acquired small/wide angle x-ray scattering (SWAXS)

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data from samples of the native and $G^{39}D$ mutant. While Guinier analysis and Indirect Fourier transformation of scattering indicated only slight difference in the shape parameters, structure reconstruction using dummy amino acids concluded that while HapR adopts a "Y" shape similar to its crystal structure, the $G^{39}D$ mutation in hinge drastically altered the DNA-binding domains by bringing them in close proximity (Figure 1). This altered spatial orientation of the helix-turn-helix domains in this natural variant provides the first structural evidence on the functional role of hinge region in quorum sensing related DNA binding regulatory proteins of *Vibrio* spp.

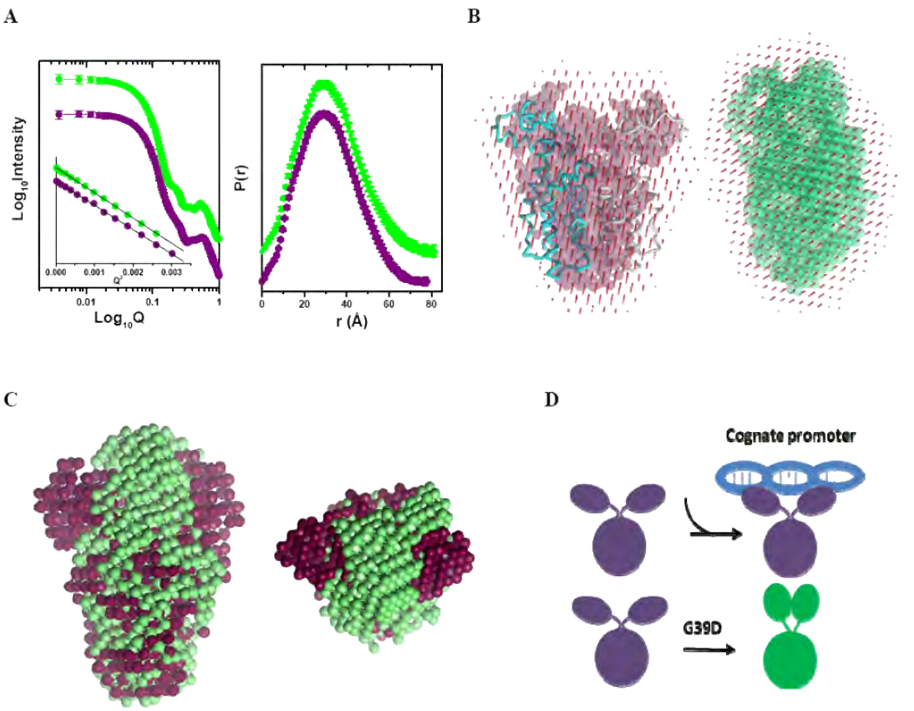

Figure 1: SWAXS of $HapR_{v2}$ and $HapR_{v2G}$: (A) SWAXS intensity profiles have been shown as function of Q ($HapR_{v2G}$: Purple; $HapR_{v2}$: Green).Inset depicts the linear region of the Guinier approximation. The Computed P(r) curves from the SWAXS data are plotted in the right panel. (B) Averaged models of $HapR_{v2G}$ (left) and $HapR_{v2}$ (right) reconstructed within shape constraints offered in the measured scattering data are presented. The model of $HapR_{v2G}$ was overlaid on the crystal structure solved for the same protein (PDB ID: 2PBX). (C) Two orthogonal views of the manual superimposition of the dummy atom models restored for $HapR_{v2G}$ and $HapR_{v2}$ highlight similarities and local differences in the global structure of the two proteins. (D) Schematic representation highlights the distortion of the hinge region upon G39D mutation which grossly alters the spatial disposition of the DNA-binding domains.

Yeast as model system to understand pathogenesis of *Vibrio cholerae*: Model systems are essentially important to understand the mechanism of virulence determinants as well as interaction of pathogens with their hosts. Recently, non-mammalian lower eukaryotes such as free living protozoa and nematodes as well as higher eukaryotes such as budding yeast *Saccharomyces cerevisiae* have been considered as model systems for numerous pathogenic bacteria. Of these, *Saccharomyces cerevisiae* has been exploited extensively as model genetic system to identify and characterize the function of virulence factors especially those translocated by T3SSs. We observed that ectopic expression of VopF, a type III effector protein of *Vibrio cholerae*, causes toxicity in the budding yeast *S. cerevisiae*. Interestingly, VopF positive *Vibrio cholerae* also exerts lethality on *Candida albicans.* Additional studies are necessary to unravel the mechanism of toxicity.

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The interactions of Erp (Exported Repetitive Protein) with macrophage proteins: New insights into the mechanisms of virulence of *M. tuberculosis*

Survival of *M. tuberculosis* within macrophages requires the interaction between proteins of macrophage and the infecting bacteria. Our laboratory initiated working on these novel and important protein-protein interactions of *M. tuberculosis* with macrophages with an aim to understand their relevance in the pathogenesis of Tuberculosis. The secretory proteins out of the *Mtb* protein repertoire play a major role in host-pathogen interactions. One such secreted virulence factors is Erp and the response of Erp to the host/macrophage is not explored. Thus, as a beginning in this direction, we initiated the protein-protein interaction between Erp, a virulence determinant required for growth in cultured macrophages and *in vivo*, with the macrophage proteins. Initially we explored the interactions of Erp with macrophage proteins using Y2H technique. Erp was used as bait and mouse macrophage cDNA library as prey. We found many interacting partners of Erp in macrophages, which were sequenced and then assigned functions, depending on the homologies with the known sequences. Out of these interacting partners, the interactions of Erp with 101 and 103 proteins (arbitrary number given by us), was confirmed by GST pull-down assay. We observed that His-tagged Erp was precipitated specifically by GST-tagged 101 and also by GST-tagged 103 under *in vitro* conditions. This shows that Erp interacts with 101 and 103 directly and the possibility of third partner mediating their interaction is ruled out. This is the first report showing the direct interaction of Erp with 101 and also of Erp with 103. 101 and 103 are macrophage proteins. 101 is reported to be present in Endoplasmic Reticulum and 103 in the cytoplasm.

These interactions under *in vivo* conditions were further investigated by Co-Immuno-precipitations. FLAG-tagged Erp, HA-tagged 101 and HA-tagged 103 were cloned into their respective eukaryotic expression vectors. Next, FLAG-tagged Erp construct was co-transfected alongwith either HA-tagged 101 or HA-tagged 103 in BHK cells or CCL39 cells. We could successfully immunoprecipitated Erp using FLAG antibodies. HA-101 and HA-

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103 were found to be co-immunoprecipiated alongwith Erp, which shows that Erp is interacting with 101 and also with 103 under *in vivo* conditions. These interactions have been confirmed by three independent experiments. The domains of Erp responsible for the interaction with 101 and the domains of 101 involved in the interactions with Erp were identified using CoIPs. Upon deletion of N-terminus (ΔNH2), Erp fails to interact with 101 and upon deletion of C-terminus of 101, the interaction between Erp and 101 is abrogated. ΔNH2-Erp also failed to interact with 103.

Subsequently, the localization of Erp with 101 and 103 were examined in BHK and THP cells using confocal microscopy. CFP-tagged Erp and either YFP-tagged 101 or YFP-tagged 103 were transfected in BHK and THP cells. 101 has been reported previously to be present in Endoplasmic Reticulum (ER). Erp was found to be present in cytoplasm and also in ER. Erp co-localized with ER-Tracker. Erp was also found to be co-localized with 101 in ER. 103 is a cytoplasmic protein. Erp was also observed to be co-localizing with 103. ΔNH2-Erp was mainly localizing in the nucleus. Localization of ΔNH2-Erp supports the CoIPs data.

Further, we explored the effect of Erp on the activities of 101 and 103. Both are well studied enzymes with good enzyme assays. Lysates of BHK cells were treated with purified recombinant Erp to study the effect of Erp on their activities. Erp was found to up-regulate 101 and 103 by 1.5 fold and 5.0 fold, respectively. ΔNH2-Erp failed to up-regulate either 101 or 103, attesting to CoIPs and localization data.

Our data suggests that Erp is interacting directly with multiple proteins in macrophages and modulates their activities. Now our efforts are on to understand the role played by Erp on 101 and 103 activity in the macrophages upon infection and thus, to understand the molecular basis of the survival mechanism(s) employed by *M. tuberculosis*. In addition, we are interested in understanding the role and regulation of Erp in mycobacterium as well.

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Understanding bacterial ecology and adaptation using comparative and evolutionary genomics

Bacteria constitute 80% of Earth's biomass. They are found in diverse places like vertebrate gut, plant leaves, soil, etc. Understanding how they have adapted to diverse habitats and co-evolved with their hosts from millions of year is a fundamental question and grand challenge. One of the pre-requisite for carrying out systematic ecological and evolutionary study is understanding the phylogenetic and taxonomic relationship of bacteria at finest possible level. Other critical requisite is selection of appropriate group of bacteria with diverse habitats and adaptation, instead of fashionable bacteria. Our first favorite bacterium in this regard is *Stenotrophomonas maltophilia* is found in soil, water, plant rhizosphere, industrial wastes and hospital settings (and emerging as one of the major opportunistic human pathogen). *Stenotrophomonas maltophilia*, which is non-phytopathogenic and taxonoimcally closely related to *Xanthomonas*. Apart from being a model group for undertaking basic studies in adaptation, ecology and evolution, both these bacteria of agriculturally, industrially and medically significant.

Resolving the phylogenetic and evolutionary relationship of *Xanthomonas* and *Stenotrophomonas* strains using complete *rpoB* gene sequence: The phytopathogenic genus *Xanthomonas* comprises numerous species and pathovars described primarily on their host and tissue specificities. *Stenotrophomonas maltophilia* has undergone several classifications from *Pseudomonas* to *Xanthomonas* and finally to *Stenotrophomonas*. In this context, we investigated the phylogenetic and taxonomic status of these members using the complete RNA polymerase beta-subunit (*rpoB*) gene sequences available from their sequenced genomes. Not only did we obtain a phylogenetic tree for xanthomonads, but *rpoB* gene sequence information has also resolved the taxonomic relationship of *X. axonopodis* pathovars, *X. albilineans* and other *Xanthomonas* strains, with the most marked evidence being that *Stenotrophomonas* is synonymous to *Xanthomonas*. This

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study has revealed the power and potential of complete *rpoB* gene sequence in taxonomic, phylogenetic and evolutionary studies on *Xanthomonas* and *Stenotrophomonas* generic complex.

SRICs – *Stenotrophomonas* specific extragenic repetitive intergenic consensus sequences: We have identified a *Stenotrophomonas* specific extragenic repetitive intergenic consensus sequences (SRICs). These sequences or elements are present only the genome of *Stenotrophomonas* strains and not in any other bacteria. Interestingly the two SRIC we have identified genome are present in more copies in the genome of clinical strain than in the genome of other environmental strains. For example the genome of clinical strain, Sma K279a contains 12 copies of SRIC1 and 40 copies of SRIC2 but on the other hand, the genome of plant endophytic strain, R551-C, contains only 2 copies of SRIC1 and 7 copies of SRIC2. This suggests that the elements, SRIC1 and SRIC2, are specifically active in the genome of clinical strains and not active in the genome of environmental strains. Such repetitive sequences are thought to be important in gene expression by increasing the stability of mRNAs or if sitting in an operon they can potentially affect expression of downstream genes. The element is associated with many regulatory genes and hence might have role in success of this strain. We are mapped this element on the genome and studying these potentially important genomic regions or associated genes. We have also designed two primers based on consensus sequence generated from aligning all the copies and will be used in molecular screening of *Stenotrophomonas* strains. We have zeroed in on *Stenotrophomonas* strains from human patients and environmental setting available at Microbial Type Culture Collection (MTCC) facility at IMTECH and international depositories. We are in the process of procuring the same and study the dynamics of this element across a collection of *Stenotrophomonas* isolates.

A multicopy orfan gene in the genome of *Stenotrophomonas*: One of the best surprises of genome sequencing is presence of large numbers of genes that do not have homolog(s) in the genome of any other bacteria. Such genes are known as ORFans and thought to be important for virulence/environmental adaptation of bacteria. During our comparative genomics work, we have identified multiple copies of an orphan gene in the genomes of three *Stenotrophomonas maltophilia* strains isolated from plant rhizosphere, ocean and clinical sample. Each of the genome has at-least six copies of this gene distributed all over the genome and located in several important gene clusters. The orphan genes show high variation among each other and have G+C content typical of *Stenotrophomonas* genome. There is no homolog of this gene in *Xanthomonas*, a taxonomically related genus or in any other bacteria. This suggests that orphan genes have might be playing important role in ecology and evolution of *Stenotrophomonas maltophilia.*

We are also majorly interested in using whole genome sequencing of bacterial strains, shotgun sequencing and amplicon sequencing of metagenomes to understand ecology and evolution of bacteria that are associated with plants and animals. In this regard, we have initiated procurement and complete genome sequencing of taxonomically and phylogenetically important bacterial strains of our interest. We are also part of multinational genome projects as experts to understand genome variation in animal and plant associated bacteria.

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Targeting promiscuous peptides to dendritic cells through Toll-Like Receptor and elicitation of enduring immunity against *Mycobacterium tuberculosis*

Development of vaccines against infectious diseases like tuberculosis: Earlier, we demonstrated a novel, yet simple cell based vaccination strategy that involves the culturing of live *Mycobacterium tuberculosis* and *Salmonella typhimurium* in macrophages, followed by drug treatment and gamma irradiation, to kill the bacteria. This approach however, worked successfully in short-term experiments but failed to impart long-lasting immunity against *M. tuberculosis* and *S. typhimurium* [J Infect Dis. 2004, US Patent 6783765)]. In extension of this study, we have now demonstrated the role of cytokines IL-1+IL-6+TNF-a *that augment T cell memory in the enhancement of long-term protection by the vaccine prepared by utilizing infected macrophages* [PLoS One, 2011]. We observed long-term generation of both central as well as effector memory CD4 and CD8 T cell pools, elicitation of mainly Th1 memory response, reduction in the mycobacterial load and alleviated lung pathology. The protection induced by the vaccine was significantly better than BCG. Importantly, the mechanism of priming of immune system by the vaccine was by generation of apoptotic vesicles containing processed mycobacterial antigens that were avidly taken by dendritic cells for priming of T cells (Figure 1).

BCG protects the childhood but not adult manifestation of TB. Thus, indicating that BCG fails to evoke long-lasting immunity, in spite of its antigenic repertoire to generate an immune response. We have demonstrated this poor induction of memory by BCG could be overcome by administration of pro-memory cytokines IL-7 and IL-15. Immunization of BCG supplemented with IL-7 and IL-15 resulted in an enduring CD4 and CD8 T cell memory response [J Infect Dis. 2010]. We could also note enhanced T cell proliferation, T helper 1 type cytokines

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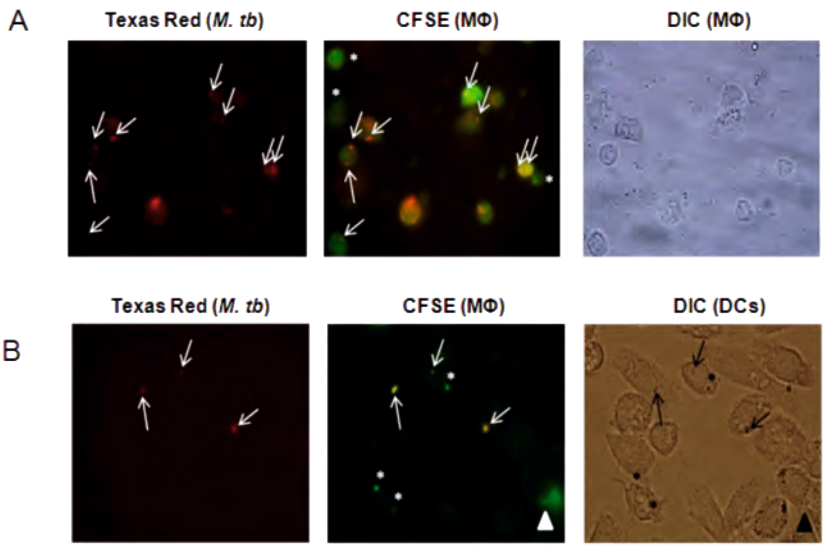

Figure 1: *Apoptotic bodies generated by infected macrophages transport antigen to bystander dendritic cells. M. tb* infected macrophages were co-cultured with Dendritic cells (DCs) for 48 h. Later, fluorescence microscopy was used to demonstrate generation of apoptotic bodies containing mycobacterial antigens and their uptake by bystander DCs. (A) Left panel, arrows indicate Texas Red labeled *M. tb*; Middle panel, *M. tb* infected macrophages (orange, mixed fluorescence) and asterisks depict uninfected macrophages (green); right panel, DIC image of macrophages (images at 100×). (B) Left panel, arrows indicate Texas Red labeled *M. tb*; middle panel, arrows indicate *M. tb* within apoptotic bodies (orange, mixed fluorescence) while asterisks indicate apoptotic bodies (green) devoid of mycobacteria; right panel, DIC image of DCs show engulfment of apoptotic bodies containing mycobacterial antigens by DCs (Images at 100×1.6×). Arrow heads indicate association of apoptotic macrophages with DCs. DIC, Differential interference contrast.

production, and an increased pool of multifunctional *M. tuberculosis* specific memory T cells in the immunized mice (Figure 2). There was a significant reduction in the mycobacterial burden in the lungs compared to BCG

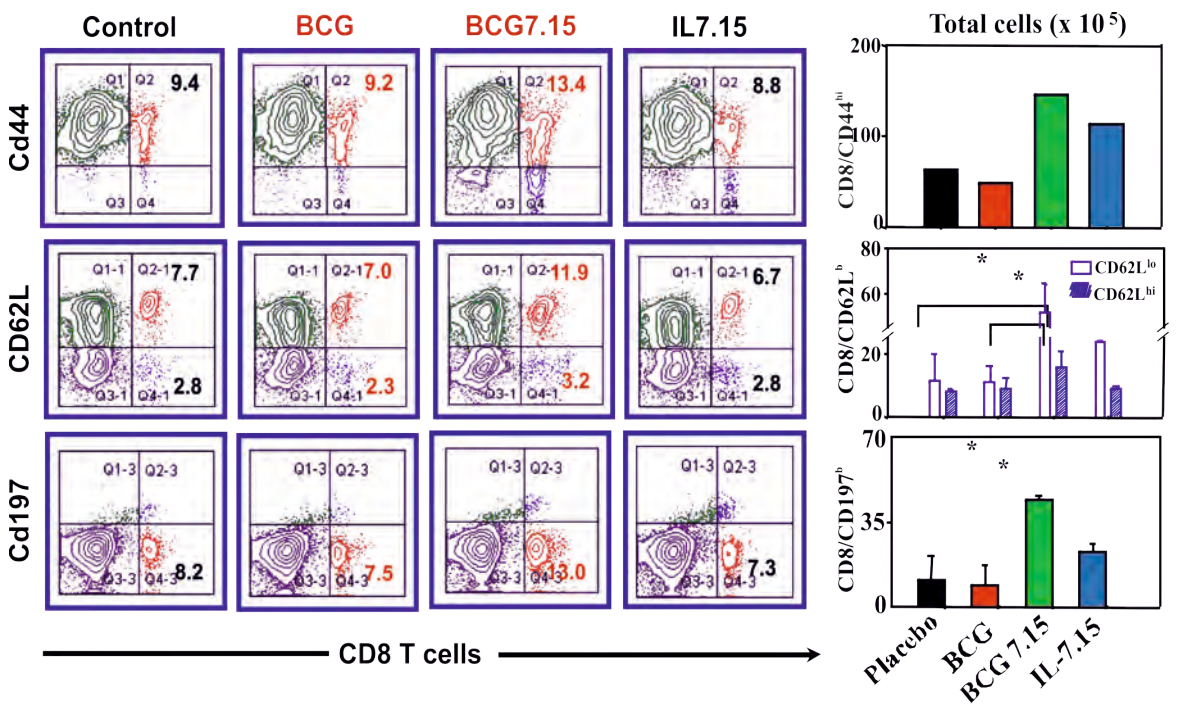

Figure 2: *IL-7 and IL-15 supplemented with BCG vaccine augments long-term sustenance and maintenance of CD8 T cells memory response*. Lymphocytes were isolated from vaccinated mice and cultured with PPD. Cells were stained after 48h with fluorochrome conjugated antibodies for CD8, CD44, CD62L and CD197 and analyzed by flow cytometry. Bar diagrams indicate total number of CD8 T-cells expressing $CD44^{hi}$, $CD62L^{hi}$, $CD62L^{lo}$ and $CD197^{lo}$ phenotype. Control (PBS) and IL7.15 (IL-7+IL-15) means placebo and cytokines injected animals, respectively. '*' indicates $p<0.05$.

immunization. The results indicate that supplementation of the BCG vaccine with IL-7 and IL-15 would substantially improve its efficacy by enhancing the T cell memory response.

Clinical trials have revealed that BCG does not confer any protection in TB-endemic areas. BCG failure in TB-endemic areas is due to masking and blocking effects of environmental mycobacteria, their impediment in antigen processing abilities of antigen presenting cells (APCs) and generation of memory T-cell response that ultimately affects BCG efficiency. Hence, any vaccine that requires extensive processing may not be quite

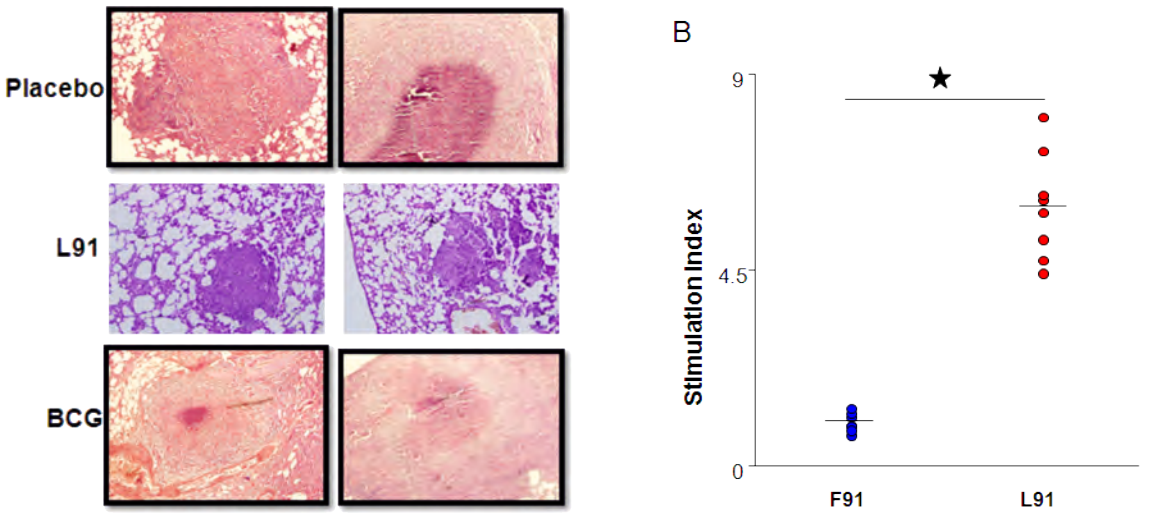

Figure 3 : L91 induces greater protection than BCG. L91 immunized Guinea pigs exhibit less pathology than BCG immunized group (A). L91 but not F91 evokes proliferation of T cells isolated from individuals of TB-endemic areas (B).

successful in these areas. To overcome this problem, we developed a unique lipopeptide (L91) by linking the promiscuous peptide (sequence 91-110) of 16 kDa antigen of *M. tuberculosis* to Pam2Cys. This lipopeptide has self-adjuvanting properties and activates dendritic cells. L91 does not require extensive antigen processing and generates enduring Th1 memory response [J Infect Dis. 2011. *in press*]. Further, L91 surmounts the barrier of MHC polymorphism and induces better protection than BCG in animal models of tuberculosis (Figure 3). Importantly, L91 activates T cells isolated from purified protein derivative positive healthy volunteers that responded weakly to free peptide (F91). In essence, L91 can be a potent future vaccine candidate against tuberculosis.

Costimulatory molecules mediated regulation of T cells and B cells : Earlier we had discovered the role of CD80 in inducing the apoptosis of B cell lymphoma by up-regulating the expression of pro-apoptotic molecules [J Biol Chem. 2002, Expert Opin Ther Targets 2008, Curr Immunol Rev. 2007]. In continuation of this observation, we currently report an alternative approach, independent of BCR, for stimulating resting B (RB) cells, by involving triggering of TLR-2 and CD40 molecules crucial for bridging innate and adaptive immunity. CD40 triggering of TLR-2 stimulated RB cells significantly augments their activation, proliferation and differentiation. It also substantially improves the calcium flux, antigen uptake capacity and ability of B cells to activate T cells [PLoS One 2011]. CD40 and TLR-2 triggered RB cells showed enhanced survival, increased amounts of activation induced deaminase (AID) and class switch recombination (CSR). Interestingly, CD40 and TLR-2 concomitant signals decreased threshold of optimum stimulation of RB cells (Figure 4). The results could also be

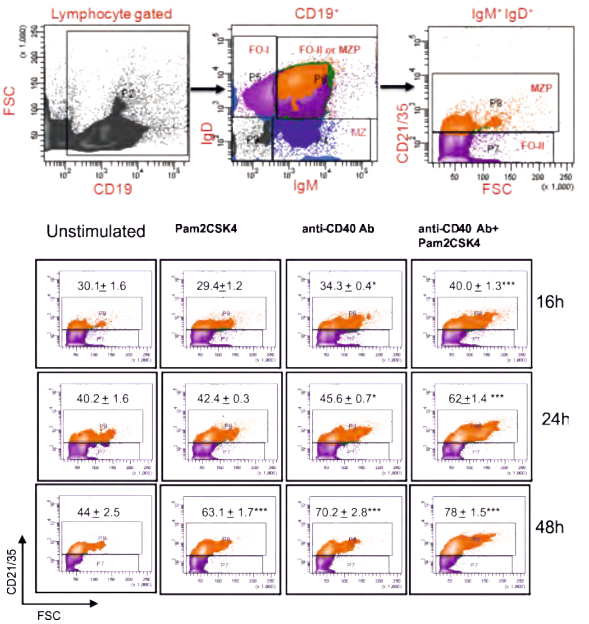

Figure 4: *Signaling through TLR2.CD40 differentiates RB cells into marginal zone precursors*. Signaling was delivered in RB cells with Pam2CSK4 and anti-CD40 Ab for 16 h–48 h. Upper panel shows the gating scheme for defining different B cell subsets: FO-I (CD19+ IgD^{hi}), FO-II (CD19+ IgDhi IgM^{lo}), MZP (CD19+ IgM^{hi} IgD^{hi} CD21/35^{hi}) and MZ (CD19+ IgM^{hi}). Lower panel shows contour diagrams of MZP in stimulated B cells at different time durations. Values indicate the average change (mean ± SEM) in percent population of CD21/35^{hi} expressing B cells. '*', '**', '***' indicates p<0.05, p<0.01, p<0.001 respectively.

corroborated well with microarray gene expression data. This study provides novel insights into coordination between the molecules of innate and adaptive immunity in activating B cells, in a BCR independent manner. This strategy can be exploited to design vaccines to bolster B cell activation and antigen presenting efficiency, leading to faster and better immune response.

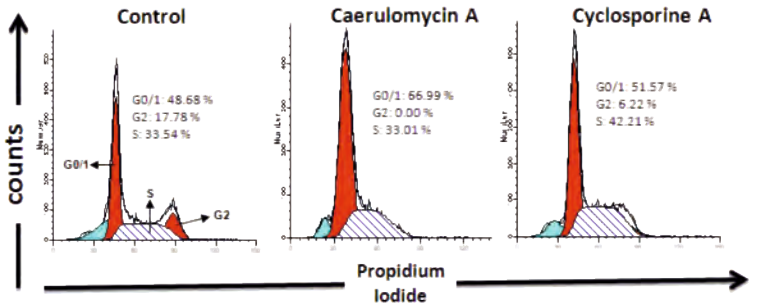

Figure 5: *'Caerulomycin A' restrains the T cell proliferation.* 'Caerulomycin A' inhibits the proliferation of T cells by arresting the cell cycle at G0/G1 phase. Cyclosporin A is used as a positive control.

Caerulomycin A: an immunosuppressive agent : Earlier we had established that 'Caerulomycin A' modulates the immune response by inhibiting the T cell activity. Dr. R. S. Jolly identified the structure of 'Caerulomycin A' and Dr. R.M. Vohra did microbiological studies [United States Patent Application No. 20070078167]. In continuation of this study, we have now demonstrated that 'Caerulomycin A' can inhibit the differentiation of naïve T cells to effector T cells and restrains T cell proliferation by arresting the cell cycle at G0/G1 phase (Figure 5).

Potential T cell epitopes of *M. tuberculosis* involved in host autoimmune pathogenesis: Antigenic determinants/epitopes present in pathogens, which resemble the host proteins that induce molecular mimicry, can potentially be a threat in the activation of the immune cells, resulting in autoimmunity. Intriguingly, autoimmune diseases have been reported to be prevalent in tuberculosis endemic populations. Further, association of *M. tuberculosis* has been implicated in different autoimmune diseases, including rheumatoid arthritis and multiple sclerosis. We have employed *in silico* tools to determine homologous antigenic regions

between *M. tuberculosis* and human proteomes. We have identified potentially cross-reactive T cell epitopes restricted to predominant class I and II alleles of human leukocyte antigen. Our study reveals many target proteins and their putative T cell epitopes that might have significant application in understanding the molecular basis of autoimmune diseases.

Publications:

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Studies on putative therapeutic/vaccine target(s) in malaria and tuberculosis

Malaria and tuberculosis, causing more than million deaths per year, have always been a challenge to human civilization, particularly in developing countries. Several initially promising strategies, applied to combat these diseases over the years, have failed to give desired results primarily because of non availability of appropriate vaccines and resurgence due to drug resistance. This emphasizes the need to search for novel strategies/molecules which are more effective in controlling such infections.

Both malaria parasite and tuberculosis causing mycobacteria are intracellular pathogens. Antigenic changes on the infected cell surface have been reported in both these infections. In malaria-infected erythrocytes, these antigens have been extensively studied but without much success vis-à-vis discovering new antigens for vaccines, primarily because of the polymorphic nature of the identified antigens. While only sporadic attempts have been made to identify and characterize such molecules in mycobacteria-infected macrophages. These pathogen/infected cell surface molecules could be attractive targets for the therapeutic intervention of the respective disease. Thus, key questions of this study have been: i) which are the functionally/structurally conserved important molecules, even otherwise minor antigenic in nature, associated with the pathogens (*Plasmodium* and mycobacteria) and infected cell, ii) what are the role(s) of the above molecules in terms of pathogen survival and pathology/immunology of the disease and, iii) can those be exploited for the development of the novel therapeutic control strategies in malaria and tuberculosis?

Studies on putative target(s) in malaria: In order to identify functionally/structurally conserved important molecules, epitopes/ mimotopes, associated with the *Plasmodium* parasite and infected cell, we have exploited multiple strategies i) selection of the target (region) based on different criteria including B cell epitope prediction using bioinformatic tools, ii) hybridoma technology employing novel immunization strategies and, iii) combination of hybridoma and peptide phage display library. In the first approach we have narrowed down,

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based on our previous work, to RhopH3 and B subunit of *P. falciparum* V-H^{+} ATPase. In the second approach, monoclonal antibodies (MAbs), generated using novel immunisation strategies, were selected based on their : a) reactivity with *P. falciparum* and *P. berghei* parasites as well as respective infected cell membranes and, b) *in vitro P. falciparum* growth inhibitory activity. Proteins recognized by these antibodies were identified by N-terminal sequencing. While in the third approach, epitopes and number of mimotopes, reactive to these antibodies, were identified using phage displayed 12 mer peptide library, which were further screened for their immunogenic potential. Also, we have isolated peptide (7 mer) phages which specifically react with: i) *P. falciparum* infected cells, ii) both *P. falciparum* and *P. berghei* infected cells and, iii) the known target protein present on infected cell membranes. These peptides were then tested, *in vitro*, for their inhibitory potential towards *P. falciparum* growth.

The N-terminal region of RhopH3 (rRhopH3$_{1-147}$) was cloned, expressed and purified. The sera generated against this fragment reacted well with RhopH3 of both *P. berghei* and *P. falciparum*, indicating the formation of antibodies against conserved epitopes. The generated antibodies were also capable of inhibiting the *in vitro* growth of *P. falciparum*. Further, lymphoproliferation assay and cytokine profile analysis showed that rRhopH3$_{1-147}$ induces the Th1 type of cell response which significantly delays the *P. berghei* infection in BALB/c mice. In our earlier report, we mentioned that the phages obtained after panning onto growth inhibitory mAbB6 (anti-RhopH3 antibody) were found to be immunogenic and the sera raised against few peptides gave the punctate fluorescence pattern, characteristic of RhopH3, with parasite infected erythrocytes as revealed by immunofluorescence assay. Epitope recognized by mAbB6 was mapped by competitive binding assays, taking custom synthesized peptides corresponding to the aligning regions in RhopH3 sequence. Further, mimotope capable of generating B6 type antibodies was also identified. Experiments are now underway to establish the vaccine potential of identified RhopH3 epitopes and mimotopes.

Subunit B of V-H+ ATPase was selected as target molecule based on its presence on *P. falciparum* infected cell surface and function (this part of the work was done with inputs from Dr. K. Ganesan, IMTECH). Sequence analysis revealed that it is conserved in different plasmodium species. Computational analysis of subunit B (494

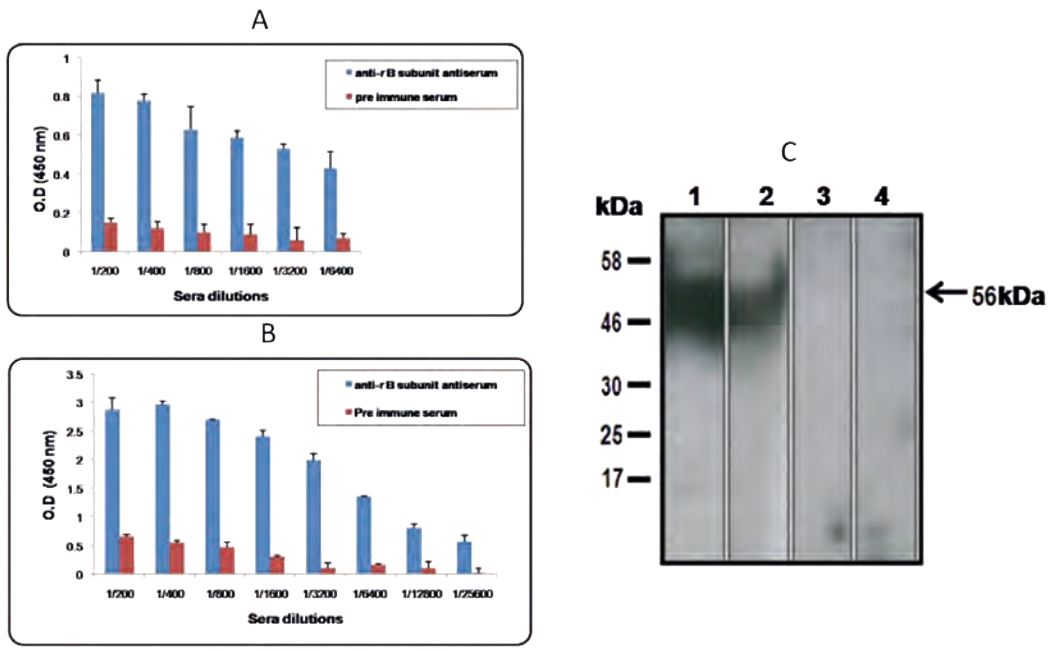

Figure 1: Reactivity of anti-rB subunit of V-H+ ATPase antiserum with *P. falciaprum* and *P. berghei* parasite lysates in ELISA (panels A & B) & in westerm blotting (panel C).

residue) of *P. falciparum* V-H+ ATPase, for conserved domains, showed the presence of walker A and B motifs, characteristic of nucleotide binding proteins. Full length subunit B of Vacuolar H+ ATPase was cloned, over e

xpressed and purified. Antiserum, generated against rB-Subunit, exhibited good reactivity with native V-H+ ATPase in ELISA and recognized ~56 kDa subunit B of V-H+ ATPase protein in both *P. falciparum* and *P. berghei* (Figure 1), thus validating the functional conserved nature of the protein. Confocal microscopy studies with anti-rB-subunit antiserum showed the presence of V-H+ ATPase on infected erythrocyte membrane in *P. falciparum* and for the first time in *P. berghei.* Screening of random peptide phage library on rB-subunit of V-H+ ATPase resulted in the selection of different peptides. The phage peptides showed good levels of binding with rB-subunit of VH+ ATPase, native *P. falciparum* V-H+ ATPase and *P. falciparum* IRBCs. Interestingly, some of these peptides inhibited *in vitro* growth of *P. falciparum*. Thus, these observations indicate the potential use of VH+ ATPase as drug target in *P. falciparum* (patent application filed).

Studies on putative target(s) in tuberculosis: Our work in this direction has focused towards the identification and characterization of putative target molecules present on mycobacterial cell wall and infected cell surface. Using *M. microti* as a model system, we first reported that infected cell surface antigenically differs from that of uninfected cells and we observed selective reactivity of few monoclonal antibodies with infected cell membranes. Also, bioinformatics analysis of different mycobacterial genomes, with emphasis on cell wall associated molecules of *M. tuberculosis* complex, shortlisted few target molecules, including cutinases and lppi. Studies are aimed to characterize these target molecules.

Many plant pathogenic fungi has been reported to be capable of producing cutin degrading esterases. In contrast, relatively few bacteria appear to possess such capability. *Mycobacterium tuberculosis* H37Rv genomic sequence revealed the presence of 7 putative cutinases. Except *cut5*, which seems to be truncated, all cutinase genes code for potential proteins of 180-250 residues, which is consistent with the size of fungal cutinases. We have cloned, over expressed and purified Cut5B form *M. tuberculosis* and full length Cut5 from *M. bovis* for their immunological and functional characterization. Also, for comparative analysis, few other cutinases and their antibodies were procured from outside. The detailed studies, using antibodies, including those generated in the lab, as well as the functional activity of the recombinant proteins, has revealed some interesting features of Cut5B, so far unreported in the literature.

Another target protein of *Mycobacterium tuberculosis* H37Rv, lipoprotein lppi (Rv 2046), was identified from the genomic database. Acylated and non acylated forms of the protein were cloned, expressed and purified. Also, a panel of monoclonal antibodies have been raised against lppi, in order to differentiate this protein from other lipoproteins as well as to characterize its presence in other mycobacteria. Besides, these MAbs will be useful while dissecting the functional role of lppi, if any. Initial immunological studies, using recombinant protein, have indicated its vaccine potential. Studies are underway to further characterize this protein for its immunological and functional properties.

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- Aithal, A., Sharma, A., Srivastava, S., Raghava, G. P. & Varshney, G. C. (2011). PolysacDB: A comprehensive database of microbial polysaccharide antigens and their antibodies (in communication).

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Patent(s):

Grish C. Varshney, K. Ganesan, Ashu Shah, Shailendra K. Gautam and Rakesh Bhatia. Peptides specific to B-subunit of vacuolar-H^+ATPase as novel drug candidates in malaria (provisinal application filed).

Participants:

Shilpy Srivastava, Ashu Shah, Surinder Batra, Deepshikha, Shailendra Kumar Gautam, Abhijit Aithal,Rakesh Bhatia

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Characterization of a membrane expressed multifunctional glycolytic protein and its role in macrophage function

GAPDH is known primarily as a cytosolic protein. Our laboratory has earlier demonstrated for the very first time that GAPDH has a novel cell surface localization in mammalian cells where it functions as a previously unknown receptor for the iron carrier protein transferrin. We have now completely characterized the internalization and recycling kinetics of this new receptor. In addition we have also discovered that apart from its role in trafficking transferrin it also serves a dual role as a receptor for the related molecule lactoferrin.

Our findings describe the presence of an entirely new uptake mechanism for the iron transport proteins transferrin & lactoferrin into macrophages, wherein these cells utilize the ubiquitous moonlighting protein GAPDH as a receptor. This mechanism provides an elegant method by which this abundant cellular protein is relocated to the membrane for this additional role. We have proposed that mammalian cell surface GAPDH represents a primitive mechanism for the uptake of iron transport proteins that has been conserved in cells. Because GAPDH is a ubiquitous protein, the broader implications of this finding are that in addition to macrophages, this may be an alternative mechanism for iron acquisition in other mammalian cells and tissues. In this context recent work from our laboratory has confirmed that this additional role of GAPDH is not limited to macrophages.

Significance of the Contribution: Our current findings are central to understanding iron metabolism in mammalian cell systems. This can be gauged from the fact that the first ubiquitous Transferrin receptor (TfR-1) was identified in the 1950's.The second Transferrin receptor (TfR-2) was identified in the 1990's which however is expressed only on certain cell types. Our recent discovery identifies a new type of ubiquitously expressed Transferrin receptor that bears no homology to the two previously known receptors. We have also established that this receptor is regulated by the levels of extracellular iron. Interestingly our work has demonstrated that

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during times of iron deficiency many cells prefer to use GAPDH for transferrin & lactoferrin delivery instead of the previously characterized receptors. Based on our findings we have proposed a comprehensive model for transferrin mediated iron uptake by mammalian cells.

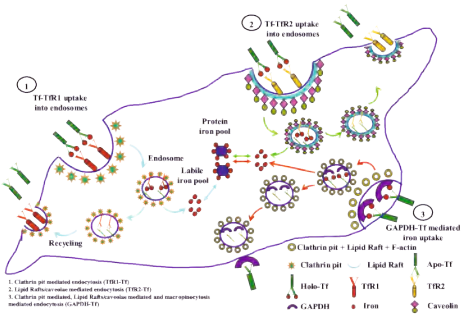

Figure 1: A comprehensive model for Tf-Fe uptake mediated by TfR1, TfR2 and GAPDH (TfR3)

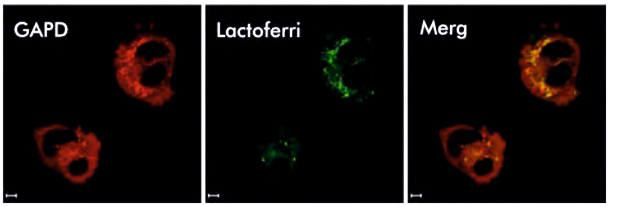

Figure 2: Trafficking of GAPDH and Lactoferrin to endosomes

Iron is also vital for the replication and survival of invading pathogens. For this purpose they must acquire host iron. To obtain host iron, successful pathogens employ several strategies. Intracellular pathogens are known to acquire iron for metabolic use from transferrin (the mammalian iron transporting glycoprotein) via the transferrin/lactoferrin -receptor endocytic route. Since iron is a critical component of living systems and is crucial for the outcome of any infection it is of vital importance to explore the role of GAPDH in iron metabolism and infection. This study would open up a new avenue of research in attempting to understand the survival mechanisms of intracellular pathogens.

Collaborator:

Chaaya Iyengar Raje

Participants:

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Understanding the role of antiapoptotic proteins in autophagosome formation in mycobacterial infection

Autophagy is an intracellular degradative process for the delivery of cytoplasmic constituents to lysosome. Recent progress has demonstrated that autophagy plays a wide variety of physiological and pathological roles which are sometimes complex. Autophagy has been linked to innate and adaptive immune response to numerous intracellular pathogens, including mycobacteria. It is tightly regulated biological process that plays a central role in tissue homeostasis, development and disease. Antiapoptotic Bcl-2 homologs downregulate autophagy through interactions with the essential autophagy effector protein, Beclin 1. Beclin 1 contains a BH3 domain, similar to that of Bcl-2 proteins, which is necessary and sufficient for binding to antiapoptotic Bcl-2 homologs and required for Bcl-2 mediated inhibition of autophagy.

Recent studies have proved that Bcl-2 family proteins also have an important role in regulating autophagosome formation. The interaction between the antiapoptotic protein, Bcl-2 and the autophagy protein Beclin 1 represents a potentially important point of convergence of the apoptotic and autophagic machinery. Recently, we have found that virulent and avirulent mycobacteria differentially regulate NF-κB dependent antiapoptotic protein Bfl-1/A1 expression (Biochim. Biophys. Acta. 2007; 1770, 649-58*)*. *M. tuberculosis* H37Rv infected THP-1 cells show prolonged Bfl-1/A1 activation than in *M. tuberculosis* H37Ra infected THP-1 cells (Biochem. Biophys. Acta. 2008; 1780, 733-742). This led us to investigate the role of Bfl-1/A1 in autophagy in mycobacteria infected cells.

Bfl-1/A1 acts as a negative regulator of autophagy in mycobacteria infected macrophages: We were interested to see the fate of *M. tuberculosis* H37Rv harboring vesicles in THP-1 cells treated with Bfl-1/A1 siRNA for this purpose, THP-1 cells were transfected with either Bfl-1/A1 siRNA or control siRNA and then infected with *M. tuberculosis* H37Rv. Bfl-1/A1 siRNA treatment knocked down the expression of Bfl-1/A1 mRNA in THP-1 cells. Next, co-localization of GFP-*M. tuberculosis* H37Rv labeled bacteria with RFP-LC3 labeled autophagosomes

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were followed by confocal microscopy. Bfl-1/A1 siRNA transfected cells showed enhanced co-localization of GFP-*M. tuberculosis* H37Rv with RFP-LC3 positive autophagosomes as compared to that in normal GFP-*M. tuberculosis* H37Rv infected THP-1 cells. Control siRNA treated cells behaved similarly as normal THP-1 cells. Similar results were obtained when human monocyte derived macrophages (MDMs) from healthy donors were subjected to co-localization study. Human MDMs treated with Bfl-1/A1 siRNA showed enhanced co-localization of RFP-LC3 with mycobacteria loaded phagosomes.

Bfl-1/A1 physically interacts with Beclin 1: Bcl-2 family proteins have been known to physically interact with

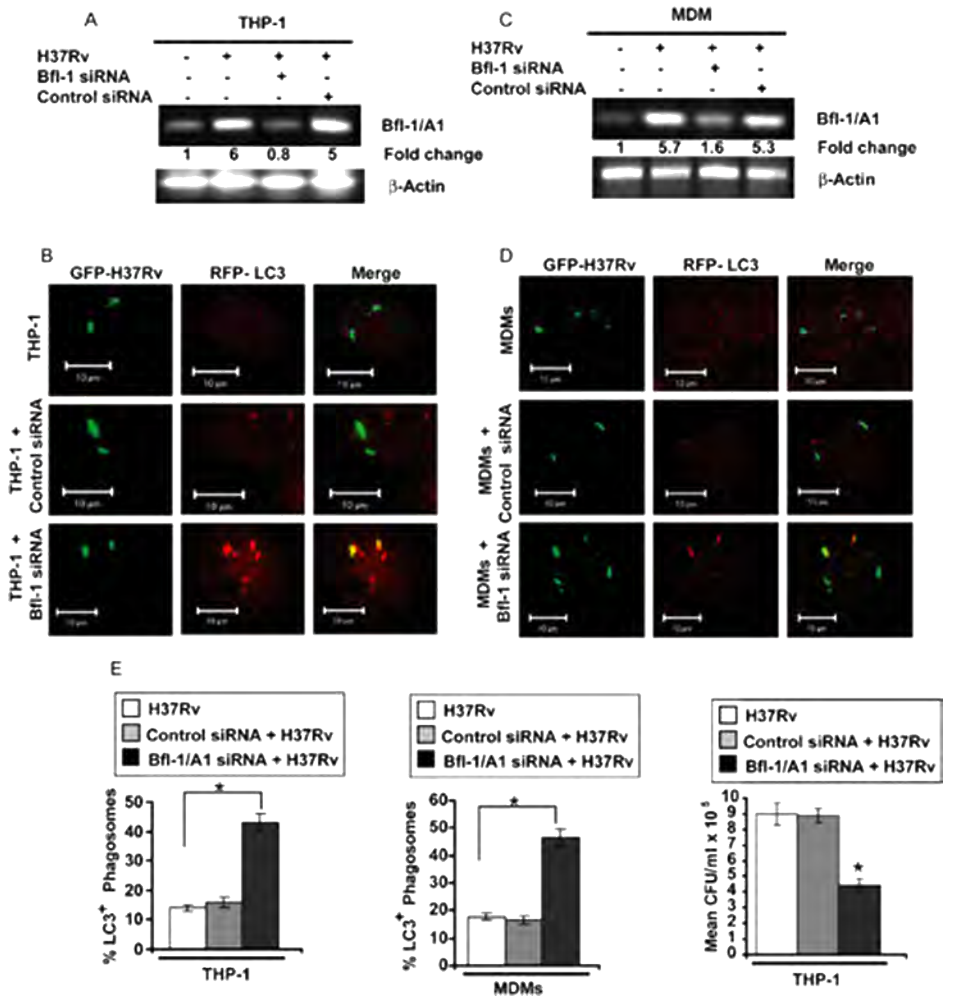

Figure 1: Bfl-1/A1 inhibition by siRNA promotes autophagosome formation of *M. tuberculosis* H37Rv infected THP-1 cells and MDMs. (A) THP-1 cells were first transfected with Bfl-1/A1 siRNA and then infected with *M. tuberculosis* H37Rv and total RNA was isolated from infected cells at 48 h post-infection. Silencing of Bfl-1/A1 mRNA was confirmed by RT-PCR analysis. (B) THP-1 cells were transiently co-transfected with RFP-LC3 and Bfl-1/A1 siRNA or control siRNA and infected with GFP-*M. tuberculosis* H37Rv for 4 h. After 48 h post-infection cells were analyzed for LC3 colocalization with GFP-labeled mycobacterial phagosomes under confocal microscopy. Quantitative analysis of percentages of mycobacterial phagosomes colocalizing with LC3 positive autophagosomes. Data are means ±SEMof three experiments (n = 100 phagosomes).*$p < 0.05$. (C and D) Similar experiments with MDMs were performed as in THP-1 above. (E) THP-1 cells were transiently transfected with Bfl-1/A1 siRNA or control siRNA and infected with *M. tuberculosis* H37Rv as described above. Colony forming units were counted after 72 h of post-infection. Data are means ± SEM of three experiments.

BH3 domain of Beclin 1, therefore we performed co-immunoprecipitation experiments to see the interaction between Bfl-1 and Beclin 1. Interestingly, our co-immunoprecipitation results showed that Bfl-1 co-precipitated with Beclin 1. Further, endogenous interaction of Bfl-1/A1 with Beclin 1 was found only in virulent mycobacteria infected THP-1 cells.

Sensitization of CTCL Lymphoma cells to TNF superfamily mediated apoptosis: Cutaneous T cell lymphomas (CTCL) are lymphoproliferative disorders of the skin. The two most common forms of CTCL are

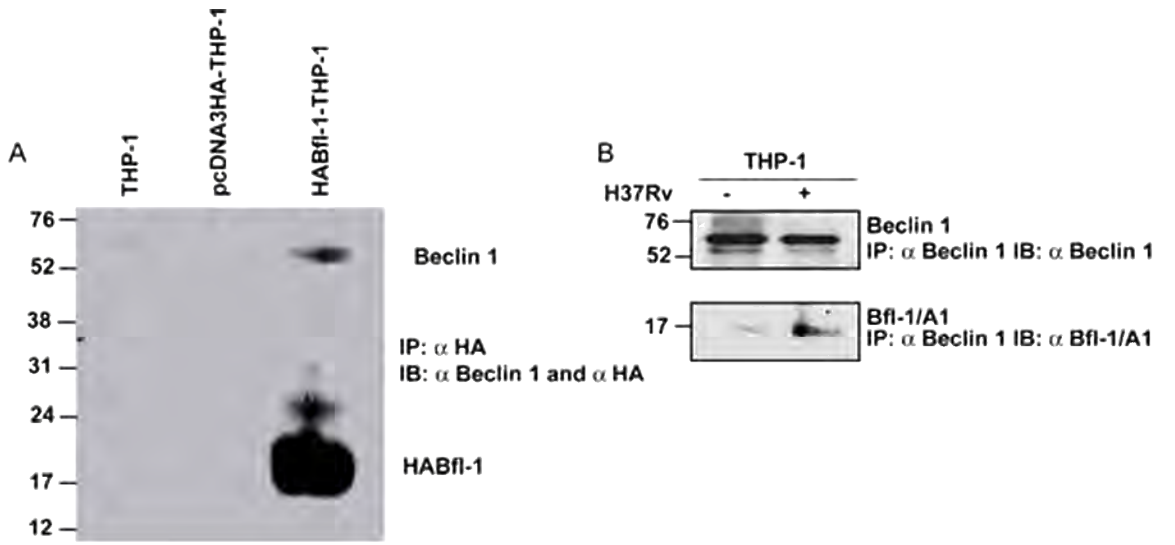

Figure 2: Bfl-1 co-immunoprecipitation with Beclin 1. (A) Lysates were prepared from HABfl-1-THP-1, pcDNA3HA-THP-1 and THP-1 cells and lysates were immunoprecipitated with anti-HA antibody. Precipitated proteins were analyzed by immunoblotting with anti-HA and anti-Beclin 1 antibody together. The position and molecular mass (kDa) of marker proteins are indicated. (B) Lysates prepared from control and *M. tuberculosis* H37Rv infected THP-1 cells after 48 h of infection were immunoprecipitated with anti-Beclin 1 antibody. Precipitated proteinswere analyzed by immunoblotting with anti-Bfl-1/A1 or anti-Beclin 1 antibody. The position andmolecularmass (kDa) of marker proteins are indicated.

Mycosis fungoides (MF) and Se´zary syndrome (SS) as its leukemic form, together they account for majority of cutaneous lymphoma. CTCL cells show defects in their death inducing signaling complex due to altered expression of death receptors which make them less sensitive toward TNF superfamily molecules. It has been reported that circulating CD4+ cells from SS patients are resistant to soluble TRAIL [Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL/Apo2L) has recently emerged as a novel anticancer agent based on i

ts ability to induce apoptosis in tumor cells, without showing toxicity to normal cells. TRAIL binds to four membrane bound receptors DR4/TRAIL-R1, DR5/TRAIL-R2, DcR1/TRAIL-R3, DcR2/TRAIL-R4 and a soluble receptor osteoprotegerin. Earlier, we reported that PTX, a methylxanthine derivative induces FasL mediated killing in human se´zary CTCL cell line, HuT-78. But its effect on TRAIL mediated apoptosis was not known.

Thus, here we were interested to see the effect of PTX on TRAIL-induced apoptosis in CTCL cell lines (HuT-78 and MyLa). We report for the first time, that PTX augments TRAIL-mediated caspase-8 activation and potently induces TRAIL-mediated apoptosis in both the cell lines by downregulating different antiapoptotic proteins and upregulating DR4 and DR5.

Pentoxifylline sensitizes TRAIL-induced cytotoxicity in HuT-78 and MyLa cells: In order to see whether PTX is able to sensitize TRAIL-induced cytotoxicity, HuT-78 cells were treated with PTX along with different concentrations of TRAIL for 24 h and cell viability was detected by live PI dye exclusion method. Our results clearly indicate that combined treatment with PTX and TRAIL significantly enhance the number of cytotoxic cells as well as apoptotic cells as evident by TUNEL assay. Combined treatment also activates caspase 8 with Bid truncation which later causes cytochrome c release from mitochondria and caspase 9 activation followed by caspase 3 activation leading to cell death.

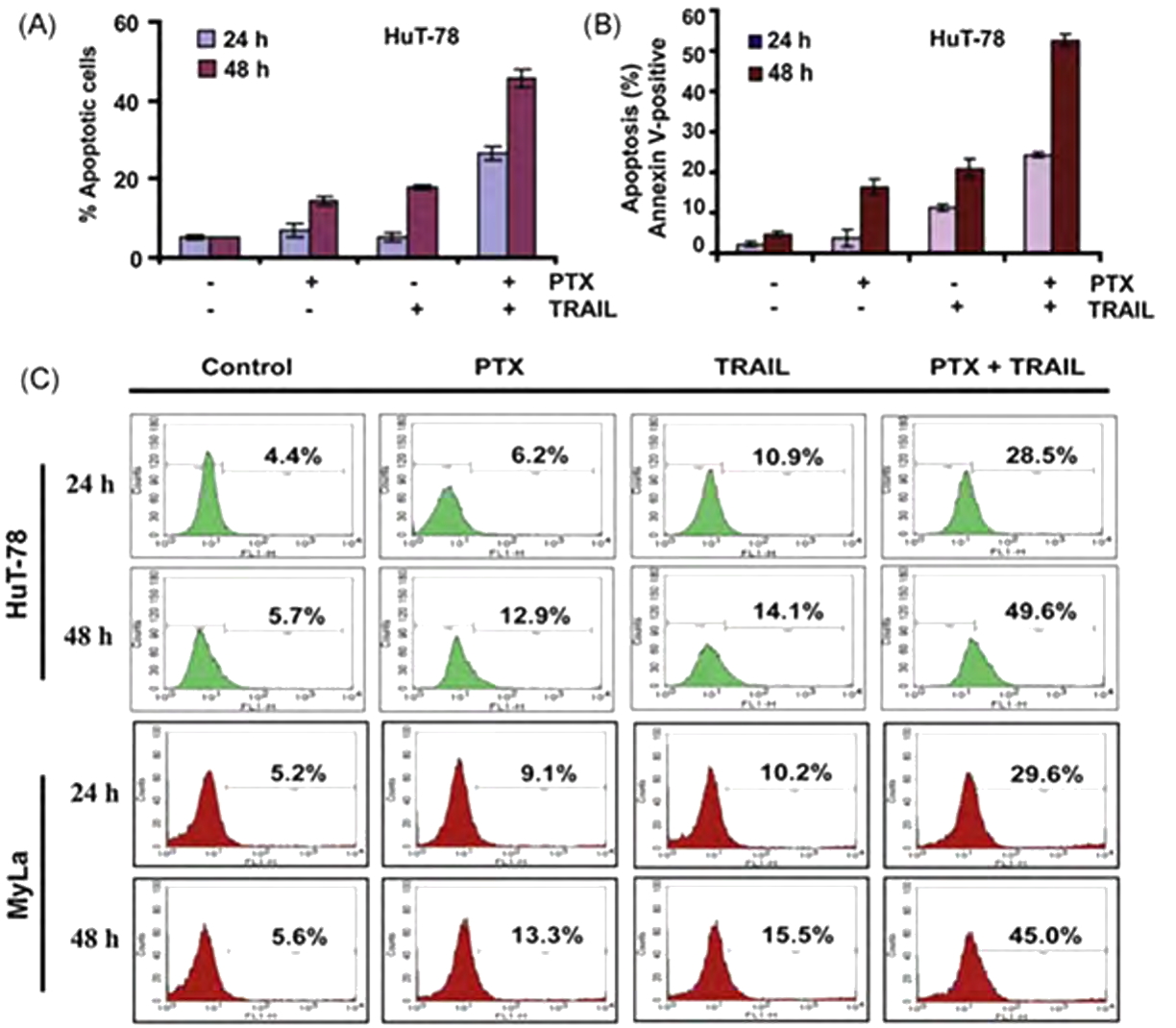

Figure 3: PTX and TRAIL combined treatment induces apoptosis in HuT-78 and MyLa cells. (A and B) HuT-78 cells were treated with PTX (3 mg/mL) or TRAIL (100 ng/mL) either alone or together for indicated time points (24 and 48 h) and apoptosis was studied by (A) sub-G1 (hypoploidic) peak analysis, data show mean values ±S.D. of three similar experiments. (B) Annexin V staining, data show mean values ±S.D. of three similar experiments. (C) HuT-78 and MyLa cells were treated similarly for 24 and 48 h and apoptosis was detected by TUNEL assay through flow cytometry at FL-1 channel, where, M1 gate demarcates apoptotic population.

TRAIL-induced augmentation of apoptosis by PTX affects Bcl-xl, c-FLIP and inhibitors of apoptosis cIAP-1, cIAP-2 and XIAP protein levels: HuT-78 and MyLa cells constitutively express high amount of various antiapoptotic proteins. Interestingly, combined treatment of PTX and TRAIL down-regulate the expression of both short and long form of c-FLIP along with the down regulation of Bcl-xL and other IAP proteins including XIAP, cIAP-1 and cIAP-2 in both the cell lines.

PTX induces upregulation of TRAIL death receptors (DRs) TRAIL-R1/DR4 and TRAIL-R2/DR5 through MAPK/JNK pathway : TRAIL surface expression was also determined with PTX treatment and we found that PTX significantly enhances TRAIL-R1/DR4 and TRAIL-R2/DR5 expression without showing any effect on decoy receptors. When we look for the mechanism of receptors upregulation we found that PTX induces death receptors via upregulating JNK/MAPK pathway. Furthermore, death receptors are found to be play important role in PTX induced TRAIL mediated apoptosis in CTCL cells.

Thus, in terms of clinical perspective PTX in combination with TRAIL may be a novel strategy for the treatment of CTCL patients.

Publications:

• Rishi, L., Gahlot, S., Kathania, M. & Majumdar, S. (2009). Pentoxifylline induces apoptosis in vitro in cutaneous T cell lymphoma (HuT-78) and enhances FasL mediated killing by upregulating Fas expression. *Biochem Pharmacol* **77**, 30-45.

• Gahlot, S., Khan, M. A., Rishi, L. & Majumdar, S. (2010). Pentoxifylline augments TRAIL/Apo2L mediated apoptosis in cutaneous T cell lymphoma (HuT-78 and MyLa) by modulating the expression of antiapoptotic proteins and death receptors. *Biochem Pharmacol* **80**, 1650-61.

• Kathania, M., Raje, C. I., Raje, M., Dutta, R. K. & Majumdar, S. (2011). Bfl-1/A1 acts as a negative regulator of autophagy in mycobacteria infected macrophages. *Int J Biochem Cell Biol* **43**, 573-85.

Participants:

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Defining the molecular mechanism of HGF/c-MET-mediated immunoregulation of dendritic cells

Dendritic cells (DC), a group of highly specialized antigen presenting cells (APCs), are known to initiate and regulate the immune responses. However, the capacity of DC to orchestrate the immune responses is largely regulated by environmental stimulation, which includes the local cytokine milieu. While proinflammatory cytokines potentiate the immunogenic presentation of an antigen by DC, immunosuppressive cytokines impart tolerogenic potential to DC. Among several immunosuppressive cytokines hepatocyte growth factor (HGF) is notable. HGF induced immunosuppression has been implicated in pathophysiology of various diseases including allergic airway inflammation where it inhibits IL-12 production and antigen presentation by DC. Furthermore, HGF plays an important role in immunopathology of malaria also where it is demonstrated as a key determining factor for establishment of parasite infection. Indeed, the lack of immunity against malaria liver stage enables parasite replication and eventual invasion of erythrocytes. This may in part be mediated by the immunosuppressive effect of HGF. Notably, HGF induces development of tolerogenic DC which further promotes regulatory T cell (Treg) development. Therefore, HGF may play a critical role in determining the type of immune response in part via regulating DC function. However, the molecular basis for immunoregulation of DCs by HGF is undefined. In this project, we have demonstrated that HGF exhibits inhibitory effect on DC activation by blocking IκB kinase (IKK) activity and subsequent NF-κB activation. Inhibition of IKK is mediated by HGF-induced activation of c-Src. Proximal signaling events induced in DCs by HGF include a physical association of c-Src with the HGF receptor c-MET and concomitant activation of c-Src. Activation of c-Src in turn establishes a complex consisting of phosphatidylinositol 3-kinase (PI3K) and c-MET, and promotes downstream activation of the PI3K/AKT pathway and mammalian target of rapamycin (mTOR). Blocking activation of c-Src, PI3K and mTOR prevents HGF-induced inhibition of IKK, NF-κB and DC activation. Notably, HGF-stimulated c-Src activation results in induction of PI3K complexes p85α/p110δ and p85α/p110δ; which is required for activation of mTOR, and consequent inhibition of IKK and NF-B activation. Our findings, for the first time, have identified the

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c-Src-PI3K-AKT-mTOR pathway that plays a pivotal role in mediating the inhibitory effects of HGF on DC activation by blocking NF-κB signaling.

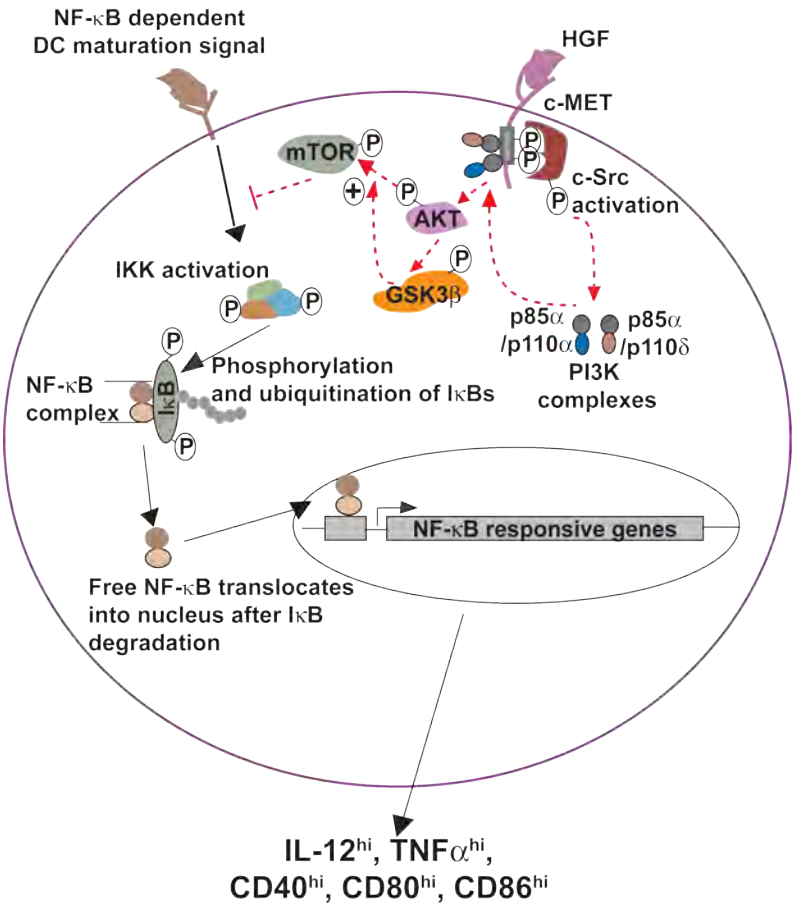

Figure 1: Mechanism of DC suppression by HGF

Regulation of sodium antimony gluconate-induced dendritic cell activation by antimony-resistant and -sensitive strains of *Leishmania donovani*: Kala-azar, a life-threatening parasitic disease caused by *Leishmania donovani*, is widening its base in different parts of the world. Till now, there is no effective vaccine against kala-azar. Therefore, antimonial drugs like sodium antimony gluconate (SAG) have been the mainstay of therapy for this disease. Recently, due to emergence of antimony-resistant parasites, SAG often fails to cure kala-azar patients, which is compounding the disaster further. It is yet unknown how antimony-resistant parasite infection, in contrast to antimony-sensitive parasite, is dealt by the kala-azar patients upon SAG treatment. This demands an understanding of the nature of host immune responses against these two distinct categories of parasites. Accordingly, our current studies have been directed to compare the impact of antimony-resistant versus antimony-sensitive parasite infection on dendritic cells (DCs). The latter upon activation/maturation initiate anti-leishmanial immunity. Our studies have demonstrated that antimony-resistant but not antimony-sensitive parasite prevented SAG-induced DC activation/maturation. Antimony-resistant parasite inhibits DCs by blocking NF-κB activation, a key signaling pathway regulating DC activation/maturation. Our studies for the first time provide both cellular and molecular basis for differential response of host cells to antimony-resistant

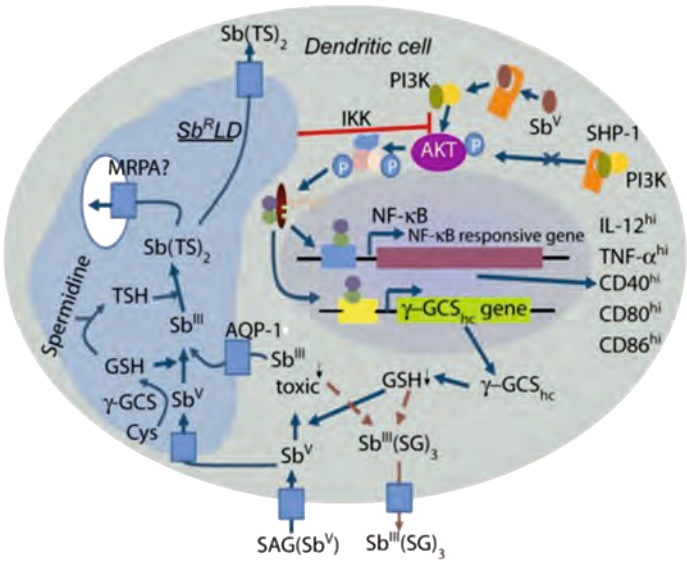

Figure 2: Proposed mechanism of supression of dendretic cell activation by antimony-resistant cells of LD.

and antimony-sensitive parasites, which may influence the outcome of the disease.

Publications:

• Haldar, A. K., Yadav, V., Singhal, E., Bisht, K. K., Singh, A., Bhaumik, S., Basu, R., Sen, P. & Roy, S. (2010). *Leishmania donovani* isolates with antimony-resistant but not -sensitive phenotype inhibit sodium antimony gluconate-induced dendritic cell activation. *PLoS Pathog* **6**, e1000907.

• Singhal, E. & Sen, P. (2011). Hepatocyte growth factor-induced c-Src-phosphatidylinositol 3-kinase-AKT-mammalian target of rapamycin pathway inhibits dendritic cell activation by blocking IkappaB kinase activity. *Int J Biochem Cell Biol* **43**, 1134-46.

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Analysis of the real time redox state of live mycobacteria using novel RoGFP based tool

Mycobacterium tuberculosis (*Mtb*) represents a major public health problem; killing ~2 million people along with 8 million new cases each year, in addition to 2 billion (1/3 of world's population) latently infected people. The ability of Mtb to persist within humans for decades without any clinical symptoms and then to reactivate causing active disease is key to its success as a pathogen which ensure its continued maintenance and viability and efficient dissemination in humans. The lack of diagnostic tools, drugs, and therapeutic vaccines against latent infection of tuberculosis is major challenge in the control of disease. During last ten years, I have exploited various multi-disciplinary approaches, new to the mycobacterial field to study the mechanisms utilized by *Mtb* to transit into latent state from actively growing state and believe that these findings will significantly contribute to the development of new vaccines, better diagnostics and novel drugs. Currently, my lab is focused on the following important issue pertaining to tuberculosis pathogenesis as depicted in Figure 1.

Utilization of RoGFP for the dissecting the mechanism of Drug action in *Mycobacterium tuberculosis*: *Mycobacterium* is exposed to a number of destructive redox active molecules during infection. The success of mycobacteria as a pathogen is dependent on its ability to sense and resist host generated oxido-reductive stress during infection. A clear understanding of molecular mechanisms utilized by *Mtb* to sense changes in the redox environment and to maintain redox homeostasis in *Mtb* is limited, primarily due to lack of a non-invasive tool, capable of directly measuring intracellular redox potential. Under this project, we have creatively developed a novel non-invasive tool. This tool exploits recently engineered redox sensitive variant of green fluorescent protein (RoGFP) to quantify the intracellular redox environment of mycobacteria in real time. Using this tool we have demonstrated that the *Mtb* is faced with oxidative stress upon treatment with Rifampicin. We are in the process of analyzing whether the RoGFP equilibrate with the mycothiol of *mycobacterium*.

Dissecting the genetic pathway utilized by *Mtb* for phenotypic switching to form biolfilms: It was recently

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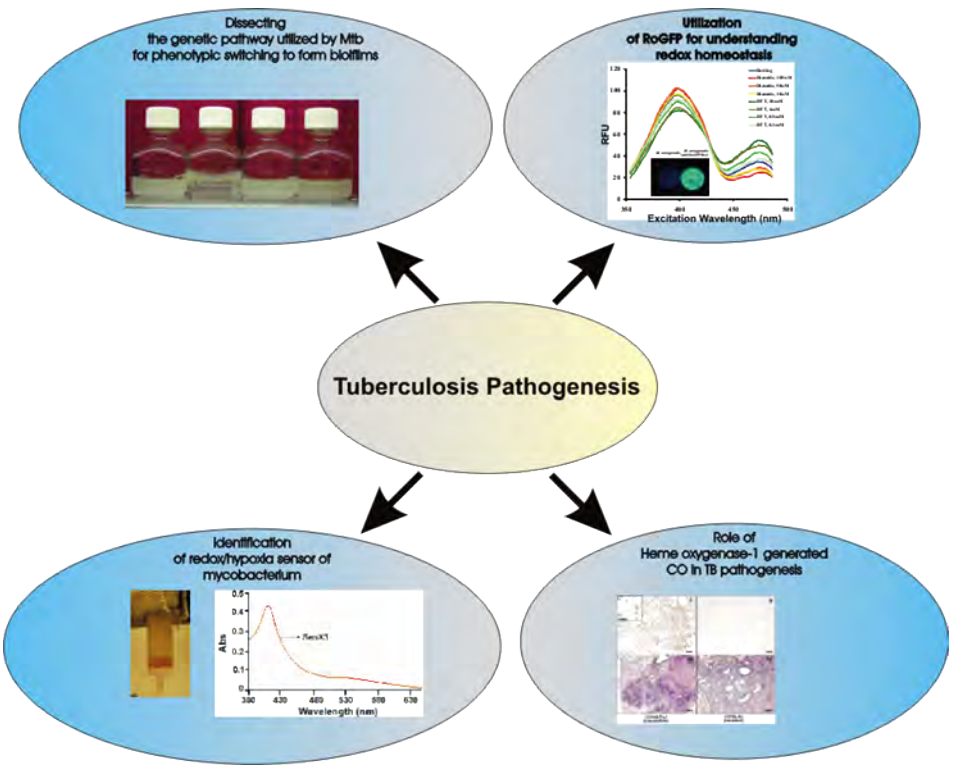

Figure 1: Tuberculosis pathogenesis aspects under study in the laboratory.

demonstrated by laboratory of Hatful at University of Pittsburg that *Mtb* is capable of forming biofilm and thus capable of switching to a phenotypic state wherein the bacilli is extremely resistant to the front line anti-TB drugs. Their report suggested that mycobacteria makes biofilm in standing culture in 4-5 months. We have been studying the effect of reductive stress on mycobacteria. Serendipitously, we have discovered that the *Mtb* exposed to stress could be phenotypically switched to form the biofilm. We have further characterized these biofilm and have identified the genetic program underlying this switching. We have identified a regulon of ~100 genes that can initiate the biofilm formation. This regulon constitutes some of the secretory proteins. Using this information, we have demonstrated that the factors that lead to biofilm formation are secreted out by *Mtb* in supernatant. We are further characterizing these secretory factors. Currently we are performing experiments to identify and characterize these proteins and to reproduce this data.

Identification of redox/hypoxia sensor of mycobacterium: It was declared by Koch in 1905 that the tuberculosis has been defeated however, the human race and science have been humbled by *Mtb* as we now know that TB cannot be eradicated in for at least another century. The reason for this is the unique capability of *Mtb* to latently infect one-third of world population, which is at 10% lifetime risk of developing tuberculosis. The mechanism behind TB latency remains unknown. However it has been realized that hypoxia and redox stress play an important role in the transition from the actively growing state to latent state of infection. However the mechanism that *Mtb* utilize to sense its microenvironment remains and understudied dimension of *Mtb* physiology. Previously, we have demonstrated that the *Mtb* sensor kinases DosS and DosT are heme based sensors that regulate the dormancy program of *Mtb*. However, the role of dormancy program in latency has been recently questioned, suggesting the presence of other sensors of hypoxia and redox stress. Towards solving this puzzle, we have identified that another sensor kinase in SenX3 of *Mtb*. We have shown that *Mtb* SenX3 is a heme

binding protein. We have further demonstrated that this heme posse's oxygen sensor like properties. We are in the process of further characterizing the mechanism of sensing by this protein.

Role of Heme oxygenase-1 generated CO in TB pathogenesis: Our work has previously demonstrated that in addition to hypoxia and NO, a third diatomic gas CO can induce the dormancy program of *Mtb*. We have further demonstrated that CO is endogenously produced by macrophages via HO-1 in response to TB infection. However the role of CO in TB pathogenesis remains unknown. Towards understanding the role of CO in TB pathogenesis, we have established that HO-1 generated CO regulates autophagy induced by rapamycin. Regulation of autophagy in response to *Mtb* infection is area of high thrust in TB pathogenesis. Now we are in the process of analyzing the role of this CO modulated autophagy in TB pathogenesis. We have recently also initiated work on understanding the role of HO-1 generated CO in apoptosis of macrophage cells infected with *Mtb*. We have obtained exciting preliminary results from these studies and these need to be further extended to analyze their role in TB pathogenesis.

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Enzymes, Abzymes & Designer whole-cell biocatalysts & identification of cellular target of novel immunosuppressive agent Caerulomycin A

Either Enantiomer, One Strain: Enzymes of opposite enantioselectivity are typically present in the whole-cell biocatalysts and they often lead to the product of low enantiomeric excess. We have developed a strategy for selective preparation of either enantiomer in high ee in a microbial whole-cell catalyzed kinetic resolution. The strategy is outlined in Figure 1.

Biocatalytic Tool Box Including a Novel Dehydrogenase for Preparation of Enantiopure (*S*)-1-Heteroaryl-1-alkanols: Hitherto, lack of substrate generality within a limited number of biocatalysts has been a major impediment in acceptability of biocatalytic methods for asymmetric reduction of heteroaryl ketones, especially in pharmaceutical industry where a wide variety of substrates are needed to be reduced in limited period of time to provide the products with high ee. We have described three new biocatalysts for the asymmetric reduction of heteroaryl ketones. Excellent substrate generality was observed within this small library of a limited number of biocatalysts. Selecting an appropriate biocatalyst from this library, we have successfully prepared a wide variety of chiral heteroaryl alkanols in enantiopure form and high yield (Figure 2). A dehydrogenase of 36.4 kDa has been purified. N-terminal sequence obtained from the purified protein by Edman degradation did not show significant similarity with any of the known dehydrogenase in database. A library of chiral heteroaryl alcohols prepared using the newly discovered biocatalysts is shown in Figure 3.

Designer whole-cell Biocatalysts. Preparation of (*S*)-Ethyl 4-Chloro-3-hydroxybutanoate (ECHB) in 100% ee: The cofactor-dependent asymmetric reduction of ketones catalyzed by alcohol dehydrogenases represents a valuable method for the synthesis of optically active alcohols.

For the competitive application of this enzymatic technology on an industrial scale, in particular when one

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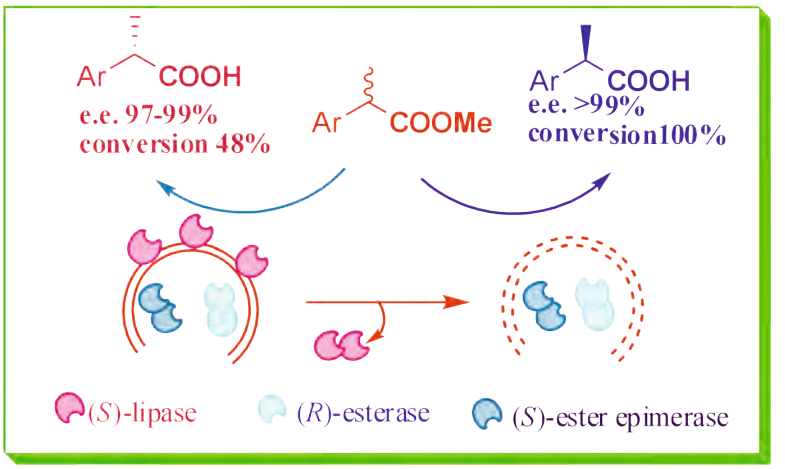

Figure 1.

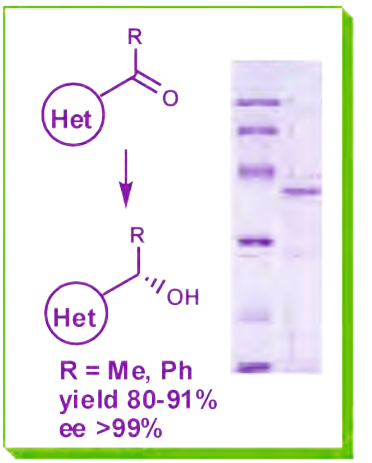

Figure 2.

considers the high efficiency of the established metal-catalyzed hydrogenation as the benchmark, it is essential to carry out biocatalytic reduction processes at high substrate concentrations. However, to date most of the biocatalytic syntheses based on the enzyme-coupled approach do not fulfill this requirement. Accordingly, only a few examples have been reported that show a potential for technical applications. We have applied a combination of (a) genetic tools to improve efficiency of biocatalyst and (b) reaction engineering tools to improve productivity to achieve significant improvement in conversion rate and concentration of the accumulated product in a dehydrogenase catalyzed reaction. We have achieved over 150-fold increased efficiency of the enzyme in the production of ECHB.

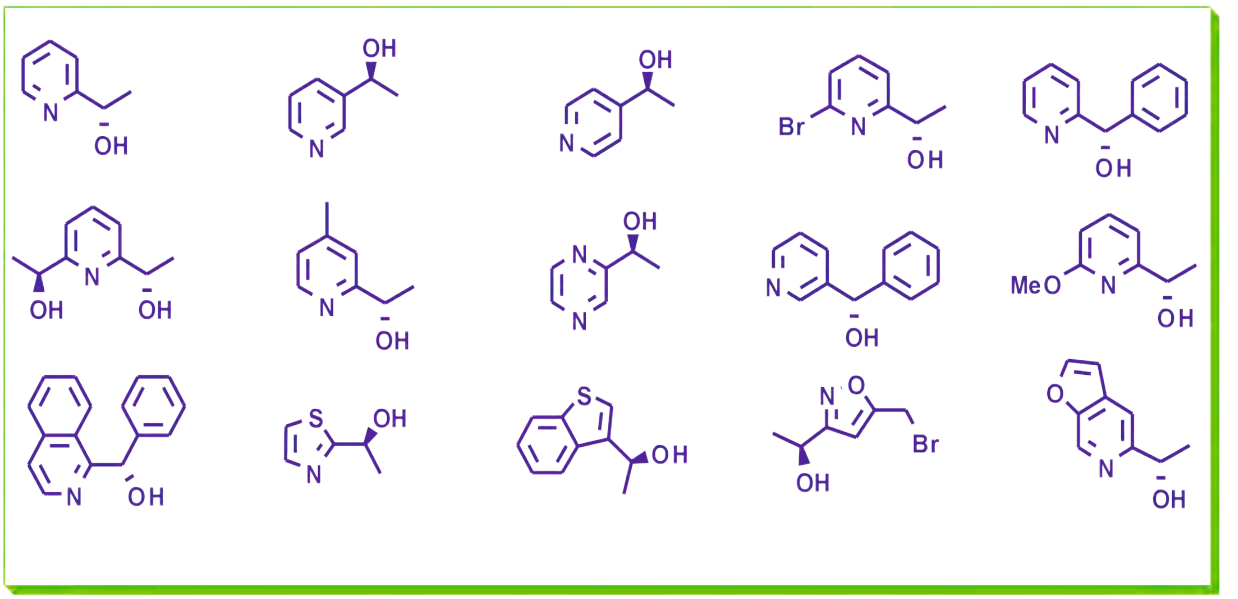

Figure 3.

Purification, cloning and overexpression of a nitrilase from a bacterium: A nitrilase from a bacterium was purified, cloned and overexpressed in *E. coli*. It was exploited for (i) monohydrolysis of symmetrical dinitriles, which is almost impossible to achieve by chemical means (ii) Regioselective hydrolysis of dinitriles and (iii) enantioselective hydrolysis of nitriles (Figure 4).

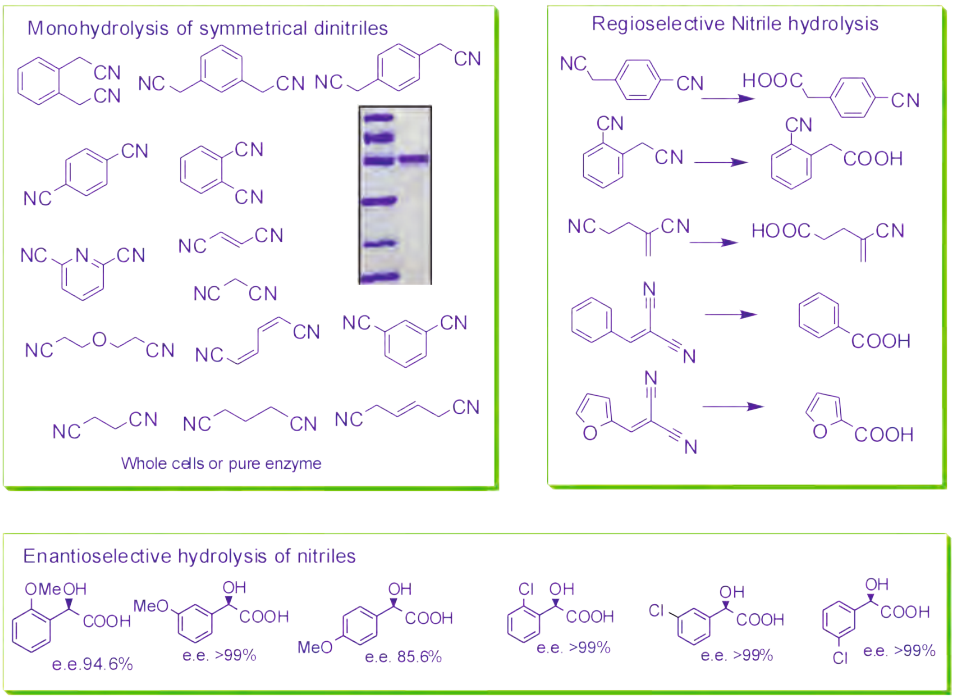

Figure 4.

Streoselective Oxidation of Penicillins to their (*R*)-sulfoxides : Hitherto, no method, chemical or biological is available for direct oxidation of penicillins to their (*R*)-sulfoxides. We have purified a novel catalase peroxidase from a bacterium, which stereoselectively oxidized penicillins to their (*R*)-sulfoxides (figure 5).

Preparation of Esomeprazole in High Ee : We have isolated an yeast, which produced esomeprazole from corresponding sulfide in >99% ee. Unfortunately, the productivity was too low to be of commercial significance (Figure 6).

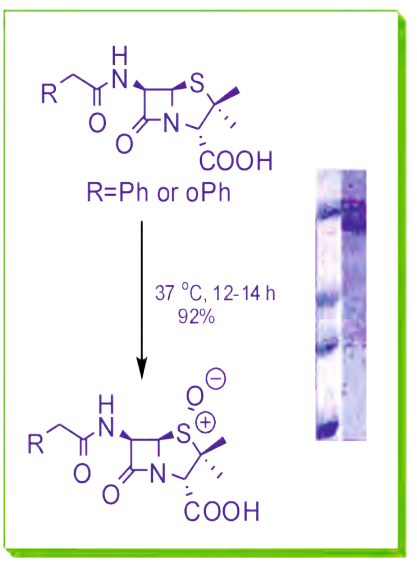

Figure 5.

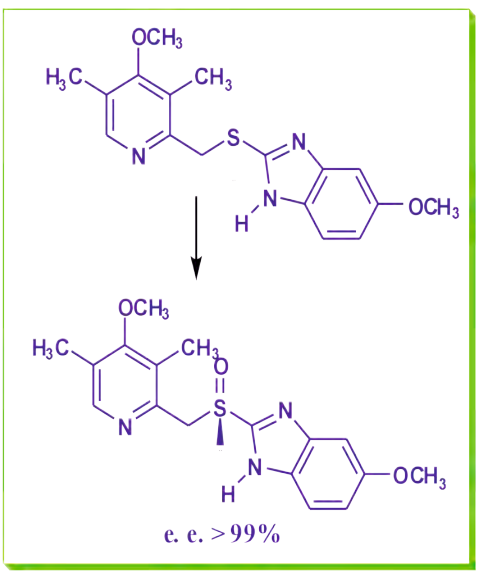

Figure 6.

Novel Chiral Shift Reagents for Cyanohydrins and Carboxylic acids: We have shown that a structure as simple as an ion-pair of (*R*) or (*S*)-mandelate and dimethylamminopyridinium ions possesses structural features, which are sufficient for NMR enantiodiscrimination of cyanohydrins. Moreover, ^{1}H NMR data of cyanohydrins of known configuration obtained in presence of mandelate-dimethylaminopyridinium ion-pair point to the existence of a correlation between chemical shifts and absolute configuration of cyanohydrins. To understand the origin of enantiodiscrimination, the geometry optimization and energy minimization of the models of ternary complexes of (*S*)-mandelonitrile/(*R*)-mandelate/DMAPH^{+} and (*S*)-mandelonitrile(*S*)-mandelate/DMAPH^{+} complexes was performed using DFT methodology (B3LYP) with a 6-311++G** basis set in Gaussian 3.0 (figure 7). Further, analysis of optimized molecular model obtained from theoretical studies suggested that (i) DMAP may be replaced with other amines, (ii) hydroxyl group of mandelic acid is not necessary for stabilization of ternary complex and may be replaced with other groups such as methyl, (iii) ion-pair should form a stable ternary complex with any hydrogen bond donor, provided its OH bond is sufficiently polarized and (iv) α-H of racemic mandelic acid should also get resolved with optically pure mandelonitrile. These inferences were experimentally verified, which not only validated the proposed model but also lead to development of a new CSA for determination of ee of carboxylic acids, and absolute configuration of aryl but not alkyl carboxylic acids.

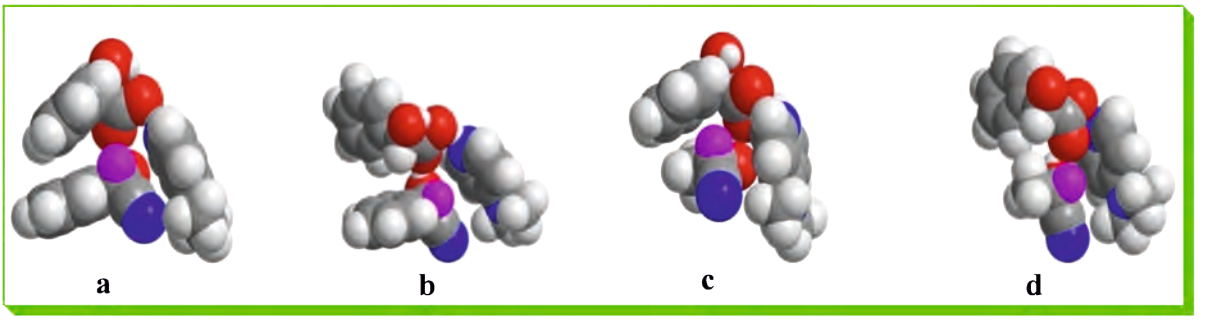

Figure 7: space-filling representations for ternary complex (a) (S)-mandelonitrile/(R)-mandelate-DMAPH^{+}, (b) (S)-mandelonitrile/(S)-mandelate-DMAPH^{+}, (c) (S)-lactonitrile/(R)-mandelate-DMAPH^{+} and (d) (S)-lactonitrile/(S)-mandelate-DMAPH^{+}. Note the screening zone of purple labeled hydrogen atom.

Characterization of Bioactives from Microbial Diversity of India : In a network program aimed at exploitation of microbial diversity of India, extracts prepared from microbes were screened for bioactivity by several partner groups. Our group has purified and identified the active component from the extract reported positive by groups of Dr. S. Majumdar and Dr. H. Nandanwar from IMTECH. Compounds identified by us, so far are shown in figure 8. All of these turned out to be compounds known to literature.

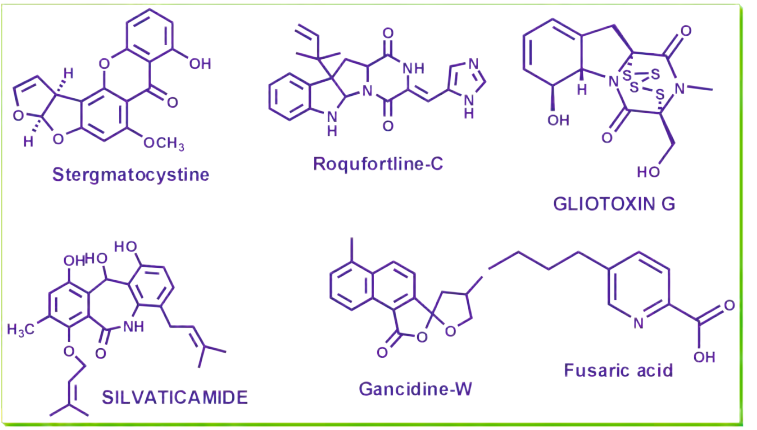

Figure 8.

Caerulomycin A: Identification of Protein Target and Description of Mechanism of Action: Recently, a collaborative effort between our group and groups of Dr. J. Agrewala and Dr. R. M. Vohra lead to characterization of Caerulomycin A as a novel immunosuppressive agent. While licensing rights to develop it for drug application were transferred to Nostrum Inc. of USA, we undertook the challenging task of identification of its molecular target. Initially we followed Activity Based Protein Profiling Approach (ABPP) and synthesized probe and tag molecules as shown below. But this approach failed to dish out the protein target. However, following an alternate approach, we have been successful in identifying cellular target of Caerulomycin A. We are continuing this lead to decipher the mechanism of action of Caerulomycin A.

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• Moon, L. S., Jolly, R. S., Kasetti, Y. & Bharatam, P. V. (2009). A new chiral shift reagent for the determination of enantiomeric excess and absolute configuration in cyanohydrins. *Chem Commun (Camb)*, 1067-9.

• Moon, L. S., Pal, M., Kasetti, Y., Bharatam, P. V. & Jolly, R. S. (2010). Chiral solvating agents for cyanohydrins and carboxylic acids. *J Org Chem* **75**, 5487-98.

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Studies on cell culture based protein therapeutics: Process development for production and nanodelivery

Biodegradable micro and nanoparticles as protein carrier are of great interest, due to their versatile route of administration, protection of protein from degradation and physiological clearance, as well as a well-defined controlled release profile. Developing a system or technology that delivers a protein of interest *in vivo* in structurally integrated form is the focus of this work. Poly(lactic-co-glycolic) acid (PLGA), a FDA approved polymer was used for the formation of micro and nanoparticles, as a delivery system for proteins. Protein inactivation, aggregation and unfolding during their encapsulation are major problems to be overcome in the development of PLGA microparticles for the delivery of protein therapeutics. The common strategies to stabilize proteins include chemical modification, protein engineering, immobilization and the use of additives such as poly hydroxyl compounds. Excipients such as polyethylene glycol (PEG) can be introduced into PLGA to adjust acidity, hydrophobicity, and permeability and also for emulsification, micronization and controlled release. Initially, BSA was used as a model protein to investigate the effects of various parameters on micro-/nano-particle preparation. Several microencapsulation methods including those based on a solid-in- oil- in-water (S/O/W) double emulsion have been proposed in which protein drugs are often applied as solid particles. However, most protein drugs have various stability problems and hence, while developing protein delivery systems, one must minimize their denaturation and aggregation during processing. The encapsulation of protein pharmaceuticals in micro-/nanospheres comprising of the bioerodible and biocompatible polyester PLGA has been widely investigated for their sustained delivery. This S/O/W method may be superior to the water-in-oil-in-water (W/O/W) emulsion method because solid-state proteins retain their activities in organic solvents due to the kinetic trapping and reduction in contact with the organic phase in PLGA blended with PEG which improve protein stability, delivery capacity and controlled release profiles.

Earlier, investigating the molecular mechanism operating behind polyethylene glycol (PEG) mediated

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stabilization of protein (BSA), we reported no significant changes in BSA conformation by the processes like lyophilization and sonication and PEG 8000 as a stabilizer at an optimum BSA:PEG molar ratio (1:0.75) stabilized BSA due to strong physical adsorption of PEG 8000 on buried hydrophobic core of BSA along with surface adsorption (Figure 1). In the present study, we have investigated the molecular mechanism of structural

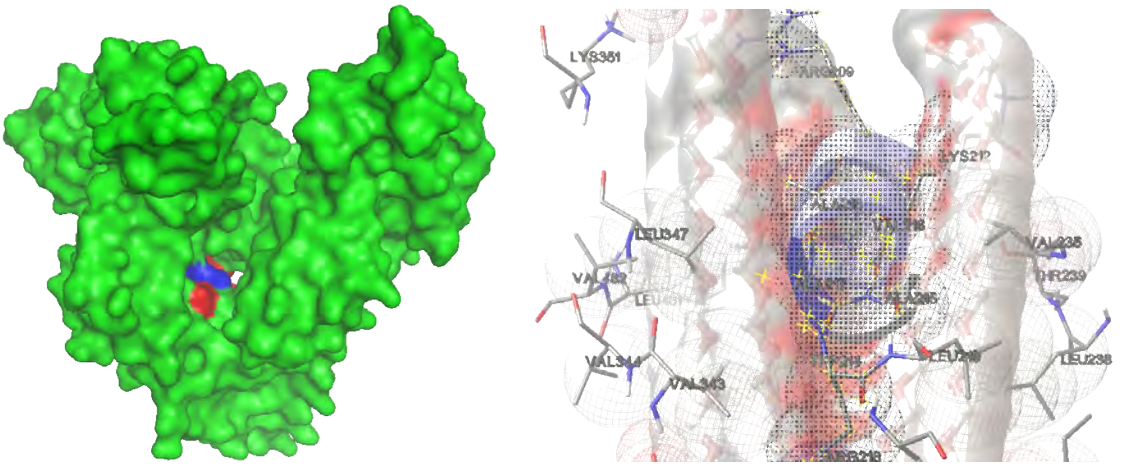

Figure 1: (a) Primary structure of BSA (PDBID 2BXA) by PyMOL viewer showing TRP-214 (blue) and other hydrophobic residues (red); (b) Docking of PEG on hydrophobic residues (209- 218) covering TRP-214 (spheres showing hydrogen bonding) (Autodock).

integrity of a model protein, BSA, in PLGA based microspheres delivery system with PEG as a stabilizer and controlled releasing agent along with chosen mixture of surfactants. The effect of surfactants was studied in three sets of formulations, each set consisting of samples prepared with/without PEG and with a constant ratio of mixture of surfactants. The primary emulsion of lyophilized BSA and BSA coated with PEG was prepared using ethyl acetate and poly DL-lactide-co-glycolide (PLGA), and secondary emulsification was accomplished by using different ratios of surfactants. The morphology of prepared microspheres was studied using dynamic light scattering (DLS) and scanning electron microscopy (SEM). BSA released from lyophilized microspheres was evaluated for the structural, conformational and thermal stability by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), fluorescence spectroscopy, circular dichroism (CD)-spectroscopy, along with differential scanning calorimetry (DSC). CD-spectroscopy showed greater increase in secondary structural content of BSA in sample containing PEG and surfactant mixture of carboxymethyl cellulose (CMC) and Tween 20 as compared to that containing mixture of CMC and Tween 80 or Tween 20 and Tween 80. The increase in the thermal stability of BSA, released from microspheres containing PEG, was indicated by the increase in peak area (ΔH) and midpoint of denaturation (T_m). In addition, the protein showed no aggregation in size exclusion chromatography-high performance liquid chromatography (SEC-HPLC) analysis whereas the fluorescence spectra showed the compactness of BSA. These results suggested macromolecular crowding due to PEG, molecular confinement because of microspheres and interfacial increase in Gibbs free energy with strong electrostatic repulsion, the last phenomenon was due to chosen surfactants that were responsible for making the protein more compact and structurally integrated (Figure 2). Our investigations proposed molecular mechanism of improved structural integrity of protein in polymer based microspheres and resulted in a process for improved protein integrity in final formulation.

In another study, PLGA (poly-L lactide D glycolide) based nanoparticles formulation for bovine insulin was

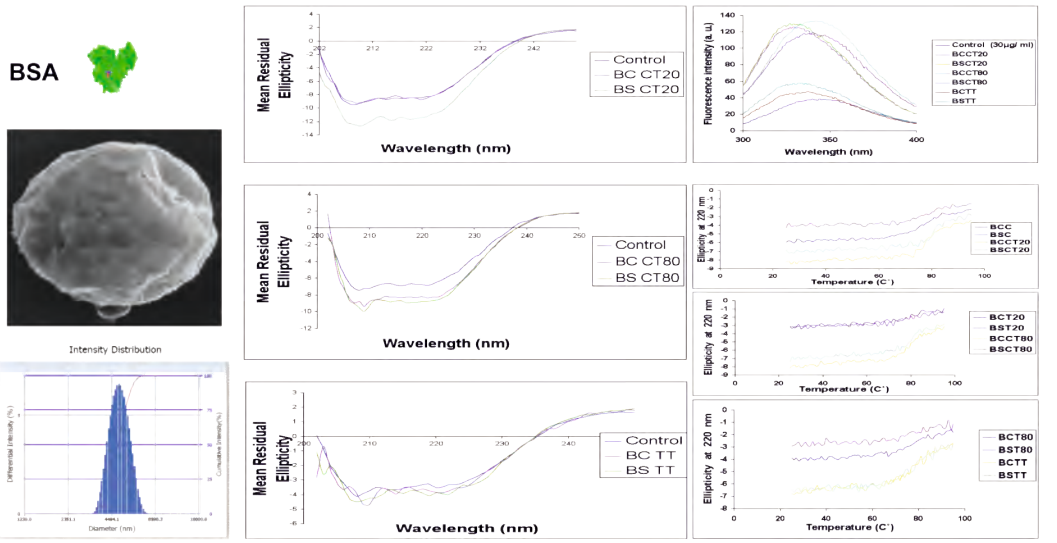

Figure 2: Schematic representation of molecular mechanism of improved structural integrity of BSA in PLGA based microspheres delivery system.

prepared. The comparative role of three kinds of surfactant systems in the inner aqueous phase system on bovine insulin for the stability, denaturation and aggregation has been evaluated. The size and morphology of prepared nanoparticles were analyzed by using dynamic light scattering (DLS) and scanning electron microscopy (SEM). The three surfactant systems chosen in nanoparticles preparation were Tween 20: Tween 80, low molecular weight poly vinyl alcohol (PVA) and high molecular weight PVA. Insulin released from lyophilized nanoparticles was evaluated for the structural, conformational and thermal stability by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), circular dichroism (CD)-spectroscopy, along with differential scanning calorimetry (DSC). The denaturation analysis of the released insulin was done by thermal-CD. The aggregation phenomenon was monitored by Thioflavin-T assay, congo red assay along with native-

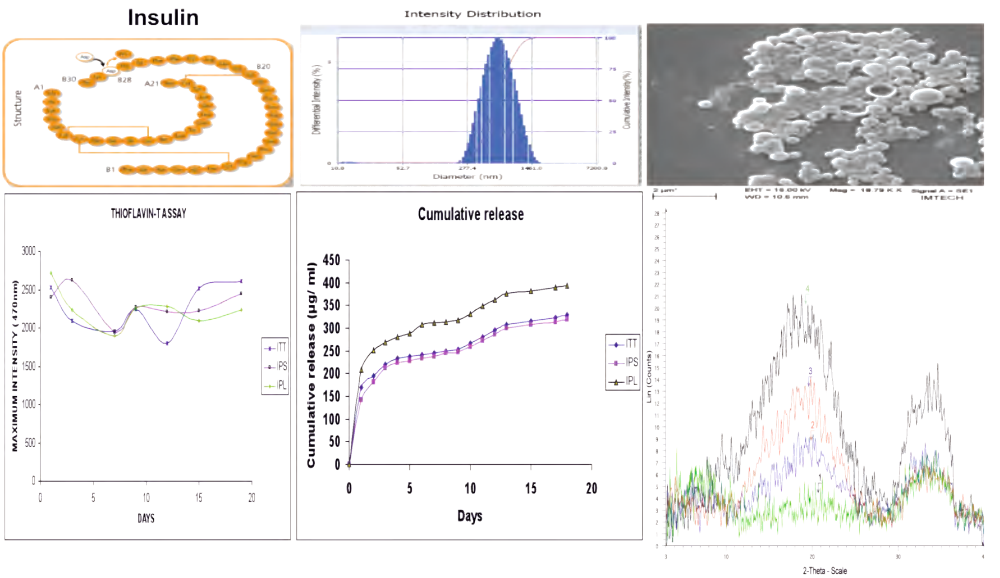

Figure 3: Schematic representation of effect of surfactants on integrity of bovine insulin in PLGA based microspheres delivery system.

PAGE. The comparative analysis of nanoparticles prepared with large PVA (out of all three compared formulations) showed the released insulin to be conformationally and thermodynamically stable without any denaturation and aggregation. The powder X-ray diffraction and DSC studies of lyophilized formulations showed that the crystallinity of the formulation is increased by the large poly vinyl alcohol leading to the stability of insulin towards the aggregation and denaturation (Figure 3:)

Biotechnology for leather-towards cleaner processing: Bioprocessing of skin or hide into leather instead of conventional chemical based technologies will amount to a paradigm shift in global leather sector. Such a technological shift is likely to gain a leadership status for the country. In order to reduce the load of sulphide in dehairing, combination of enzyme and lime/ sulphide method is used, but it still adds sulphide load to waste water. This project was initiated with primary objectives to develop bioprocessing alternatives to currently employed chemical methods in leather manufacturing technologies. In this multi-laboratory NMITLI project, we studied the use of enzymes in leather processing, specifically selection of specific proteases, capable of replacing chemical inputs in dehairing, enzymatic opening of fibre bundles without osmotic swelling by lime and other alkalies and the development of bioprocesses for selected enzymes. Initially, process optimization and scale up studies on the production of IMTECH 4 protease were carried out at 20 L fermenter scale. The medium for protease production consisted of in-expensive carbon and nitrogen sources, commercial starch as carbon source and a commercial partially hydrolyzed protein as nitrogen source along with inorganic salts such as sodium carbonate, potassium di-hydrogen phosphate and magnesium sulphate. The protease production process was scaled upto 700 L scale at CLRI fermentation scale up facility. The extracellular enzyme (alkaline proteases) was recovered using centrifugation and further concentrated by ammonium sulphate precipitation. Enzyme yields of $>$ 40,000 protease units/L of fermented broth were obtained in three consecutive batches at 700 L scale and upon downstream processing using centrifugation and ammonium sulphate precipitation, the enzyme recovery of $>$ 60% were obtained in all three cases. This ammonium sulphate precipitated enzymes were applied for leather processing at Pilot Scale in collaboration with Central Leather Research Institute (CLRI), Chennai. IMTECH 4 protease was assessed for unhairing efficiency individually and in an integrated enzyme only process where it is used along with a lipase and an amylase (Figure 4). In case of its individual use, though there was not much reduction in pollution parameters such as BOD (Biological Oxygen Demand), TDS (Total Dissolved Solids) and TSS (Total Suspended Solids), the values of COD and sulphide were significantly reduced and a higher BOD/COD ratio indicated much better degradability and treatability of waste water as compared to comical based processing (CBP) of leather. The physical characteristics of the experimental crust leather were comparable to that of control leathers. In integrated process, since the suphide was completely eliminated, the waste water did not contain sulphide. In addition, there was significant reduction in COD and high BOD/COD

Figure 4: Enzyme only leather dehairing using alkaline protease IMTECH 04 was carried out in collaboration with Central Leather Research Institute (CLRI). Chennai.

ratio in enzyme based process (EBP) indicated better degradability of waste water from EBP as compared to CBP. The comparison of leather quality showed leathers of EBP smoother than that of CBP, where as the fullness, tightness and other physical properties of leathers were comparable in both cases.

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• Jawed, A., Dikshit, K. L. & Sahoo, D. K. (2010). Enhanced production of recombinant staphylokinase in *Escherichia coli* carrying *Vitreoscilla* haemoglobin gene. *J Biotechnol* **150**, S444-S444.

Patent:

• Debendra K. Sahoo, K. L. Dikshit and Ashshad Jawed. Process for production of soluble staphylokinase using *Escherichia Coli*. Indian Patent Application No. 0062DEL2010 (1.11.2010).

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Development of process for biodegradation of textile dyes

Dyes used in the textile industry are chemically stable molecules that are resistant to fading upon exposure to sweat, light, water, oxidizing agents and microbial attack. Annually about 800,000 tons of textile dyes are consumed in the textile industry. About 15% of the total textile dyes employed in the process become a part of the textile effluents. Physical and chemical methods used for removal of dyes are adsorption, chemical transformation, incineration, photocatalysis or ozonation. These methods are not feasible at large scale Biodegradation is an environmental friendly and cost competitive alternative. Due to the increasing awareness and enforcement of environmental regulations, treatment of textile effluents before their release in to the environment is necessary. This project aims at developing a biological process for treatment of textile effluents.

Ligninolytic fungi are able to synthesize laccase (benzenediol:oxygen oxidoreductase EC1.10.3.2) that can degrade the phenylpropanoid polymer. The ligninolytic enzymes have very broad substrate specificity that renders many xenobiotic compounds including synthetic textile dyes. Laccase is a polyphenol oxidase containing multiple copper atoms. Laccase exhibits broad substrate specificity against mono and diphenols, aminophenols and their derivatives. Laccase have low redox potential hence, can catalyze many oxidation reactions with oxygen directly. Laccase is economically important enzyme because it has many applications in food and beverage industry bioremediation paper and pulp bleaching development of fuel cells and biosensors for analytical applications.

Screening for laccase producing fungi using ABTS was done (Figure 1). A laccase producing fungal strain was isolated which has been identified as *Arthrographis kalrae*, belonging to the class Ascomycetes (Figure 2 and 3).

In the presence of 400 μm copper sulphate and 400 μm of xylidine the yield of the enzyme was 9760 UL^{-1}.

The laccase from Arthrographis has been purified to homogeneity. The novel laccase is acidic protein having

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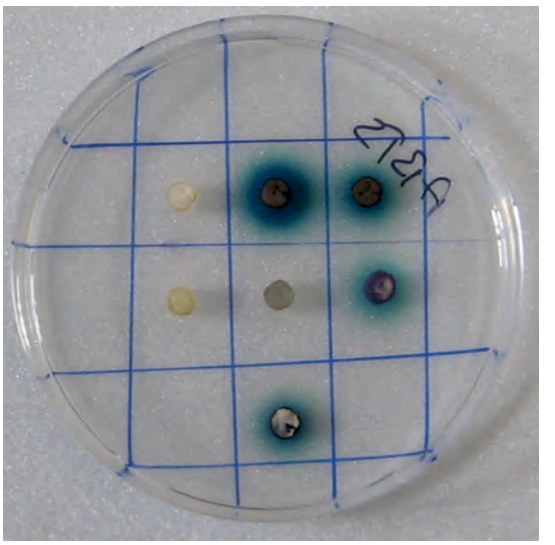
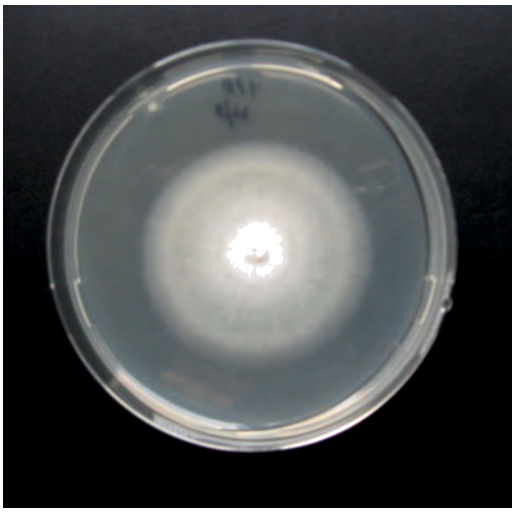


Figure 1: Screening for laccase using ABTS.



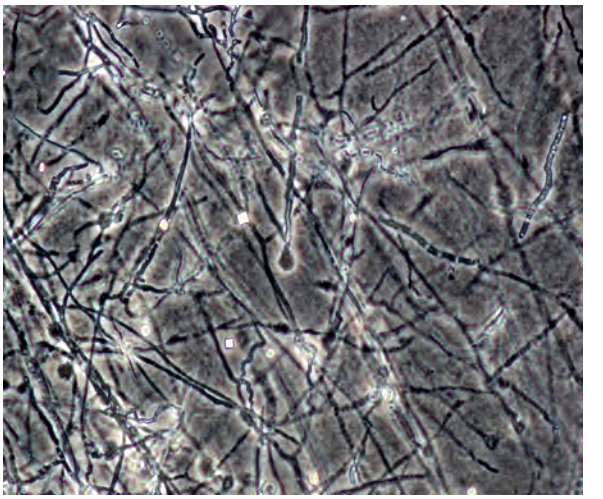


Figure (2 & 3): Morphology of *Arthrographis kalrae.*

isoelectric point (pI) below 3.5.The laccase showed optimum activity against ABTS and 2,6-DMP at pH of 3.5 and 4.0 respectively. Kinetic parameters were estimated using ABTS and 2,6-DMP as substrates. The K_m and V_{max} values were found to be 16.99 μM and 325.7-μM min^{-1} for ABTS and 257μM and 356-μM min^{-1} for 2,6-DMP respectively. The N-terminal sequence of amino acid residues the *Arthrographis* laccase was Gly-Ile-Gly-Pro-Val-Asp/Thr-Leu/Asp-Lys-Ile for 8 amino acid residues. The novel laccase from *Arthrographis kalrae* has been found effective in the degradation of various textile dyes belonging to the azo, triphenylmethane and anthroquinone groups. The broad activity against various dyes in the presence of 1-hydroxybenzotriazole as the redox mediator shows that the laccase from *Arthrographis* has potential in bioremediation of textile dye effluents (Figure 4a ,4b and 4c).

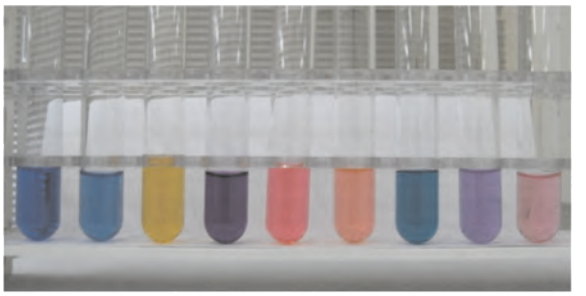
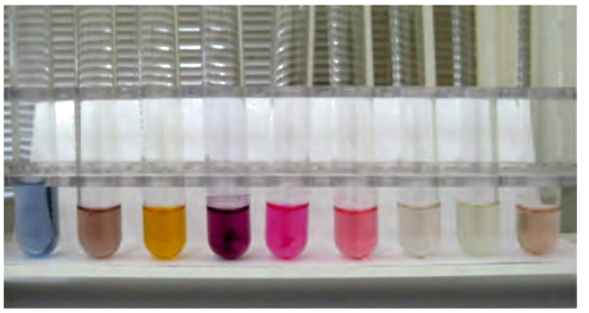


Figure 4a: Solutions (50 mg L^{-1}) of various dyes



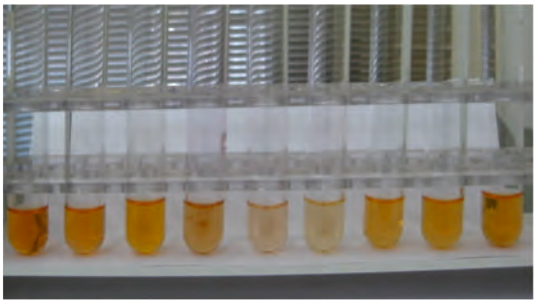


Figure 4(b, c): Degradation of various dyes by laccase 4U ml^{-1} without redox mediator (4b), and with redox mediator (4c).Dyes:1 Brilliant Blue, 2 Cibcaron Blue,3 Cresol Red, 4 Crystal Violet, 5 Rose Bengal, 6 Erythrosine,7Malachite Green,8 Remazol Brilliant Blue, 9 Methyl Red

Patent:

- Novel laccase enzyme and its applications. Indian Patent Application No.1951DEL2010 (17.08.2010)

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New bioactives from microbes: Hopes and remedies

Owing to technical improvements in screening programs, and separation and isolation techniques, the number of natural compounds discovered exceeds 1 million. Among them, 50-60% produced by plants (alkaloids, flavonoids, terpenoids, steroids, carbohydrates, *etc*.) and 5% have a microbial origin. Of all the reported natural products, approximately 20-25% show biological activity, and of these approximately 10% have been obtained from microbes. Microbes have made a phenomenal contribution to the health and well-being of people throughout the world. In addition to producing many primary metabolites, such as amino acids, vitamins and nucleotides, they are capable of making secondary metabolites, which constitute half of the pharmaceuticals on the market today. Furthermore, from the 22,500 bio-active compounds that have been obtained so far from microbes, 45 % are produced by actinomycetes, 38 % by fungi and 17 % by unicellular bacteria. The increasing role of microorganisms in the production of antibiotics and other drugs for treatment of serious diseases has been dramatic.

Why new antimicrobials?: The development of drug resistance in microbes has become a major problem and requires much research effort to combat it. This resistance increasingly limits the effectiveness of current antimicrobial drugs. In 2004, more than 70 % of pathogenic bacteria were estimated to be resistant to at least one of the currently available antibiotics. The so-called 'superbugs' (organisms that are resistant to most of the clinically used antibiotics) are emerging at a rapid rate. *Staphylococcus aureus*, which is resistant to methicillin, is responsible for many cases of infections each year. Penicillin-resistant *pneumococci* and vancomycin-resistant *enterococci* are equally serious problems. More than 60% of sepsis cases in hospitals are caused by Gram -ve bacteria. Among them, *Pseudomonas aeruginosa* accounts for almost 80% of these opportunistic infections. Many such strains susceptible to gentamicin, tobramycin and amikacin, have developed resistant.

Mycosis is a condition in which fungi pass the resistance barriers of the human or animal body and establish infections. In most cases, these infections are not life threatening. However, when they are deeply invasive and

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disseminated, they lead to more serious infections, particularly in critically ill patients, elderly people and those who have conditions that affect the immune system. Patients with burns, neutropenia, pancreatitis or after organ transplantation (40% of liver transplants, 15-35% of heart transplants and 5% of kidney transplants) are also predisposed to fungal infection. Approximately 40% of deaths from nosocomial infections are caused by fungi, and 80% of these are caused by *Candida* and *Aspergillus*. Pulmonary aspergillosis is the main factor involved in the death of recipients of bone marrow transplants.

Besides being used in antibacterial, antifungal and antiviral infections, microbes are also used as enzyme inhibitors *(e.g.* β-lactamase, α-glucosidase, protease, lipase, *etc*.), anti-tumor drugs, gastrointestinal motor stimulator agents, hypocholesterolemic drugs, *etc*. Most of the Gram +ve and Gram -ve bacteria have developed the resistance against second generation and third generation penicillin due to wide range of β-lactamases secreted by them. To combat the β-lactamase resistance, the potent inhibitors for β-lactamase are essentially needed. Similarly, α-glucosidase inhibitors are useful for the control of carbohydrate-dependent diseases, such as diabetes, obesity and hyperlipemia. These diseases are the great concern for today's lifestyle.

One additional reason for developing new antimicrobial is related to their own toxicity, includes mild reactions such as upset stomach, vomiting and diarrhea (cephalosporins, macrolides, penicillins and tetracyclines), rash and other mild and severe allergic reactions (cephalosporins and penicillins), sensitivity to sunlight (tetracyclines), nervousness, tremors and seizures (quinolones). Some side effects are more severe and, depending on the antibiotic, may disrupt the hearing function (aminoglycosides), kidneys (aminoglycosides and polypeptides) or liver (rifampin).

Our interests: The current interest of my group is exploitation of microbial diversity explored from untouched niches in various parts of India for new antibiotic, better substitute for existing antibiotic, enzyme inhibitors, *etc*. Secondary metabolites have exerted a major impact on the control of infectious diseases and the development of pharmaceutical industry. Probably, the most important use of secondary metabolites has been as anti-infective drugs. In 2000, the market for such anti-infectives was US $ 55 billion and in 2007 it was US $ 66 billion. The present focus is to find out an alternative antimicrobial drug especially active against MRSA, *vancomycin resistant Enterococcii* (VRE) and aminoglycoside-resistant *P. aerugenosa.* Due to increasing resistance against azole-type and polyne-type of antifungal, our focus is to evolve new antifungal antibiotic with less toxicity, less side effects compare to reported ones.

Another research area we are focusing is “enzyme inhibitors as therapeutic agents”, that includes β-lactamase inhibitors and α-glucosidase inhibitors. Besides, some known β-lactamase inhibitor such as clavulanic acid, tazobactam and sulbactam, the candidates with less toxicity and side effect having higher potency are essentially needed. Potent candidates against third-generation-penicillin-resistant bacteria and clavulanate-competitive agent may be a breakthrough in this research. Similarly, α-glucosidase inhibitor with wider specificity to cure obesity and as an anti-diabetic agent is our parallel research.

Where we are: We have generated the library of more than 6000 organic extracts from diverse microbes isolated from unexplored niches. These extracts are exploited for various targets set up in the lab such as antimicrobials for broad spectrum bacteria, Methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant Enterococcii (VRE), extended spectrum β –lactamase (ESBLase), α-glucosidase and azole resistant-*Candida albicans.*

We have developed *in vitro* (micro-titre plate based) as well as agar-plate-based assays for β-lactamase and α-glucosidase inhibitor. We have also, evaluated *in-vitro* screening assays for tyrosinase and trypsin inhibitor and

Figure1: Schematic for sample collection, isolation, antibiotic assay and result interpretation, Graph 1 is for number of antibacterial screened for 3 yrs; Graph 2 is number of antifungal categorized w.r.t. their zone of inhibition size; Graph 3 is number of enzyme inhibitor (α-glucosidase, β-lactamase, protease) categorically.

completed the exploratory project on, "screening for enzyme inhibitors in skin care technology", with Procter & Gamble, Bangalore.

Multi-drug resistance is serious problem in tuberculosis and other bacterial infectious disease. It is mostly due to cell-wall barrier, bio-film formation at the target and efflux of drugs from target. Efflux pump inhibitors stops the efflux of drugs and restores at the site of action to give complete therapeutic efficacy. So, we have set up an assay to screen efflux pump inhibitors, recently.

In terms of output some promising bioactive compounds against MRSA, azole-resistant *C. albicans,* α-glucosidase inhibitor, ESBLase inhibitor, bio-film inhibitor and bio-film enhancer, are under purification and characterization stage. Bringing novel bioactive molecule on the public platform is a big task. We are highly hopeful about our efforts which will make it true.

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Screening and process development for microbial exopolysaccharides

Many microorganisms synthesize exopolysaccharides (EPS), which either remain attached to the cell surface or are found in the extracellular medium in the form of amorphous slime. In the natural environments in which the microorganisms are found, such polymers may either be associated with virulence, as in the case of plant or animal pathogens, with plant microbial interactions or even protect the microbial cell against desiccation or attack by bacteriophages and protozoa. In both natural and man-made environments, the EPS play a major structural role in 'biofilms', the normal habitat of many microbial communities, in which varying numbers of prokaryotic and eukaryotic microorganisms grow while attached to solid-liquid interfaces.

Several such microbial polysaccharides are now widely accepted products of biotechnology, while others are in various stages of development. The uses of such polymers vary widely - some are employed because of their unique or superior physical properties relative to traditional plant polysaccharides. In this category the major examples are xanthan, from *Xanthomonas campestris*, and gellan from *Sphingomanas pauciniobilis* strains .

The microbial products always have to compete against other natural or synthetic polymers, which may be inferior in their physical or ecological properties but are nevertheless much cheaper to produce and market. Alteration of the chemical properties of the original exopolysaccharide may also greatly enhance their value and extend their range of applications, as exemplified by the dextran-derived Sephadexe products. Although we now have a better idea of the relationships that exist between structure and functional properties, it is still difficult to predict which microbial polymers will prove worth developing. Many initial reports in the literature have proved wildly overoptimistic. On the other hand, two products have proved to be valuable are pullulan and microbial cellulose.

Pullulan is one such EPS which is being mainly produced by yeast like fungus (*Auerobasidium pullulans*). It is a homopolymer consisting of repeating maltotriose subunits which are linked through α-1,6 –glycosidic linkages.

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This is a water soluble random coil glucan and this unique linkage pattern has endowed it with distinctive physical traits. It has adhesive properties and it can be used to form fibers, compression molding and strong oxygen impermeable films. It also can be derivatized easily to alter its solubility or to provide suitable reactive groups. These have made pullulan a potential candidate for several food as well as pharmaceutical applications.

Pullulan is not degraded by most amylases, but specific pullulanase enzymes (isolated from sources including *Enterobacter aeroxenes*) can be used to hydrolyse the polysaccharide to its component maltotriose units and thus provide a useful means of preparing these oligosaccharides.

Microbial cellulose is perhaps the more surprising, given the universal availability and cheapness of plant cellulose. In contrast to its role in the wall of plants, cellulose is produced as an exopolysaccharide by *Acetobacter xylinum* and other, mainly Gram negative, bacterial species. It is excreted into the medium where it rapidly aggregates as microfibrils, yielding a surface pellicle. Bacterial cellulose is essentially a high-value speciality chemical with specific applications and usage. Some is produced commercially as a source of highly pure polymer in the so-called cellulose-I form (60% Iα:40% Iβ), free from lignin and other non-cellulosic material. The fibrils form a unique ribbon 3-8 nm thick and approximately 100 nm wide, which differs in morphology from other native celluloses. Bacterial cellulose also forms the basis for high-quality acoustic-diaphragm membranes, in which the distribution of the fibrils containing a parallel orientation of the glucan chains yields fibres possessing high tensile strengths. Bacterial cellulose can also be used as a binder for ceramic powders and minerals and as a thickener for adhesives. Although less-pure material can be used for similar purposes in the mining industry, the cost remains relatively high in comparison with other, competing, polymers. Furthermore, an area for potential development lies in the applications for cellulose-synthetic copolymer. In addition to these technical applications, bacterial cellulose forms part of the traditional fermented food 'Nata de Coca'.

The primary goal of the project is to screen several strains for their capability of production of pullulan and cellulose and to develop a suitable process for the above mentioned microbial polysaccharides. Upon screening the strains which will produce significant quantity of the polymer they will be selected for process development study.

During screening, we have obtained five yeast-like fungal isolates from flowers of wild plants whoose phenotypic and molecular characterization showed that they are related to *Aureobasidium pullulans*. Compared to other isolates, an osmotolerant and non-pigmented isolate *A. pullulans* RBF-4A3 produced 26.35 g l^{-1} of melanin-free exopolysaccharide (EPS) in 96 h at 30°C in 5% glucose containing medium (Figure 1). At higher concentrations

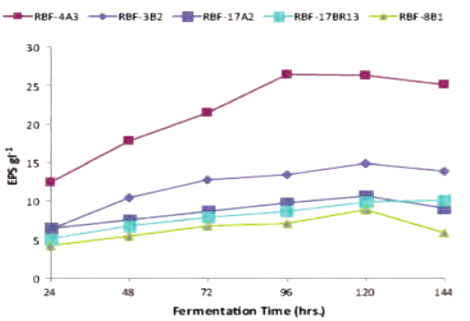

Figure 1: Exopolysaccharide production by five flower isolates of *A. Pullulans* in 5% w/v) glucose containing medium 30°C.

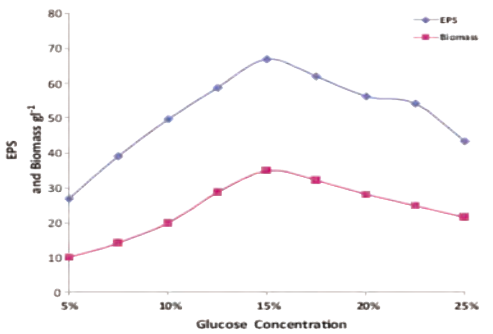

Figure 2: Effect of different concentrations of glucose on exopolysaccharide and biomass production by *A. pullulans* 4A3 at 96 h time at 30°C.

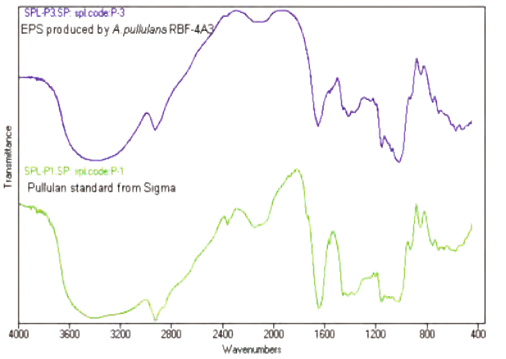

Figure 3: FT-IR spectra of exopolysaccharide produced by *A. pullulans* RBF-4A3, and standard pullulan from Sigma.

of glucose (7.5-25% (w/v)),the EPS produced by this organism increased from 34.68 to 66.79 g l^{-1} up to 15% (w/v) glucose, with a productivity of 16.69 g l^{-1} per day. Beyond 15% (w/v) glucose concentration, the EPS production decreased gradually to 43.29 g l^{-1} at 25% (w/v) glucose (Figure 2). Fourier-transform infrared (FTIR) spectroscopy confirmed that chemical structures of the exopolysaccharide produced by *A. pullulans* RBF-4A3 and standard pullulan were identical (Figure 3). This is the first report of pullulan production at 15% (w/v) concentration of glucose by and osmotolerant strain of *A. pullulans*. At present, process and media composition optimization for pullulan production and screening for cellulose producing microbes are under progress in our laboratory.

Publications:

Prasad, G. S., Choudhury, A. R. & Saluja, P. (2011). Pullulan production by an osmotolerant *Aureobasidium pullulans* RBF-4A3 isolated from flowers of *Caesulia axillaris*. *Carbohydrate Polymers* **83**, 1547-1552.

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Studies on isolation and characterization of retinal protein bacteriorhodopsin from solar salterns for Nano device applications

Halophiles grow in marine salterns and hyper saline lakes, such as the Dead Sea and the Great Salt Lake, where the salinity is more than ten times that of the normal seawater. *Halobacterium salinarum* produces large quantities of the heptahelical transmembrane protein bacteriorhodopsin (bR). The polypeptide chain of fully processed bacteriorhodopsin contains 248 aminoacid residues has a molecular weight 26 kDa. Biosynthesis of bacteriorhdoopsin in *Halobacterium* is induced by low oxygen tension and high light intensity. Large quantity of bR is in demand for research and development but only small quantity in the range of 8-10 mg / liter of protein is available due to the difficulty in cultivating *Halobacterium* species and purification of bR protein. Till now bacteriorhodopsin is produced in the small quantities in laboratory scale. For industrial applications larger amounts are necessary and inexpensive process optimization is required for obtaining high yield bacteriorhodopsin with unique spectral properties.

bR is the key protein for the halobacterial photosynthetic capabilities, enabling the organisms to use light energy directly to drive bioenergetics processes by the generation of a proton gradient. It is also one of the very rare molecules that occur in crystalline form in nature. The intrinsic properties of bR such as long term stability of the protein to thermal and photochemical degradation, wavelength independent quantum yields, and ability to form thin films with excellent optical properties makes an outstanding candidate for the development of electrochemical and optical based biosensors. The purification of bR from *Halobacterium* cells requires different stages such as harvesting, disruption of cells by osmolysis, sucrose density separation, removal of salts and sucrose by dialysis, protein concentration, detergent solublization and gel filtration chromotography to obtain monomeric bR.

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Bacteriorhodopsin is of special interest as it is a nonchlorophyll- based type of phototrophy which generates chemical energy (ATP) from light energy. However, very little is known about the diversity and distribution of

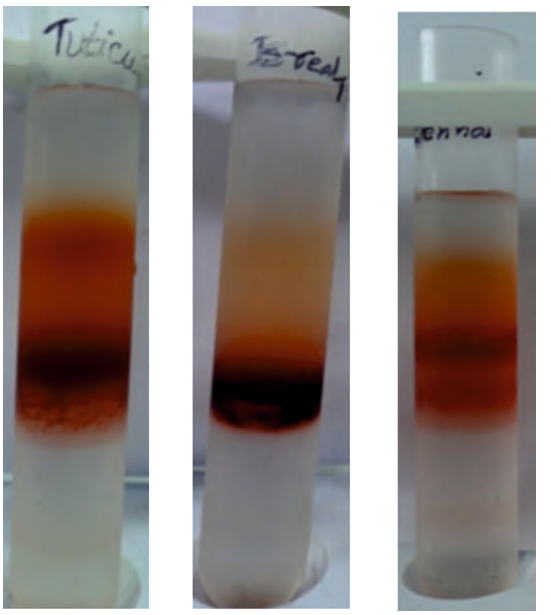

Figure 1: Pigment fractionation of isolates from solar saltern samples.

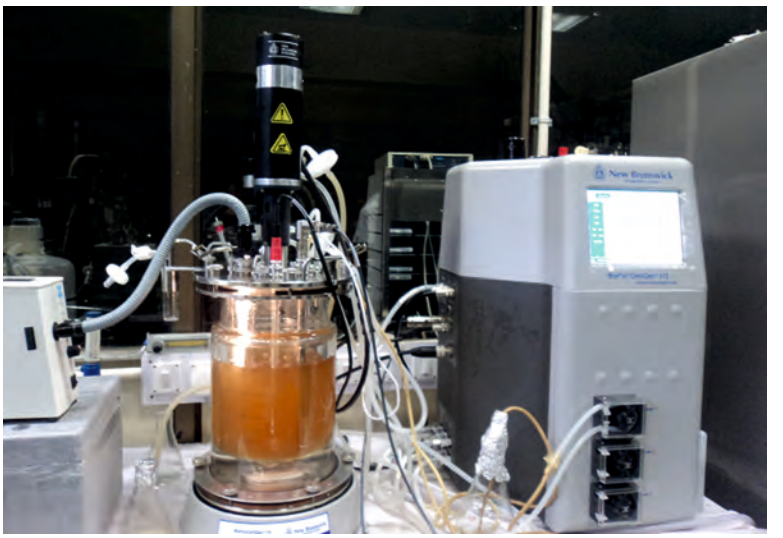

Figure 2: Large scale cultivation of halobacterium species in photobioreactor for bacteriorhodopsin production.

rhodopsin genes in hypersaline environments. The shallow crystallizer ponds and other hypersaline ecosystems represent the high concentration of salt in the range of 3-5 M supporting halophilic microorganisms which results in low concentration of oxygen in the Hypersaline environment. The dominant halobacterial groups under such conditions hints that rhodopsin-based energy generating photosystems may play a significant role in the success of these organisms in such ecosystems. Bacteriorhodopsin have been developed into superb model systems by structural biologists, photophysiologists and biophotonist for the last two decades for Nanodevice applications.

Our laboratory is focusing on identifying the presence of bop genes codes for the expression of bacteriorhodopsin protein through culture independent and culture dependent approach. These genes act as a model systems to understand the diversity and distribution of rhodopsin genes from solar salterns samples collected from Chennai, Tuticorin and Israel. Taxonomy studies using 16S rRNA gene archaeal primers have shown that some of the isolates belong to haloarchaea. Development of PCR Based molecular tools for identification of bop gene from solar salterns community DNA indicates that rhodopsin like genes present in haloarchaea isolated from Indian solar salterns. Earlier work in our lab had identified some haloarchaeal strains like *Haloferax alexandrinus*, *Haloarcula argentinensis* from Israel and Chennai solar salterns. So there is an clinching evidence that presence of microbial rhodopsins in the haloarcula genus from Indian solar salterns and this is the first report for the rhodopsin gene characterization from such a hypersaline environments in India.

Publications:

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Neural and hybrid filters for noise-affected microbial fermentations

Under practically relevant conditions, large bioreactors are often subject to disturbances (or noise) from the environment. Common examples are the fluctuations seen in the feed rates of substrates and in the concentrations of dissolved oxygen and the evolution of carbon dioxide. Although ideally any noise may be undesirable, it is practically difficult to remove noise entirely.

The noise experienced by large bioreactors often varies with time and it may contain components of different variances. Conventional noise filters, such as of the cusum, low pass, and Kalman types, are too inflexible to be effective under varying noise conditions. Moreover, they require a flexible and reliable model of the biological process, which is difficult to formulate under nonideal conditions. Filters that are independent of, or only partly dependent on, the fermentation model and are sufficiently auto-adaptable to changing environmental disturbances offer a viable alternative to conventional algorithmic filters.

From this perspective, different configurations of noise filters comprising partially neural networks and partially of mathematical models were designed and applied to two fermentation systems. One was the production of poly-β-hydroxybutyrate (PHB) by *Ralstonia eutropha*, either as a single culture or as a co-culture with *Lactobacillus delbrueckii*, in fed-batch fermentation. The other was the production of ethanol by *Saccharomyces cerevisiae* in continuous cultures. The two systems also differed in the nature of the outputs. The PHB system consistently generated monotonically varying concentration profiles whereas *S. cerevisiase* may produce either monotonic or oscillating outputs. In both cases excessive noise resulted in chaotic behavior.

Detailed explorations across different conditions showed that auto-associative neural networks in conjunction with simple mathematical models were more effective than pure neural networks or fully mathematically oriented filters (algorithmic filters are of this kind). A key issue was the determination of the best combination of the two components of a hybrid filter. This was observed to be problem-dependent and there is yet no general strategy to

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determine an optimal hybrid model. Detailed results and discussion on both fermentation applications are available in the publications.

Bacterial chemotaxis under the influence of diffusion perturbations and environmental noise: In their natural habitats and in the spatially nonuniform environments in large bioreactors, bacteria such as flagellated *Escherichia coli* navigating in response to chemical stimuli experience noise from the environment in addition to those within the cells. Environmental noise here is similar to that studied in Area-1 for fermentations by *R. eutropha* and *S. cerevisiae*; it comprises a mixture of Gaussian noises of different variances.

Environmental noise adds to that emanating inside the cells and at the binding sites on the cell walls. Intra-cellular noise may be present in the expression of different proteins by corresponding genes, in the concentrations of ribonucleic acids and in the metabolic reactions leading to desired products. In addition, chemotactic cells also experience noise in the process of binding between ligands of the chemoattractant molecules and their chemoreceptors projecting outward from the cytoplasm.

Bacterial cells have, through evolution, developed mechanisms to filter out intra-cellular noise and ligand binding noise, the former through negative feedback loops and the latter through an integral filtering method. Both kinds of filters have been modeled. Less analyzed are filters for environmental noise. Therefore this aspect was the focus of the present research. Drawing on prior studies of bioreactors, hybrid neural filters were used for environmental noise. Since all sources of noise interact, a simulation flow diagram proposed earlier was expanded to include external Gaussian noise.

As for microbial fermentations, chemotactic performance was optimized by manipulating the design parameters of the filters. It was observed that the incorporation of an environmental filter into the feedback schemes of intra-cellular and ligand binding filters could enhance the average distance traversed by the cells by up to 30%. Considering that environmental noise is a ubiquitous feature of bacterial chemotaxis in many real situations and that hybrid neural filters are inexpensive software devices, significant benefits can accrue by employing external filters.

An interesting observation common to all the studies discussed here was that the best performance was obtained by allowing optimally filtered noise to enter the microbial system. In other words, neither the complete elimination of noise nor excessive noise is desirable. This seemingly unusual observation has been explained in terms of stochastic resonance among different sources of filtered noise.

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Computer-aided drug and vaccine design

Annotation of genomes/proteomes : Presently, genomes of more than 1000 organisms have been sequenced or are in advanced stages of sequencing. This has posed a major challenge for bioinformaticians to annotate these genomes for predicting the genes and the repeat regions. We are actively working to analyze the data and derive rules for genome annotation. Numerous softwares and web servers have been developed to predict gene and repeat regions in genomic DNA of organisms. To eventually suggest function of proteins based on sequence content, a large number of programs/web servers have been developed for predicting subcellular localization of proteins. Following is a brief description of major programs developed by our group: ESLpred employs SVM based approach for predicting subcellular localization of Eukaryotic proteins. PSLpred is a method for predicting subcellular localization of prokaryotic proteins, HSLpred, RSLpred and TBpred are organism specific methods developed for predicting subcellular localization of human, rice and mycobacterial proteins.

Biological interactions of proteins: Function of a protein depends on its interaction with other proteins and ligands. Our group has developed methods for predicting DNA binding proteins and DNA interacting regions in proteins. In addition, methods have been developed for predicting RNA interacting regions in RNA binding proteins. Our group is developing methods for predicting various kind of protein interactions.Following are a few examples i) protein-nucleotide interactions (*e.g*. ATP, GTP, NAD, FAD), ii) protein-protein interactions, iii) protein-carbohydrate interaction (mannose), iv) protein-peptide interaction.

Drug targets and inhibitors: One of the important challenge for bioinformaticians is to discover effective drugs *in silico* particularly against drug resistant strains of pathogens. Our group is working since the last number of years to assist researchers interested in the field of drug discovery. Prediction of the function of proteins is important for searching targets suitable for drug design. Our group has developed a number of novel methods for classifying and predicting receptors (G-protein coupled receptor (GPCR), nuclear receptors), toxins and virulent proteins. These methods are playing an important role in drug development as they allow prediction of important class of proteins like GPCR (more than 50% drugs in market are against GPCR). Additionally, methods

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have been developed for predicting secretory proteins like 'Pseapred' for secretory proteins of malaria, 'SRTpred' for non-classical secretory proteins.

Protein Structures Prediction: The prediction of structure of a target/protein is one of major challenge in drug development. Our group has developed methods for predicting secondary structure (regular as well as irregular), super secondary structure (*e.g*. beta-hairpins, beta-barrels) and tertiary structure (*ab initio* methods for bioactive peptides). The performance of their best secondary structure prediction method was ranked within the top 5 methods in the world, according to the community wide competitions like CASP, CAFASP and EVA. These are the only servers from Asia that participate and compete successfully in most of the international competitions. Recently, we have developed a *de novo* method "PepStr" for predicting tertiary structure of peptides which is very popular among structural biologist

Computational Resources for Drug Discovery (CRDD): It is an *in silico* module of Open Source for Drug Discovery (OSDD) which is a forum with a vision to provide affordable healthcare to the developing world. The CRDD web portal provides computer resources related to drug discovery on a single platform. In addition to provision of resources to the community, we also are developing softwares and web servers in the field of drug discovery. For the first time our group has developed a number of on-line tools for predicting inhibitors against novel drug targets of *M. Tuberculosis.* All these tools are free for academic use, and we hope more such free tools will be developed in the field of drug discovery in future to lower the cost of drug discovery process.

Epitope-based vaccine design: Since 1995, subunit vaccine design has become an integral part of vaccine design in which immunogenic region of the protein is used instead of a complete protein as vaccine candidate. Therefore, identification of immunologically active regions/epitopes recognized by T/B cells plays a crucial role in subunit vaccine design. Experimental methods for the identification of such regions include overlapping peptide synthesis, random cloning, and display libraries. Though accurate, these methods are both cost and labor-intensive. Therefore, computation prediction of such sites based on sequence and structure feature of protein is of great value. In order to overcome some of the problems faced by experimental biologists a project "Computer-aided Subunit Vaccine Design based on Epitope" was initiated. The aim of this project was to develop better methods for predicting potential vaccine candidates. Following are the major problems addressed by our group:

Adaptive immunity: In 2001 our group initiated the computer-aided vaccine design work, there were only limited number of methods available at that time. It was felt that the experimental data was scattered. In order to overcome this, we compiled the data from literature and other public resources. The following two databases were developed i) MHCBN: a comprehensive database of T-cell epitopes, MHC binders, TAP bonders and non binders having >25,000 peptides sequences and ii) BCIPEP: a database that experimentally determines B-cell epitopes of varying immunogenicity. After creating databases, next challenge was to develop comprehensive methods. In this direction, our first step was to develop promiscuous MHC class I/II binders using virtual and quantitative matrices. Most of the existing methods of epitope prediction are around MHC binders. Thus, we developed methods, which consider other component of antigen processing for example proteasome cleavage sites, TAP binders. In addition to T-cell epitopes, we developed a method for predicting B-cell epitopes. Recently, our group developed a method for predicting conformational B-cell epitopes in protein from its amino acid sequence.

Innate Immunity: Despite tremendous efforts only limited success has been achieved so far, as epitopes alone fail to elicit a robust and sustained immune response, thereby defeating the main goal of vaccination *i.e.* to provide strong and long lasting immunity. Therefore, in order to elicit an optimal immune response against a pathogen, there is a need to stimulate innate and adaptive immune response simultaneously. In order to

understand innate immunity, we developed a number of methods likes AntigenDB (a database of antigens and their function), PRRDB (a database of pattern-recognition receptors and their ligands) AntiBP/AntiBP2 (prediction/classification of antibacterial peptides), and DESTAM (designing of stable antibacterial peptides).

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Nitish K. Mishra, Mamoon Rashid, Firoz Ahmed, H. Rahman, Deepak Singhla, Arun Sharma, Bharat Panwar, Jagat Chauhan, Ravi, Rahul Kumar, Kumardeep, Sandeep Dhanda, Harinder, Sudhher Gupta, Surendra Vikram, Atul Tyagi, Gaurav Mittal

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Studies on protein-DNA recognition and membrane proteins by molecular simulations

In silico methods, particularly molecular simulations provide understanding of motion of domains, flexibility of active site residues, conformational transitions and mechanisms of the enzyme reactions, permeation of ions and solutes through channels *etc*. Simulations can also provide details not available through experiments, for instance, pressure distributions inside membranes. In our lab, we are making an attempt to understand protein-DNA recognition and permeation through membrane proteins using computational techniques.

Protein-DNA recognition: DNA replication, repair, packaging, modification are direct outcome of protein-DNA interactions. For about half a century, concerted efforts have been made to characterize protein-DNA interactions at atomic level. It seems nearly impossible to derive rules for protein-DNA recognition based on protein and DNA sequence. Large amounts of sequence information for genes from genome analyses are presenting a great challenge in the field of bioinformatics. PDB statistics reveals a gap between the number of experimentally determined structures of proteins and their complexes with nucleic acids. Even with this available sequence and structural data of protein-DNA complexes, our knowledge is limited as far as understanding of mechanisms of their intermolecular recognition is concerned. Computational approaches offer a prompt and lucrative means of investigating these interactions in a high throughput fashion. We have generated a dataset of proteinDNA complexes by taking information from well known public databases such as Protein Data Bank (PDB) and Nucleic Acid Databank (NDB). This dataset has been analyzed to understand role of base-amino acid propensities underlying direct and indirect recognition of protein-DNA interactions. A set of charged residues (such asparagine, arginine, lysine *etc.*) have been found to be involved in interactions predominantly as compared to others. The complex structure of protein and DNA can be predicted by docking methods, thus complementing the experimental techniques employed for co-complex determination. We have performed a detailed and systematic comparison of different macromolecular docking programs for docking protein and DNA involving docking and scoring accuracies. There are differences in the potential of these programs to

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Balvinder Singh has obtained his Ph.D. (1999) in Molecular Biophysics from Panjab university, Chandigarh. He then worked as scientist at the Centre for Cellular and Molecular Biology, Hyderabad before joining IMTECH in 2002.

sample "near native" structure. The methods employing desolvation and electrostatic energies have been observed to perform better in terms of docking and scoring docked conformations. Role of protein architecture in influencing the outcome of docking using these programs has also been analyzed.

Molecular dynamics simulation of protein-DNA complex of homeodomain bound to DNA has been carried out with different all atom force fields to evaluate their relative performance. The results show grossly similar behavior of protein-DNA complex in different force fields. Subtle differences have been observed in non-bonded contacts between protein and DNA with different force fields. Particularly, hydrogen bonding between protein and DNA showed difference among mainly used force fields. Variations in hydration pattern of protein-DNA complex with different force field are quite apparent. Overall, all four force field have been observed to simulate the crystal structure to an extent. Further evaluations of these force fields for different protein-DNA complexes with longer durations of simulations are being carried out to conclusive establish the differences.

Aquaporins: Aquaporin family members facilitate the rapid transport of water as well as small uncharged solutes across the cell membranes. Aquaporins have been linked to various physiological disorders like brain edema, lung edema thereby making them potential drug targets. Aquaporin family members share a conserved domain and highly conserved motifs such as asparagine-proline-alanine (NPA) and ar/R constriction site formed by highly conserved arginine, histidine, phenylalanine residues and a less conserved cysteine residue. Aquaporin family has been subdivided into two subfamilies: aquaporins that are selective for water only, for eg. aquaglyceroporins like AQP3, *E. coli* GlpF *etc.* that in addition to water, also transport some uncharged solutes such as glycerol, urea, ammonia *etc*. Despite high structural similarity, aquaporin family members exhibit diversity in substrate specificity. Thus, it is an important question what determines the substrate specificity. Understanding the structural basis of substrate specificity would require their three dimensional structures. Till date, only structure of aquaglycerolporin that is permeable to substrate other than water (*i.e*. glycerol) is

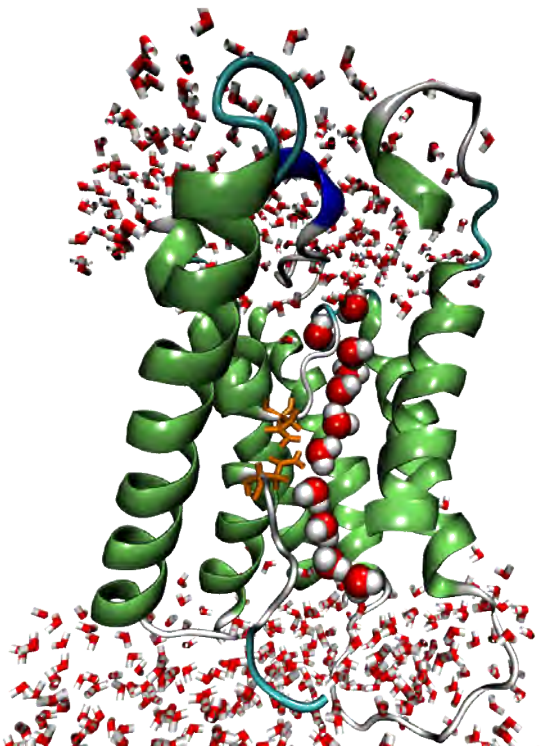

Figure 1: Water in Aqp channel during MD.

available in the database.

We have attempted the modeling of aquaporin structures that permeate ammonia, urea *etc.* employing homology based method and have carried out molecular dynamics to understand their permeation. We are analyzing different kinds and types of amino acid residues at the critical sites along the path of permeation. This will establish their roles and need during the permeation of different solutes through aquaporin channel. In addition, we have carried out molecular dynamics studies so as to understand the role of phosphorylation in the regulation of water channel.

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Development of bioinformatics tools for RNAi

Short- interfering RNAs (siRNA) suppress sequence specific gene expression through a highly coordinated cellular process known as RNA interference (RNAi). In RNAi, ribonuclease (RNase) III–like enzyme called Dicer cleaved the long dsRNA into 19-21 mer siRNAs. Then siRNAs are incorporated on the RNA-induced silencing complex (RICS) to degrade the mRNA in sequence specific manner. RNAi technology has been widely used to study and validate gene function through selective knockdown of their target mRNA. Selection of effective siRNA is one of the crucial steps in designing gene silencing experiments. The performances of the existing siRNA efficacy prediction methods are far from satisfactory. In this study, we have developed a method "siRNApred" a support vector machine based method for predicting efficacy of siRNA.

The main dataset used in this study for developing SVM models consists of 2182 siRNAs obtained from single experimental under homogeneous condition (Huesken D. *et al.* Nat Biotechnol 2005). The SVM models have been developed using nucleotide frequencies, binary pattern of nucleotides and their hybrid (which combines nucleotide binary pattern and frequencies) to achieve a maximum correlation of 0.72. In addition, SVM models have been developed on an alternate dataset, which consists of 581 siRNAs derived from heterogeneous experimental conditions (Saetrom P. *et al.* Biochem Biophys Res Commun 2004). We achieved a maximum correlation of 0.56 on this dataset using hybrid model. The performances of models developed in this study were comparable or better than existing methods on the same datasets. Based on this study, a web server has been developed for predicting efficacy of siRNA (http://www.imtech.res.in/raghava/sirnapred/) Figure1.

Research Interests: Our lab has been involved in the Computational Biology research with emphasis on developing prediction algorithms and databases for biological problems using Machine learning techniques viz. Support Vector Machine (SVM) and Artificial Neural Network (ANN). We have initiated our work by developing methods/web server viz. *VirusSLpred*- Development of SVM based method for predicting the sub-cellular localization of viral proteins within host and virus-infected cells; and *siRNApred*- SVM based method for

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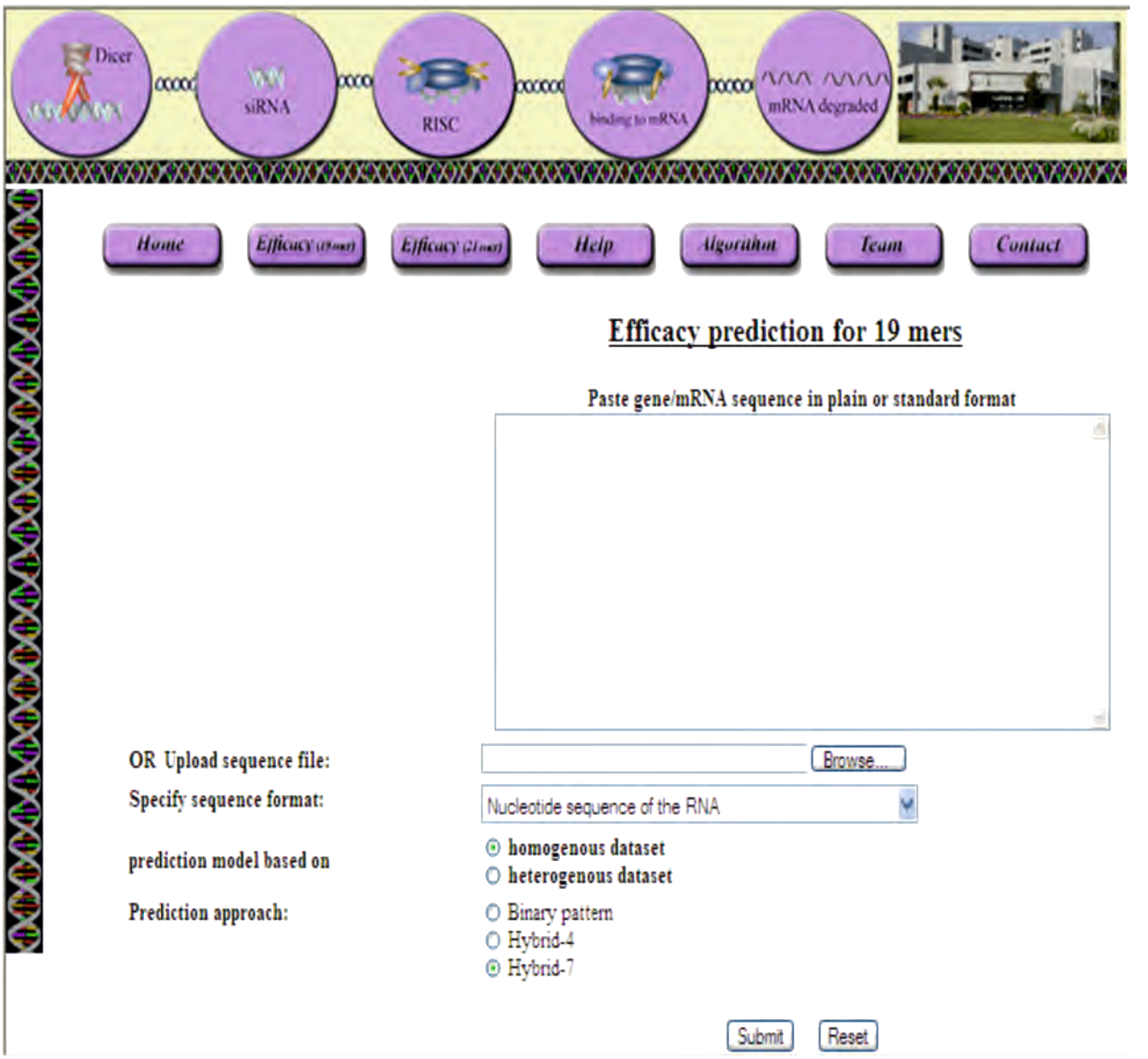

Figure1: siRNApred Webserver: a support vector machine based method for predicting efficacy of siRNA

predicting efficacy value of siRNA.

Earlier, at National Centre for Disease Control (NCDC) (previously known as National Institute of Communicable Diseases) Delhi, I have over 8 years wet lab experience in molecular diagnosis, strain differentiation, drug resistance and genotyping of important epidemic/outbreak causing pathogens in India *viz*. HIV, Dengue, influenza, Hepatitis, SARS, Polio, and *M. tuberculosis, Yersinia pestis, Bacillus anthracis etc*. under biosefety requirements (BSL-2/3).

Further, our focus is on the molecular epidemiology of emerging/re-emerging viruses using genomic approaches besides the development of bioinformatics tools for RNAi and also to experimentally validate the predicted viral siRNA so as to develop siRNA based antiviral therapeutics.

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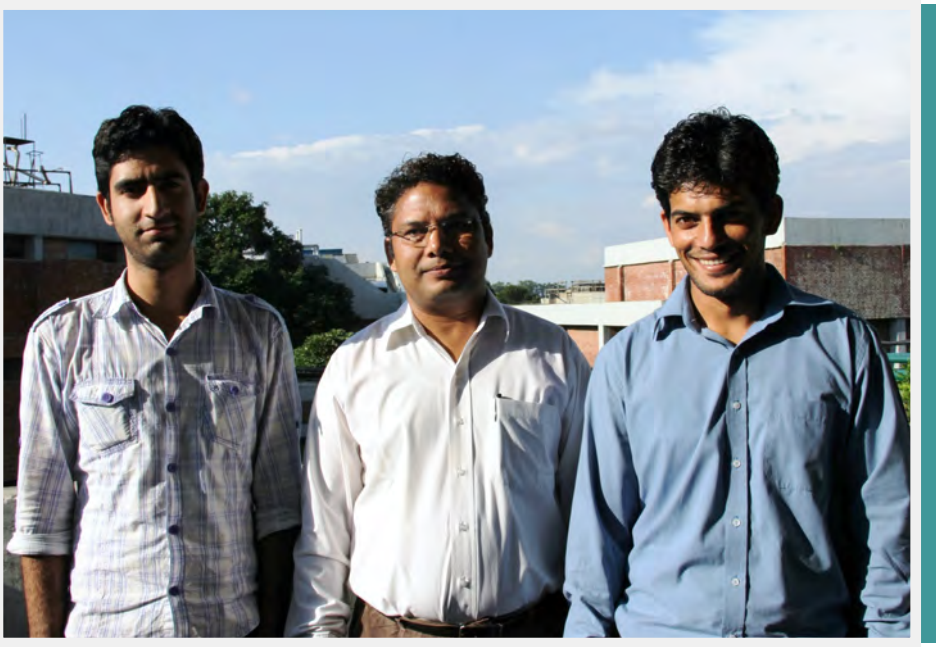
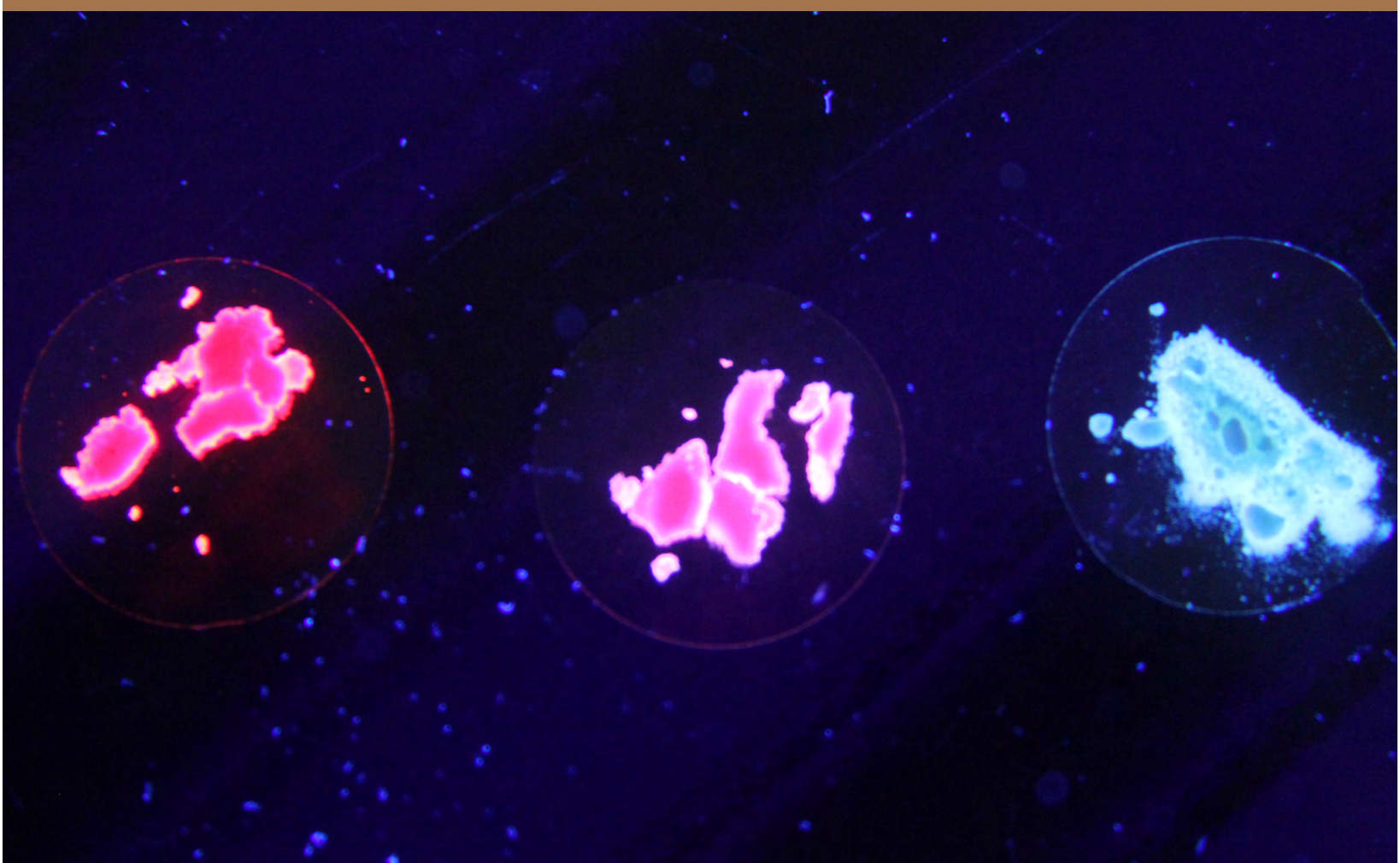
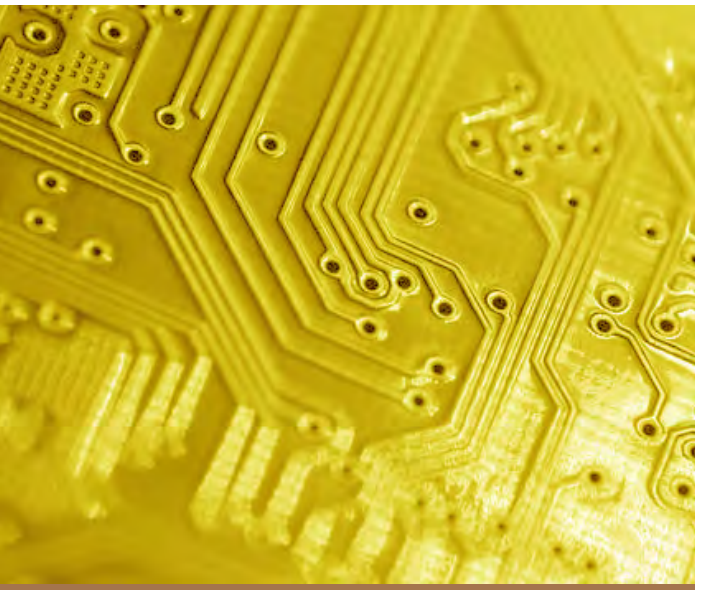




biosensors and nanotechnology



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Nanomaterial based immunobiosensor systems for specific and sensitive detection of environmental pollutants

Nanomaterials are expected to play a key role in many conventional and emerging technologies such as immunodiagnosis and therapeutic. A highly interdisciplinary programme for investigating the synthesis, assembly, properties and commercial application of nanomaterials interfacing with biomaterials (antibodies) has been initiated at IMTECH for the development of immunosensing techniques for clinical/environmental applications. These highly sensitive immunosensing techniques enabled measurement of concentration of biological molecules, biological structures, microorganisms *etc.*, by translating a biochemical interaction at the probe surface into a signal that can be measured. The design of biochemical detection systems that use antibodies as recognition elements has generated considerable interest from both the scientific and industrial communities, as the potential applications for this type of biosensor technology are very broad and include diagnostics, detection, process control, and environmental monitoring. The potentially high sensitivity of antibody-based systems makes them ideal in environments where speed and accuracy are a priority. Biosensor group at the Institute of Microbial Technology, Chandigarh is working in the field of development of antibody based biosensor kits for the different environmental and clinical applications.

Mission and goals of the group:

- Development of enzymatic and antibody based biosensors for clinical / environmental applications.
- Synthesis of nanoparticles (inorganic, polymer and semiconductor) for immunodiagnosis and therapeutic applications.
- Design and development of antibody based immunobiosensors for monitoring various organic/inorganic/biomolecular substances.

C. Raman Suri obtained his Ph.D. (1995) in Biotechnology from Panjab University, Chandigarh after joining IMTECH in1986.

Competencies:

• Generation of specific antibodies (IgG and IgY types) against toxins (pesticides, aflatoxin *etc.*), narcotic drugs (heroin, morphine, cocaine *etc*.) and surface antigens (outer membrane protein, biomarkers etc) for clinical diagnosis.

• Optimization of biomolecular immobilization techniques for a stable coupling of the antibodies to the transducer surface without compromising their biological activity.

• Characterization of the bio-interface upto the molecular resolution by different physical techniques, such as Scanning Electron Microscope (SEM), Scanning Tunneling Microscope (STM), Atomic Force Microscopes (AFM), Ellipsometer *etc.*

• Development and standardization of *immunobiosensor* systems (Optical, Microgravimetric, Micromechanical, Microfluidic and Dipstick).

• Stability analysis of immobilized enzyme/antibody for biosensor probes.

• Synthesis of nanoparticles (inorganic, semiconductor and polymer based) for immunodiagnosis and therapeutic applications.

Biosensor group at IMTECH has successfully developed some sensitive immunosensing kits for the detection of environmental pollutants, mainly pesticides *i.e.*, Low cost LASER ablated electrochemical, Immunochromatographic, dipstick Micromechanical cantilever based BIOMEMS and Quartz crystal based microgravimetric immunosensor formats respectively.

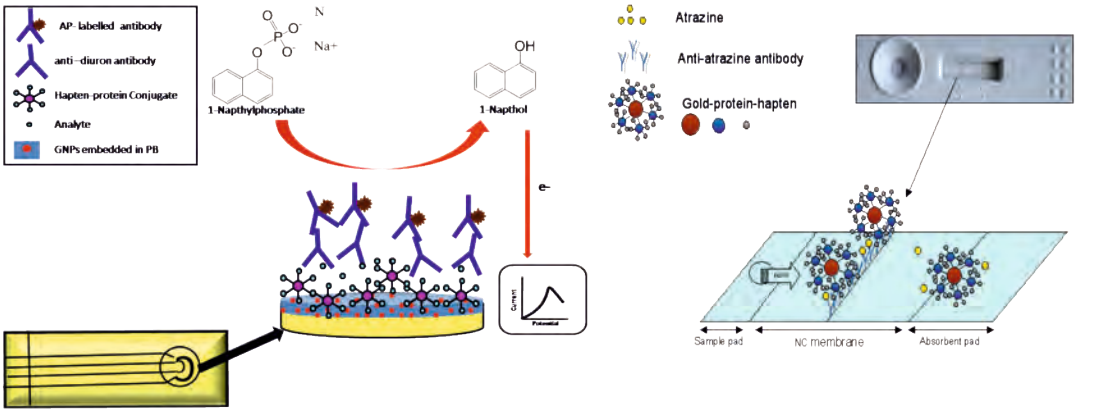
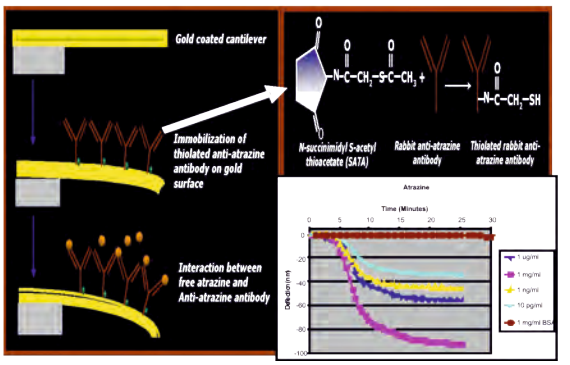
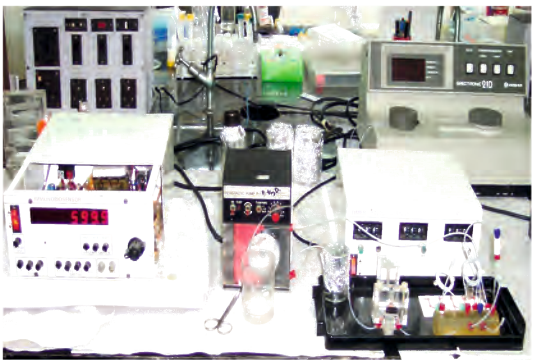


Fig. 1





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Participants:

Vijender Bhalla, Priyanka Sharma, Yogesh Nangia, Adity Chopra

Dr. C. Raman Suri's group

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Bioinformatics Centre

A National Facility supported by Department of Biotechnology (DBT): The Bioinformatics Centre (BIC) at the Institute of Microbial Technology (IMTECH) is playing multiple roles that include i) serving as a national facility under Biotechnology Information Systems Network (BTISnet) programmee of DBT; ii) R&D activity in the field of Bioinformatics and Computational Biology; iii) Computational Resources for Drug Discovery (CRDD); iv) maintaining infrastructure for protein modelling/engineering and iv) providing Information Technology related services to IMTECH. Following is a detailed description of its major activities:

National Facility: The Government of India established Biotechnology Information System Network in 1987, first of its kind in the world. Initially, nine Distributed Information Centres (DICs) were established all over India with the objectives to create infrastructure for information dissemination in the field of biotechnology. BIC at IMTECH, Chandigarh is one of the DICs supported by DBT under the BTIS programme. It has shown consistent growth in different areas of bioinformatics whether in carrying out peer reviewed research or providing services, databases & softwares world wide. The Centre also provides services in the field of protein modeling, protein engineering, biocomputing and networking. BIC staff have trained a large number of students and scientists over the years by conducting national and international conferences/training workshops. They train students of IMTECH as well as nearby universities; BIC provides/distributes education and research related material to public via its website; providing infrastructure for R & D in bioinformatics. This Centre has a adopted policy of Open Source i.e. all software and databases developed at the Centre are available free for public use. BIC has developed more than 100 softwares and databases which are heavily used by the public (more than 80,000 hits per day).

Research in Bioinformatics: Presently, more than 10 students are pursuing their Ph.D. in the field of bioinformatics at the centre. Major research activities of BIC staff are as follows:

Structure and function characterization of proteins using *in-silico* techniques: Protein modeling and engineering are the thrust areas of BIC. Structure prediction techniques like homology-modeling and fold recognition are used routinely. Molecular simulations help comprehend structural stability, domain motion and interactions of substrates with active sites of enzymes. BIC staff is also collaborating with structural labs and other labs in the institute to get a deeper insights into the biological phenomenon by validating bioinformatics with wet lab data. We have worked in collaboration on proteins like TET aminopeptidase, and PhoP binding to DNA.

Computer-Aided Vaccine Design: One of the major challenges in the post-genomic era is to design personal medicine with minimum side effects. Traditional vaccines were based on whole pathogens which were costly and toxic. Designing epitope or peptide-based vaccines is a need of the hour. Computational prediction of epitopes save time, cost & labour when compared to experimental techniques. One of the major activities at BIC

is “Computer-aided Subunit Vaccine Design based on Epitopes”. A large number of methods and databases have been developed over the years that include five reference databases in immunoinformatics on immunogens and immunogenic peptides, methods for MHC class II binders for large number of alleles, prediction techniques for B-cell epitope, identification of CTL epitopes, MHC I binders, TAP binders and cleavage sites and, peptides and proteins involved in innate immunity.

Annotation of genomes/proteomes: BIC staff is extensively involved in identification of genes and prediction of their structures. Following are the major softwares developed at BIC i) FTG program for locating genes in prokaryotic genomes; ii) EGPred for predicting eukaryote genes and its structure; iii) GWFASTA for genome wise similarity search. The protein sequence databases are also growing exponentially due to progress in sequence techniques. The BIC group developed *in-silico* methods for annotating proteomes like ESLpred/PSLpred/VirusSLpred for predicting subcellular localization of eukaryotic/prokaryotic/viral proteins and RSLpred/HSLpred for predicting subcellular localization of rice/human proteins.

Drug targets: A number of novel methods have been developed at BIC for predicting drug targets. Most of the existing drugs have been developed against G-protein coupled receptors (GPCR), nuclear receptors, toxins and virulent proteins.

Chemoinformatics: This is a new activity at this center for designing or predicting potential inhibitors against novel drug targets. In this subject first docking energies are used as descriptors for developing QSAR modules. In past few years, docking based QSAR models have been developed against novel drug targets of *M. tuberculosis.* Researchers at BIC, developed free web servers in the field of chemoinformatics.

Computational Resources for Drug Discovery (CRDD): BIC staff is actively participating in development of CRDD, an important in silico module of “Open Source for Drug Discovery (OSDD); which is a mega project of CSIR. The CRDD web portal provides computer resources related to drug discovery on a single platform. CRDD; it provides computational resources for researchers in the field of computer-aided drug design allows users to discuss their problem with other members, it maintains wikipedia related to drug discovery and development of free web servers in the filed of chemo and pharma-informatics.

Infrastructure for protein modeling/engineering: BIC is creating and maintaining state-of-the-art infrastructure for protein modeling. In addition, BIC staff is working in the field of protein modeling, structure prediction, docking, protein-protein interaction and DNA-protein interaction. BIC is equipped with state-of-art computers ranging from desktop to workstations and servers. Most of the softwares needed for protein modeling work are available at centre. These include, molecular modeling and docking, sequence-structure analysis and molecular graphics softwares. BIC also has workstations and servers which provide computing power in teraflops (the range of supercomputers), as well as a number of graphics work stations.

Information Technology related services: BIC is also playing the role of an IT hub by providing swift, IT related services to IMTECH and the scientific community on the web. BIC established the email facility, LAN, internet facility and IMTECH website since 1990, 1994, 1996 and 1998 respectively. IMTECH campus is now networked, having LAN connection via wired Ethernet and Fiber Optics, Access Points for wireless and DSLAM/ADSL. In

addition, BIC provides support for maintaining computers, networks, softwares to all IMTECH staff. BIC is also playing important roles in general computerization of IMTECH including automation of store/purchase departments using the software COMPASS.

Participants:

G.P.S. Raghava, Balvinder Singh, Manoj Kumar, Harvinder Jassal, Harminder Singh, Paramjit Lal, Sandeep Kumar.

Microbial Type Culture Collection & Gene Bank (MTCC) an International Depositary Authority (IDA)

The Microbial Type Culture Collection and Gene Bank (MTCC), a national facility established in 1986 is funded jointly by the Department of Biotechnology (DBT) and the Council of Scientific and Industrial Research (CSIR), Government of India. The main objectives of this national facility are to act as a depositary, to supply authentic microbial cultures and to provide related services to the scientists working in research institutions, universities and industries. Presently, the MTCC has five sections, the Actinomycetes, Bacteria, Fungi, Yeasts and Plasmids and collectively hold over nine thousand cultures. MTCC was recognized as an International Depositary Authority (IDA) under Budapest Treaty on 4th October, 2002 by the World Intellectual Property Organization (WIPO), Geneva, Switzerland thus becoming the first IDA in India.

Objectives:

- Procurement of cultures and *ex-situ* conservation of microorganisms.
- Provide authentic microbial cultures to industries as well as academic and research institutes.
- Provide identification, freeze drying and other microbiology related services.
- Act as a depositary of patent cultures.
- Research on microbial diversity, taxonomy and related areas.
- Impart training, organize workshops in the areas related to culture collection, microbial taxonomy and diversity.

Progress report:

Service related activities: MTCC is a member of World Federation of Culture Collections (WFCC) and registered with World Data Centre of Microorganisms (WDCM). MTCC is the Designated National Repository (DNR) for Microbial cultures by National Biodiversity Authority (NBA) India. Over the last 20 years MTCC has established itself as a unique collection for microbial resources in India. It has about 22,000 microbial cultures (actinomycetes, bacteria, fungi, yeasts and plasmids) in its collections, of which about 10,000 are available for public distribution. Information about these cultures is available at the website http://mtcc.imtech.res.in. The collections includes type strains, strains used for teaching purposes, genetic stock and cultures used for various applications. More than 80% of MTCC general collection are of Indian origin and from various ecological niches of India. In addition, about 12,000 potentially important cultures of bacteria and fungi are also maintained by MTCC as part of various research projects. New cultures are regularly added to the MTCC collections from exploration studies carried out by MTCC staff. Researchers from other organizations who use microbial cultures for their research also deposit the cultures in MTCC after publication to make them available for other researchers. Cultures are normally preserved under liquid nitrogen, -70°C and by freeze-drying, the fungi are also preserved in mineral oil. Viability and some key characteristics of the organisms are periodically checked so that cultures continue to represent the original deposits. Relevant information about the strains held in the MTCC

is computerized for easy search analysis and retrieval.

Since, its inception in 1986, MTCC has strived, to provide quality services to its customers. Any researcher who avails the services of MTCC is given a unique customer number and using that number, the entire transactions of that customer can be retrieved. At present, MTCC has a database of 6,930 customers who rely on the services offered by MTCC. Most of the research organizations, academic institutions, pharmaceutical, biotech, food industries involved in microbiology and biotechnology related work depend on MTCC for their microbial cultures and identifications. During the last two years, MTCC has supplied more than twelve thousand microbial cultures to different customers. Details of the cultures supplied are given in Figure 1.

Last year, MTCC had initiated the MTCC tracking system (MTS), a process of sending email acknowledgment to

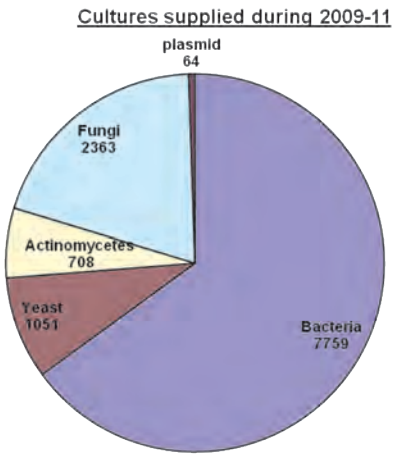


Figure 1: Details of cultures supplied by MTCC during 2009-11

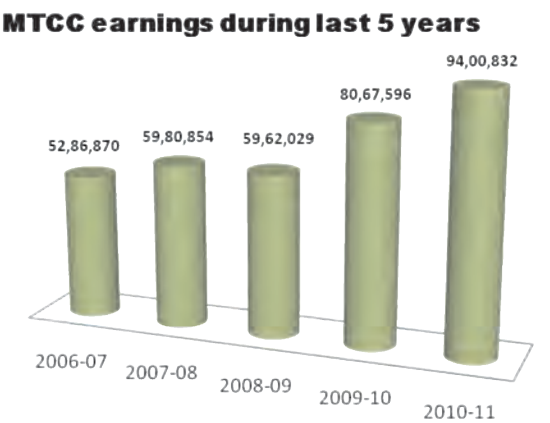


Figure 2 : Revenue generated by MTCC from services during last 5 financial years

customers about the receipt of their request and possible date of completion of the work. Any problem with the customers request is intimated to the customer to rectify the same at the earliest, so that corrective measures can be taken. The advantage of this system is that entire information about the transactions can be obtained at one place and can be viewed on daily, weekly or monthly basis.

Besides supplying the authentic microbial cultures, MTCC also helps the researchers in identification, freeze-drying, fatty acid methyl ester analysis *etc*. of their microbial cultures. MTCC receives several requests for identification of microorganisms by phenotypic and sequence based characterization. During the last two years about 1,000 microbial cultures were identified using phenotypic, sequencing and fatty acid methyl ester analysis methods. As a Designated National Repository (DNR), MTCC receives several microbial cultures for general deposit from researchers all over India. Most of the requests received for general deposit are related to description of novel taxa or part of some publications or research projects. Microbial cultures that are properly identified and sent with relevant data to MTCC are reexamined by MTCC staff for their authenticity before accepting them for deposit.

MTCC has the mandate to accept deposits of actinomycetes, bacteria, fungi, plasmids and yeasts for patent purposes. It accepts three kinds of confidential deposits *viz*., 1. Safe deposit : Confidential deposit service for

those valuable cultures for which patent protection has not been sought, if requested, the culture can be transferred to a patent deposit at a later date. 2. Indian patent deposit : Valid for filing patents in India, and 3. Deposits under Budapest Treaty : Valid for filing patents in all the PCT countries (including India). Several researchers approach MTCC with queries related with these deposits for advice. MTCC regularly receives microbial cultures for safe deposit, Indian patent deposit and Budapest Treaty deposits. At present, there are more than 600 microbial cultures in the MTCC patent collection, and about 30% of these are from industries.

As a culture collection supported by Government of India through Council of Scientific and Industrial Research (CSIR) and Department of Biotechnology (DBT), MTCC charges a moderate fee for the services offered. Revenue generated from service related activities of MTCC during the last five financial years is highlighted in Figure 2.

Organization of workshop: As part of the mandate, MTCC periodically organizes the workshops related to microbial diversity and characterization. One such workshop entitled "Characterization of Microbial Communities Associated with Polluted Environments using Polyphasic Approach" was organized from 7th-16th February, 2010. The main objective of workshop was to provide hands-on experience to the participants in the basic and advanced tools of molecular taxonomy and bacterial systematics. It included studies pertaining to the microbial diversity in soil and their characterization. Although microbial diversity of soil is very large, polluted environments exhibit selective microbial diversity since it allows only selected organisms to grow. However, all microbes are not cultivable in laboratory and thus they are called as "uncultivable". Therefore, this workshop was focused on the methods involved in characterization of cultivable and non-cultivable microbes. Many strains isolated during the workshop were also found to act as potential tools in bioremediation.

Besides IMTECH scientists, several renowned speakers from India and abroad delivered lectures related to different aspects of the workshop and conducted hands-on experiments. International speakers participated in the workshop were Dr. T. Itoh (Head, Archaea & Extremophiles, JCM, Japan), Prof. H. J. Busse (University of Vienna, Austria), Prof. A. Oren (The Hebrew University of Jerusalem, Israel) and Prof. Gary S. Sayler, (Director, CEB, USA).

Research activity: In the initial years, MTCC mainly focussed on providing the quality services to the customers. Since the last one decade, MTCC scientists are actively involved in research activities related to microbial diversity and applications, microbial taxonomy and environmental biotechnology. More than 50 novel taxa of bacteria, actinomycetes and yeasts have been described by the MTCC scientists. More than 100 research papers related to microbial diversity and environmental biotechnology were published. MTCC scientists are also involved in various CSIR network projects and projects from other funding agencies such as Department of Science and Technology (DST), Department of Biotechnology (DBT). MTCC has now established itself as a leading centre in microbial taxonomy and diversity studies and is renowned worldwide for its quality services and research activity. During the last two years MTCC scientists have published 32 research papers.

Participants:

Scientists :

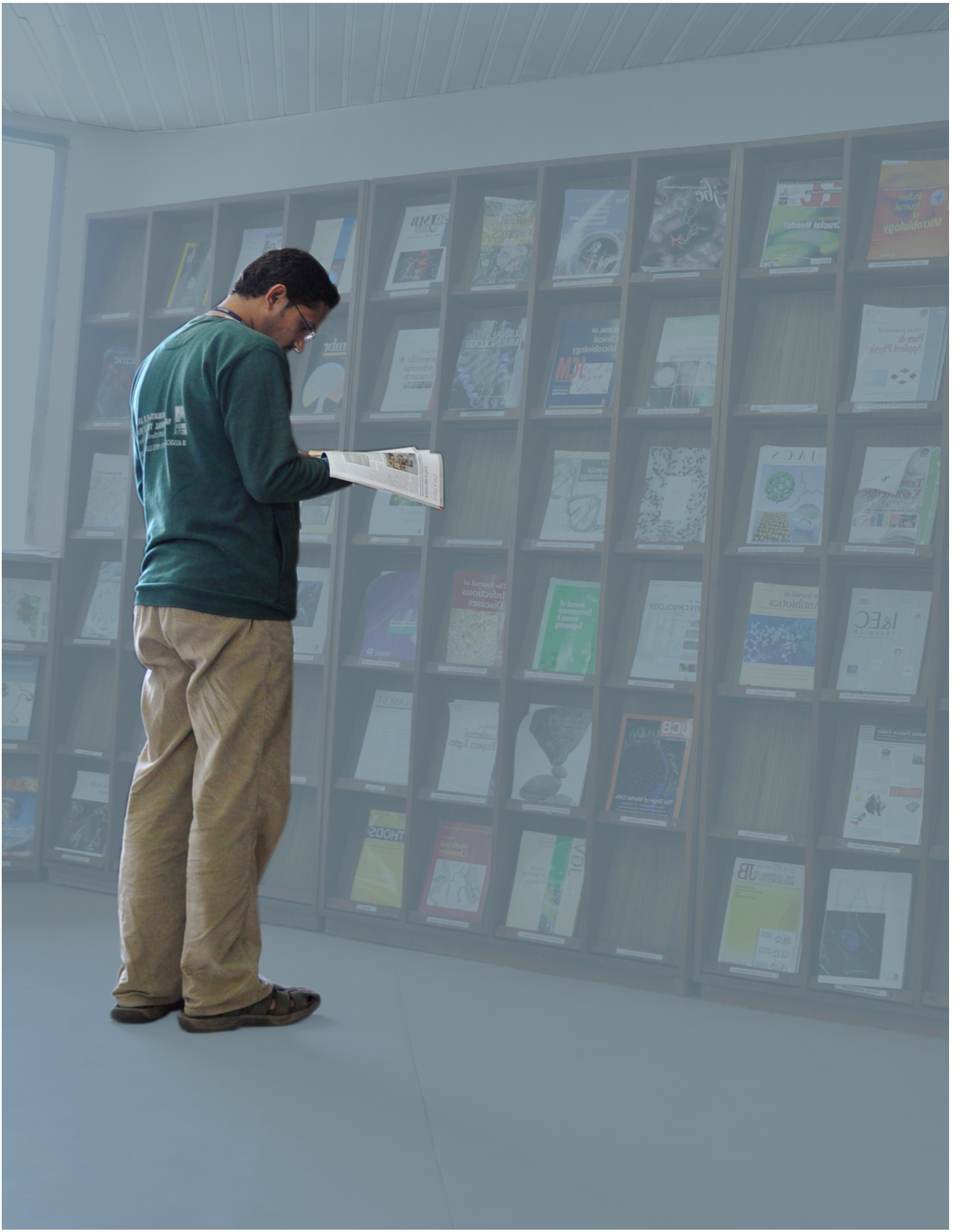
Late R. K. Jain (ex-Head MTCC, Bacteria and plasmids section), G. S. Prasad (Yeast section), D. Ananthapadmanaban (Filamentous fungi section), S. Mayilraj (Actinomycetes section), K. Suresh (Bacteria and anaerobic bacteria section), B. D. Shenoy (Filamentous fungi section), P. Anil Kumar (Bacteria and cyanobacteria).

Technical Staff :

Dhan Prakash (Technical officer), Sumit Mittal (Technical assistant), Upendra Singh (Technician), Paramjit (Technician), Malkit Singh (Technician), Girja Ditya (Technician), Manoj (Technician), Ramesh Singh (Lab attendant).

Administrative staff :

Sushil Kumar (Sr. Stenographer), Kavita (Jr. Stenographer).



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Intellectual Property

INDIAN PATENTS GRANTED

Title	Inventor	Comp. Filing Date	App. No.	Grant Date	Patent No.
A process for the preparation of strain deficient in protease enzymes.	Aggarwal P, Bachhawat AK	27/03/2003	0469DEL2003	02/06/2009	234499
An improved process for the preparation of plasminogen activator protein.	Girish Sahni, Rajesh Kumar, Chaiti Roy, Kammara Rajagopal, Deepak Nihalani, Vasudha Sundaram, Mahavir Yadav	20/02/2003	0160DEL2003	24/02/2010	238877
An improved process for the intercellular over-production of streptokinase using genetically engineered strain of *E.coli.*	Vinay Venkatrao Vyas, Govindan Rajamohan, Ramandeep, Kanak Lata Dikshit	20/02/2004	00403DELNP2004	31/03/2010	239733
A process for the preparation of recombinent growth hormones of economically important animals indigenous to the indian sub-continent with dairy and veterinary importance.	Mukhopadhyay U, Sahni G	31/10/2001	1111DEL2001	20/08/2010	242286
A method for the oxygen regulated production of recombinant staphylokinase.	Govindan Rajamohan, Monika Dahiya, Ranjana Pathania, Kanak Lata Dikshit	31/03/2004	0647DEL2004	20/08/2010	242278
A process for bioremediation of p-nitrophenol contaminated soil and other sites.	Rakesh Kumar Jain, Sumeed Labana, Gunjan Pandey	29/09/2004	1871DEL2004	30/12/2010	245068

FOREIGN PATENTS GRANTED

TITLE	Inventor	Grant Date	Patent No.	Country
A novel method for the oxygen regulated production of recombinant staphylokinase and its derivatives useful for thrombolytic therapy.	Rajamohan G, Dahiya M, Pathania R, Dikshit KL	28/04/2009	7524644	US
The vaccine for the treatment of tuberculosis and other intracellular infectious diseases.	Agrewala JN, Sharma N	10/06/2009	ZL 01802209.X	CN
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Methods for identifying genes that increase yeast stress tolerance and use of these genes for yeast strain improvements.	Rekha Puria, Rohini Chopra, Kaliannan Ganesan	09/09/2009	1910536	EP
A novel method for the oxygen regulated production of recombinant staphylokinase and its derivatives useful for thrombolytic therapy.	Rajamohan G, Dahiya M, Pathania R, Dikshit KL	20/11/2009	2373281	RU
Process for the intracellular over-production of streptokinase using genetically engineered strain of *E. coli*.	Vinay Venkatrao Vyas, Govindan Rajamohan, Ramandeep, Kanak Lata Dikshit	05/01/2010	7642069	USB
A process for high cell density fermentative production of intercellular recombinant streptokinase.	Vinay Venkatrao Vyas, Govindan Rajamohan, Ramandeep, Kanak Lata Dikshit	19/05/2010	1704229	EP
Mycobacterial peptide deformylase.	Rahul Saxena, Pradip K Chakraborti	30/06/2010	2009/01273	ZA
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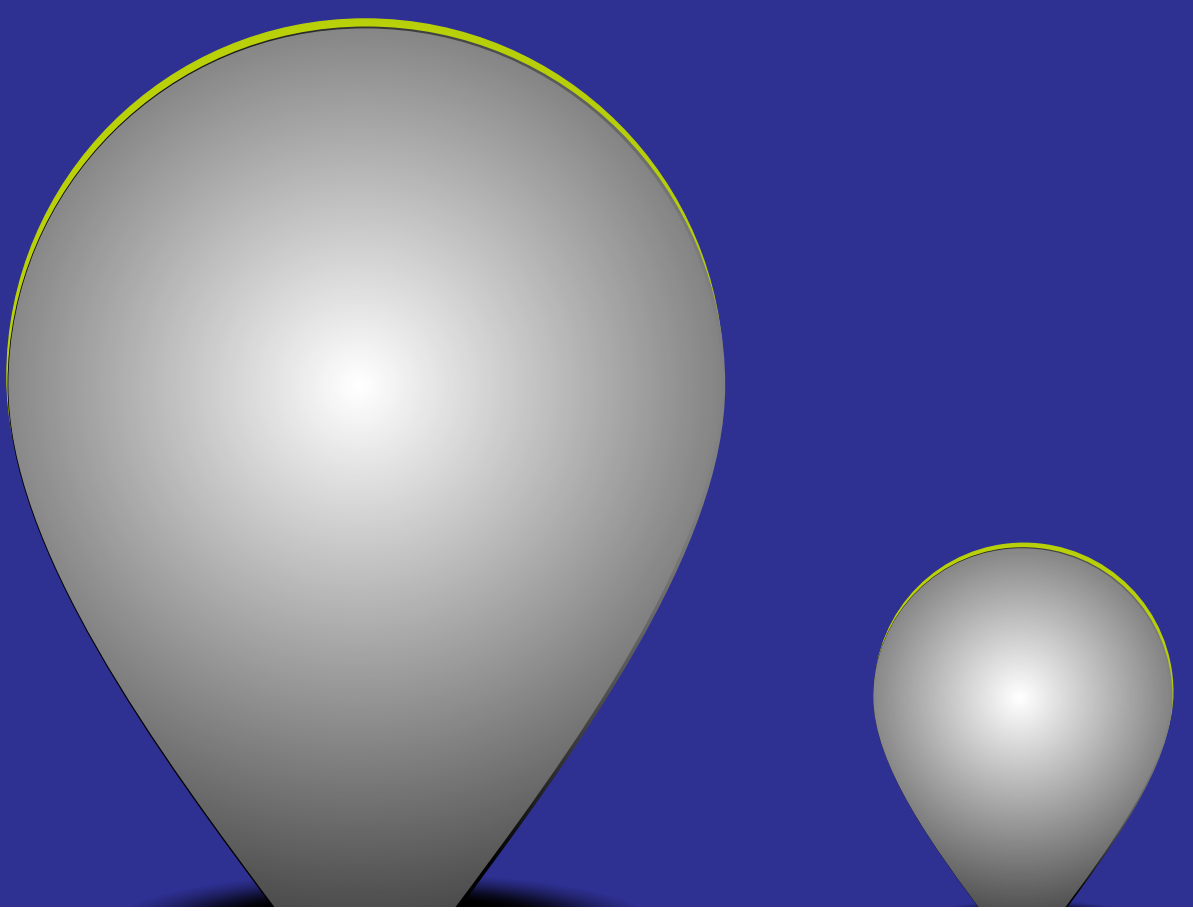
Title	Inventor	Prov. Filing Date	Application No.
A novel construct and strain for oxygen regulated production of recombinant staphylokinase and its derivatives useful for thrombolytic therapy.	Rajamohan Govindan, Dahiya Monika, Pathania Ranjana, Dikshit Kanak Lata	31/03/2010 (Comp. Divisional)	0788DEL2010
A novel construct and strain for oxygen regulated production of recombinant staphylokinase and its derivatives useful for thrombolytic therapy.	Rajamohan Govindan, Dahiya Monika, Pathania Ranjana, Dikshit Kanak Lata	31/03/2010 (Comp. Divisional)	0789DEL2010
A process for production of suluble staphylokinase using recombinant *Escherichia coli*.	Debendra K Sahoo, Kanak Lata Dikshit, Arshad Jawed	11/01/2010	0062DEL2010
Protein fusion constructs possessing thrombolytic and anticoagulant properties.	Neeraj Maheshwari, Girish Sahni	05/08/2010	1845DEL2010
Method of isolation of laccase enzyme from *arthographis* sp.	Vijay Chintaman Sonawane	17/08/2010	1951DEL2010
Targeting promiscuous peptides to dendritic cells for generating long-lasting immunity and development of vaccines	Javed Naim Agrewala, Uthaman Gowthaman, David Jackson, Weiguang Zeng	14/09/2010	2172DEL2010

FOREIGN PATENTS FILED

Title	Inventor	Comp. Filing Date	Application No.	Country Code
Creation of a meso-active thermo-stable chimera through transplantation of the entire active surface of a mesophile enzyme onto its thermophile homolog.	Divya Kapoor, Sanjeev Kumar, Shubbir Ahmed, Swati Sharma, Manish Dutt, Balvinder Singh, Karthikeyan Subramanian, Purnananda Guptasarma	01/05/2009	2009-535190	JP
Creation of a meso-active thermo-stable chimera through transplantation of the entire active surface of a mesophile enzyme onto its thermophile homolog.	Divya Kapoor, Sanjeev Kumar, Shubbir Ahmed, Swati Sharma, Manish Dutt, Balvinder Singh, Karthikeyan Subramanian, Purnananda Guptasarma	05/05/2009	2668690	CA
Creation of a meso-active thermo-stable chimera through transplantation of the entire active surface of a mesophile enzyme onto its thermophile homolog.	Divya Kapoor, Sanjeev Kumar, Shubbir Ahmed, Swati Sharma, Manish Dutt, Balvinder Singh, Karthikeyan Subramanian, Purnananda Guptasarma	06/05/2009	2007318868	AU
Creation of a meso-active thermo-stable chimera through transplantation of the entire active surface of a mesophile enzyme onto its thermophile homolog.	Divya Kapoor, Sanjeev Kumar, Shubbir Ahmed, Swati Sharma, Manish Dutt, Balvinder Singh, Karthikeyan Subramanian, Purnananda Guptasarma	25/06/2009	200780048162.0	CN
New staphylokinases carrying amino and carboxy-terminal extension and their peg (polyethylene glycol) conjugated forms.	Satish Singh, Kanak Lata Dikshit	03/12/2009	302/2009	BD
New staphylokinases carrying amino and carboxy-terminal extension and their peg (polyethylene glycol) conjugated forms.	Satish Singh, Kanak Lata Dikshit	04/12/2009	12/631617	US
New staphylokinases carrying amino and carboxy-terminal extension and their peg (polyethylene glycol) conjugated forms.	Satish Singh, Kanak Lata Dikshit	04/12/2009	PCT/IB2009/007645	WO
New staphylokinases carrying amino and carboxy-terminal extension and their peg (polyethylene glycol) conjugated forms.	Satish Singh, Kanak Lata Dikshit	05/12/2009	14831	GC

Cysteine variants of streptokinase and its covalently modified forms.	Shekhar Kumar, Neeraj Maheshwari, Girish Sahni	29/09/2010	PCT/NA1644/2010	EG
Cysteine variants of streptokinase and its covalently modified forms.	Shekhar Kumar, Neeraj Maheshwari, Girish Sahni	29/09/2010	Pi2010004574	MY
Cysteine variants of streptokinase and its covalently modified forms.	Shekhar Kumar, Neeraj Maheshwari, Girish Sahni	30/09/2010	MX/A2010/010759	MX
Cysteine variants of streptokinase and its covalently modified forms.	Shekhar Kumar, Neeraj Maheshwari, Girish Sahni	14/10/2010	2010/07356	ZA
Cysteine variants of streptokinase and its covalently modified forms.	Shekhar Kumar, Neeraj Maheshwari, Girish Sahni	27/10/2010	09727334.6	EP
Cysteine variants of streptokinase and its covalently modified forms.	Shekhar Kumar, Neeraj Maheshwari, Girish Sahni	30/11/2010	200980120111.3	CN



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awards & honours

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Awards & Honours

Awards & Honours

Name	Details of Award/Honour	Date conferred
G. P. S. Raghava	Thomson Reuters Research Excellence - India Research Front Award.	April 24, 2009
Shanmugam Mayilraj	Raman Research Fellow.	June 1, 2009
Pawan Gupta	DST-ITS Travel Award.	August 25, 2009
Javed N. Agrewala	Elected Fellow of Indian National Science Academy.	December 1, 2009
G. P. S. Raghava	NASI-Reliance Industries Platinum Jubilee Award in Biological Sciences.	December 14, 2009
Pradip K. Chakraborti	Elected as a Fellow of Indian Academy of Sciences.	January 1, 2010
Srikrishna Subramanian	Selected as Associate Editor of BMC Bioinformatics.	April 16, 2010
Pawan Gupta	ICMR-Travel Award.	April 28, 2010
Pawan Gupta	Innovative Young Biotechnologist Award.	May 12, 2010
Pawan Gupta	U.S. Civilian Research & Development Foundation Travel Award.	August 1, 2010
G. P. S. Raghava	J C Bose National Fellowship.	September 1, 2010
Pradip K. Chakraborti	Fellow of Indian National Science Academy.	December 29, 2010

memorable events

memorable events

Memorable Events

• To commemorate CSIR Foundation Day, the Institute organized a special lecture on September 26, 2009 which was delivered by Prof. N. Sathyamurthy, Director, Indian Institute of Science Education & Research, Mohali on the topic "Atoms & Molecules in a Confined Environment".

• The Institute of Microbial Technology (IMTECH), Chandigarh continued to celebrate its Silver Jubilee Celebrations on Jan. 24th, 2010 also by organizing several events, the most important among those being a Silver Jubilee Foundation Day Lecture by Prof. Prof. D. Balasubramanian, Director of Research, L.V. Prasad Eye Institute, Hyderabad on the topic "From Bench to Bedside - The Story of Translational Research in India since Independence".

• To commemorate CSIR Foundation Day, the Institute organized a special lecture on September 26, 2010 which was delivered by Prof. T.P. Singh, DBT-Distinguished Biotechnology Research Professor, Department of Biophysics, All India Institute of Medical Sciences, New Delhi on the topic "Unexpected Surprises Revealed by Protein Structures".

• As a part of their celebration of the 25th year of its foundation, the Institute of Mircobial Technology, Chandigarh successfully conducted from January 22-24, 2010 an International Conference on "Understanding and Managing Pathogenic Microbes" in which more than 300 participants from both India and abroad participated.

• The Institute of Microbial Technology (IMTECH), Chandigarh celebrated its Foundation Day on Jan. 24th, 2011 by organizing several events, the most important among those being the visit of Hon'ble Shri Pawan Kumar Bansal, the Union Minister for Parliamentary Affairs, Science and Technology & Earth Sciences and Vice President CSIR. After assuming his charge as Minister of Science and Technology this was Mr. Pawan Kumar Bansal's first visit to any CSIR laboratory.

• The Bioinformatics Centre successfully conducted from November 15-19, 2010 an Indo-Russian Workshop on "Predictive Biology Using Systems and Integrative Analysis and Methods" which was attended by more than 50 participants from India and 10 from Russia.

• The ninth Prof. B.K. Bachhawat Memorial Lecture was delivered on November 20, 2010 by Dr. C.M. Gupta, Distinguished Biotechnology Research Professor (DBT), C.D.R.I., Lucknow on the topic "An Unconventional form of Eukaryotic Actin with Conventional Functions".

• Institute of Microbial Technology, Chandigarh successfully conducted from March 28-30, 2011 an International Workshop on "Computational Aspects of Working with Genomes" in which more than 100 participants from both India and abroad participated.

Public Functions & Official Orations

Sh. Pawan Kumar Bansal addressing the IMTECH staff on 24 January 2011.

Incubator facility for Biopharmaceuticals being inaugurated by Sh. Pawan Kumar Bansal on 24 January, 2011.

A sapling being planted by Sh. Pawan Kumar Bansal in IMTECH on 24 January, 2011.

Dr. Girish Sahni presenting a memento to Dr. C.M. Gupta on the occasion of ninth Prof. B.K. Bachhawat Memorial Lecture.

Dr. Girish Sahni presenting a memento to Dr. T.P. Singh, Distinguished Professor, AIIMS on the occasion of CSIR Foundation Day, 26 September, 2010.

Prof. N. Satyamurthy, Director IISER, Mohali presiding over the IMTECH foundation day celebrations on 24 January, 2011.

Prof. Samir Brahmachari, DG-CSIR, delivering the IMTECH foundation day lecture on 24 January, 2011.

Dr. Girish Sahni interacting with delegates of U.M.P.M-2010 held from 22-24 January, 2010.

Delegates of Indo-Russian workshop on "Predictive Biology Using Systems and Integrative Analysis and Methods" held from 15-19 November, 2010.

Pat Mc Fadden, Member of Parliament U.K., interacting with Dr. Naresh Kumar on 15 Feburary, 2010.

Blood Donation camp being organized at IMTECH on 23 October, 2009.

IMTECH's winning team of 41st S.S.B.M.T. (outdoor) finals Cricket tournament held at C.S.I.O. Chandigarh on 19-21 Feburary, 2010.

CSIR Technofest 2010

Council of Scientific and Industrial Research (CSIR), New Delhi organized a CSIR mega showcase, titled **'CSIR Technofest 2010'** during the India International Trade Fair (IITF) organized by Industrial Trade Promotion Organization (ITPO), Ministry of Commerce, during 14-27 November, 2010 at Pragati Maidan, New Delhi in which IMTECH actively participated and showcased its R&D achievements in Biology Sector related to Health Care along with other CSIR labs and the Theme Pavilion on **Healthcare** was the Platnium Award Winner. The exhibition offered CSIR/IMTECH a unique opportunity to highlight its achievements to public and it also learnt from industry their R&D needs in the specialized areas.

Glimpses of 'CSIR Technofest 2010'

स्पिक मैके SPIC MACAY

Society for promotion of Indian Classical Music And Culture Amongst Youth

heritage 2011

at Chandigarh (celebrating foreign students in india)

Indian Council for Cultural Relations

Shankar Brothers (Shehnai) in IMTECH auditorium on May 22, 2010.

Pt. Vishwa Mohan Bhatt (Mohan Veena) at IMTECH auditorium on 31 January, 2011.

Pt. Hariprasad Chaurasia (Flute) at IMTECH auditorium on 1 Feburary, 2011.

Shashank Subramaniam (Carnatic Flute) at IMTECH auditorium on 4 Feburary, 2011.

external linkages

external linkages

Technology Transfers and Tie-ups

Nostrum Pharmaceuticals Inc., USA obtains Worldwide Licensing Rights from IMTECH for Clinical Development of New-Generation Thrombolytic Molecules

Nostrum Pharmaceuticals Inc., USA, a privately-held company based in Edison, New Jersey, obtained the worldwide license to develop and commercialize new (Third and Fourth) generation thrombolytic molecules. The license was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh on November 24, 2010.

Dr. Nirmal Mulye and Dr. Girish Sahni signing a 150 m$ licensing agreement in New Delhi in the presence of Honorable Mr. Kapil Sibal and Professor Samir K. Brahmachari on November 24, 2010.

Designing of these new generation thrombolytic molecules by Dr. Sahni's team at IMTECH was based on long-term basic research which has been converted into applied work. Earlier, Girish Sahni and co-workers had developed technologies for the production of vitally needed streptokinase at affordable costs in India. At the present time, products such as 'STPase' (Cadila Pharm. Ltd., Ahmedabad), and 'Klotbuster' (Alembic) and 'Lupiflo' (Lupin) have successfully been launched in the market, the latter through transfer of technology to Shasun Chemicals & Drugs Ltd., Chennai.

"Licensing of these molecules to Nostrum is a proud achievement for IMTECH, especially since these are the third- and fourth-Generation molecules that IMTECH has licensed out to Nostrum, and should fill an acute need in the world, especially the developing countries where effective but affordable treatments for circulatory disorders such as heart attacks and stroke are a crying need, and where the more expensive option of surgical intervention via Cath Labs is not an easy option".

Dr. Mulye and Dr. Sahni signed the licensing agreement in New Delhi at a function that was attended by the Honorable Mr. Kapil Sibal, Minister of Science and Technology, Government of India, and Professor Samir K. Brahmachari, Director General, CSIR. The function was also attended by various dignitaries within the Indian scientific establishment.

Mr. Sibal, the Minister of Science and Technology, acknowledged that this agreement was once again of great importance whereby IMTECH and CSIR have been able to license for clinical development new molecules for therapeutic purposes. Mr. Sibal praised and congratulated Dr. Girish Sahni and his scientific team at IMTECH that developed these molecules. He expressed "hope and confidence that Nostrum and its Symmetrix subsidiaries will nurture this project well and develop it into a blockbuster drug".

Prof. Brahmachari also complimented the scientific team lead by Dr. Girish Sahni at IMTECH for developing these molecules. Development of technology for Clot-specific Streptokinase *ie.* Work on upscaling and human testing (Phase-I) is in progress (Nostrum Pharmaceuticals Inc., USA). Development of technology for Recombinant Stapylokinase- Work on Phase II is also in progress (Strides Arcolab Ltd., Bangalore).

IMTECH's Technology for Recombinant Streptokinase Commercially Launched for Indian Market

IMTECH's Technology for Recombinant Streptokinase was commercially launched for Indian Market on July 10, 2009 by the Honorable Mr. Prithviraj Chavan, Minister of State for Science and Technology, Government of India, and Professor Samir K. Brahmachari, Director General, CSIR. The function held at CSIR headquarters was also attended by various dignitaries within the Indian scientific establishment, and the event was covered by the press and television media.

The technological package (recombinant clones, fermentation parameters and down-stream processing to obtain drug-quality product) was successfully transferred in the year 2002-03 to a leading drug industrial house M/S Shasun Chemicals & Drugs Ltd., Chennai which specializes in bulk drug manufacture.

The product was launched for the Indian markets under the brand names 'Lupiflo' and 'Klotbuster' being marketed by Lupin Chemicals Ltd. and Alembic Chemicals Ltd. respectively.

Commercialization of recombinant streptokinase by Shasun Drugs & Chemicals Ltd., Chennai is a proud achievement for IMTECH, especially for the scientific team led by Dr. Girish Sahni, including Drs. D.K. Sahoo and K.L. Dikshit that developed this technology over several years of meticulous research. Moreover, this is the second molecule that IMTECH has successfully developed and commercialized. The first was for the production of "Natural Streptokinase" which is India's first indigenously produced thrombolytic drug, sold by M/s Cadila Pharmaceuticals Ltd., Ahmedabad under the brand name 'STPase'.

lectures

lectures

Lectures Delivered/Papers Presented

Name	Date	Topic	Venue, City, Country
G. Rajamohan	April 9, 2009	Group A Streptococcal GAPDH, an Anchorless surface protein, SDH, is a novel transcriptional regulator.	Center for Microbial interface Biology, The Ohio State Universtity, Columbus, U.S.A.
G. Rajamohan	May 17-21, 2009	Group A Streptococcal GAPDH, an Anchorless surface protein, SDH, is a novel transcriptional regulator.	Philadelphia, Pennsylvania, U.S.A.
Javed N. Agrewala	July 17-18, 2009	Differentiation and migration of T cells in different part of body during infection.	Devi Ahilya Vishwavidyalaya, Indore, India.
Dibyendu Sarkar	July 26-31, 2009	Molecular probing of promoter-regulator interactions provide insights on novel functions of *M. tuberculosis* PhoP.	Salve Regina University, Newport, U.S.A.
Girish Sahni	August 8, 2009	Design of new Clot-buster drug.	Huazhong Agricultural Univ., Wuhan, China.
Girish Sahni	August 11, 2009	Use of protein genetic engineering approaches for the designing of improved biomolecules.	University of Science & Technology, Hefei, China.
Jagmohan Singh	September 1-5, 2009	Proficient Imprinting of mat1 locus by the catalytically dead Pola in fission yeast.	Cold Spring Harbor Laboratory, New York, U.S.A.
Vijay Sonawane	October 1, 2009	Potential of ligninolytic fungi and their enzymes for bioremediation.	PES Institute of Technology (PESIT), Bangalore, India.
Javed N. Agrewala	October 4-6, 2009	IL-7 and IL-15 promote long-lasting T cell memory against BCG.	Singapore.
R. Kishore	October 21, 2009	NMR on Biomolecules.	NIPER, Mohali, Punjab, India.
Manoj Kumar	October 26-28, 2009	Protein Sequence: Identification and Analysis and Sequence alignment & Phylogenetic Analysis.	Himachal Pradesh University, Summer Hill, Shimla, India.
Javed N. Agrewala	November 2-4, 2009	Novel vaccination strategies against tuberculosis.	Indian Habitat Centre, New Delhi, India.
Charu Sharma	November 2-4, 2009	The mechnaisms employed by mycobateria for their survival in	Indian Habitat Centre, New Delhi, India.

		macrophages.	
Debendra K. Sahoo	November 4-6, 2009	Production and delivery of biopharmaceuticals: application of tools of 'omic' sciences for bioprocess analysis and process development.	EU-India S&T co-operation days, Jawaharlal Nehru University, New Delhi, India.
Girish Sahni	November 6, 2009	New challenges to onventional biology: Translation of scientific concepts for betterment of life.	DAV College, Chandigarh, India.
Javed N. Agrewala	November 14, 2009	Lipidated promiscuous peptides for elicitation of protective immunity.	Indian Institute of Sciences, Bangalore, India.
Javed N. Agrewala	November 15, 2009	Targeting lipidated peptides to bolster dendritic cells activation.	Hamdard University, New Delhi, India.
Rajagopal	November 21-23, 2009	National convention for food science and technology.	NIMHANS, Bangalore, India.
Dibyendu Sarkar	January 22-24, 2010	Molecular probing of promoter regulator interaction(s) provide insights into the mechanism of action of *Mycobacterium tuberculosis* PhoP.	IMTECH, Chandigarh, India.
Pushpa Agrawal	January 22-24, 2010	Novel stress regulated redox system of *Mycobacterium tuberculosis* H37Rv.	IMTECH, Chandigarh, India.
K. L. Dikshit	January 22, 2010	Unusual mode of nitric oxide detoxification by truncated hemoglobin, HbN, of *Mycobacterium tuberculosis.*	IMTECH, Chandigarh, India.
Pradip K. Chakraborti	January 22-24, 2010	Exploring N-terminal methionine excision pathway: role of three Rs in mycobacterial peptide deformylase.	Understanding and Managing Pathogenic Microbes, IMTECH, Chandigarh, India.
Grish C. Varshney	January 22-24, 2010	Combined application of monoclonal antibodies and phage display system in search of novel vaccine and drug targets in malaria.	Understanding and Managing Pathogenic Microbes, IMTECH, Chandigarh, India.
K. L. Dikshit	February 6, 2010	Microbial hemoglobins and their diverse functions.	Department of Biochemistry, Delhi University South Campus.
K. Ganesan	February 7-16, 2010	DNA sequencing and troubleshooting.	IMTECH, Chandigarh, India.
G. S. Prasad	February 15, 2011	Deposition of Microbial cultures - need and procedures.	IMTECH, Chandigarh, India.
G. S. Prasad	February 27, 2010	Microbial diversity and modern trends in their analysis.	C.K.Thakur College, Navi Mumbai, India.

Girish Sahni	March 17, 2010	Refashioning of old drugs to smarter molecules: A useful paradigm for biotechnology.	GNDU, Amritsar, India.
Balvinder Singh	April 22, 2010	Protein structure and its classification.	North-Eastern Hill University, Shillong, India.
G. S. Prasad	May 18, 2010	Depositions of microbial cultures under Budapest Treaty.	Technology Information, Forecasing and Assessment council (TIFAC), New Delhi, India.
Dibyendu Sarkar	May 31, 2010	Novel structural features contribute to expanded regulatory capabilities of virulence-associated response regulator PhoP from *Mycobacterium tuberculosis..*	Institut Pasteur, Paris, France.
Pawan Gupta	June 13-18, 2010	Retinoic acid-induced sequential phosphorylation, HDAC3 mediated PML recruitment and SUMOylation of nuclear receptor.	Carefree, Arizona, U.S.A.
Pradip K. Chakraborti	July 1-2, 2010	Tr2 to suppress Oct4 expression. Understanding mycobacterial N-terminal methionine excision pathway.	Indian Academy of Sciences, Bangalore, India.
Girish Sahni	July 4, 2010	The design of Target-specific clot busters through protein engineering approaches.	The International Workshop on Industrial Biotechnology, Cairo, Egypt.
Neeraj Khatri	July 20, 2010	Animal Experimentation: CPCSEA guidelines.	Rayat & Bahra Institute of Pharmacy, Sahauran, Mohali, India.
Girish Sahni	July 31, 2010	Smarter cardiac clot busters from an old 'work horse' an expedient paragdigm for drug development.	PGIMER, Chandigarh, India.
C. R. Suri	August 22-27, 2010	Synthesis and bio-functionalization of gold nanoparticles for immunodiagnosis applications.	Moscow, Russia.
K. L. Dikshit	August 23, 2010	An unusual flavohemoglobin from *Mycobacterium tuberculosis.*	University of Antwerp, Antwerp, Belgium.
Debendra K. Sahoo	September 14-18, 2010	A novel organic solvent-stable alkaline protease from newly isolated Stenotrophomonas maltophilia: production, purification and characterization.	14th International Biotechnology Symposium and Exhibition, Palacongressi, Riminy, Italy.
Debendra K. Sahoo	September 14-18, 2010	Enhanced production of recombinant staphylokinase in *Escherichia coli* carrying *Vitreoscilla* haemoglobin gene.	14th International Biotechnology Symposium and Exhibition, Palacongressi, Riminy, Italy.
Manoj Raje	October 4-8, 2010	Use of nanoparticles in quantative	I.H.B.T., Palampur, India.

		detection of molecules in cells & tissues: Optimization of immunogold labelling in TEM.	
P. B. Patil	October 12, 2010	Biodiversity Informatics of Plant Associated Bacteria.	Indian Institute of Spice Research, Calicut, Kerala, India.
G. S. Prasad	October 12-16, 2010	Diversity of yeasts from high temperature regions of India, and pullulan production by an osmotolerant *Aureobasidium pullulans.*	National Institute of Technology and Evaluation, Chiba, Japan.
G. S. Prasad	October 15, 2010	Service and research activities of Microbial Type Culture Collection and Gene Bank (MTCC).	National Institute of Technology and Evaluation, Chiba, Japan.
Pradip K. Chakraborti	October 25-28, 2010	Intermolecular phosphotransfer is crucial for efficient catalytic activity of nucleoside diphosphate kinase.	Heidelberg, Germany.
Girish Sahni	November 11, 2010	Emerging opportunities for biotechnologists of the future.	Thapar University, Patiala, India.
Pushpa Agrawal	November 12-14, 2010	Redox homeostasis in *Mycobacterium tuberculosis* H37Rv: WhiB like proteins form unusual and novel redox network.	Madurai Kamraj Univeristy, Madurai, India.
Balvinder Singh	November 23, 2010	Using Molecular Modeling and dynamics simulation studies to understand structure and function of *Mycobacterium tuberculosis* proteins.	National Institute of Pharmaceutical Education and Research, Mohali, India.
P. Anil Kumar	November 25-26, 2010	Polyphasic Taxonomy- Importance in systematics.	North-Eastern Hill University, Department of Biotechnology and Bioinformatics, Shillong, India.
Jagmohan Singh	December 4-6, 2010	Dynamics of Swi6/Hp1 and Clr4/Suv39H1 recruitment during heterochroamtin assembly in fission yeast.	Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India.
Girish Sahni	December 9, 2010	Soil Metagenomics: Implications of next-generation sequencing technologies on microbial diversity research & soil ecology.	Braunschweig, Germany.
Dibyendu Sarkar	December 13-15, 2010	Multiple mechanisms of transcription regulation by the virulence-associated response regulator PhoP from *M. tuberculosis.*	Indian Institute of Science, Bangalore, India.
Srikrishna Subramanian	December 20-24, 2010	Protein fold recognition from sequence and structure.	Indian Institute of Science, Bangalore, India.
Girish Sahni	January 18, 2011	Stiff challenges for Indian bio-pharma industry and some rays of	Biotech Summit CII, Pune, India.

		hope.	
G. S. Prasad	January 24-26, 2011	The use and exchange of microbial genetic resources for food and agriculture.	University of Catholique de Louvain, Brussels, Belgium.
Saumya Raychaudhuri	January 27-29, 2011	International Symposium on Molecular and pathophysiological research on enteric pathogens.	Kolkata, India.
K. L. Dikshit	January 31, 2011	A novel flavohemoglobin from *Mycobacterium tuberculosis* exhibiting antioxidant activities.	CDFD, Hyderabad, India.
Javed N Agrewala	February 7-10, 2011	Caerulomycin A suppresses the function of both T cells and B cells.	Dubai. U.A.E.
Girish Sahni	February 8, 2011	Converting biosimilars into 'bioleaders': opportunities and challenges in biotech arena.	IIM, Ahmedabad, India.
Charu Sharma	Feburary 17, 2011	Interaction of Mycobacterium virulence factors with macrophage proteins.	Panjab University, Chandigarh, India.
S. Karthikeyan	February 21-23, 2011	Structural characterization of enzymes involved in Riboflavin/ FAD biosynthesis pathway: An antibacterial drug target.	IIT, Bombay, India.
Manoj Kumar	February 22, 2011	Artificial neural networks applications in neuro-bioinformatics research.	Panjab University, Chandigarh, India.
C. R. Suri	February 24, 2011	Synthesis and bio-functionalization of Au-nanoparticles for biomedical imaging/diagnosis applications.	Tanjavur, TN, India.
C. R. Suri	March 11, 2011	Immunosensing techniques for pesticides analysis.	New Delhi, India.
Debendra K. Sahoo	March 21-23, 2011	Why do BioProcess Engineers get so obsess with oxygen transfer in microbial cell culture.	World Congress on Biotechnology, Hyderabad, India.
Pradip K. Chakraborti	March 24, 2011	Molecular insight into the functionality of bacterial kinases.	ACTREC, Navi Mumbai, India.
P. B. Patil	March 29, 2011	Phylogenomics - The first and critical step in comparative genomics.	Institute of Microbial Technology, Chandigarh, India.

distinguished visitors

distinguished visitors

Distinguished Visitors

Distinguished Visitors

Date	Visitor name
November 25, 2009	Dr. Yashveer Singh Anton Paar, Osterreich, Austria.
January 22, 2010	Dr. Patrick Brennan Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, U.S.A.
January 22, 2010	Dr. A. M. Chakrabarty University of Illinois at Chicago College of Medicine Chicago, Illinois, U.S.A.
January 22, 2010	Dr. R. K. Poole Department of Molecular Biology and Biotechnology, University of Sheffield, United Kingdom.
January 22, 2010	Dr. Robert Clubb Dept. of Chemistry & Biochemistry, University of California Los Angeles, Los Angeles, U.S.A.
January 22, 2010	Dr. Y. Av-Gay Division Of Infectious Diseases, Faculty of Medicine, The University of British Columbia, British Columbia, Canada.
January 22, 2010	Dr. Tanya Parish Centre for Infectious Disease Institute for Cell and Molecular Science, School of Medicine and Dentistry, London, UK.
January 22, 2010	Dr. David Sherman Seattle Biomedical Res. Inst., Seattle, U.S.A.
January 22, 2010	Prof. C.J. Dorman School of Genetics and Microbiology, Moyne Institute of Preventive Medicine, Trinity College, Dublin, Ireland.
January 22, 2010	Dr. John D. Mac Micking Section of Microbial Pathogenesis, Boyer Centre for Molecular Medicine, Yale University School of Medicine, New Haven, CT, U.S.A.
January 22, 2010	Dr. Thomas Griffith University of Lowa Hospitals and Clinics, Lowa, U.S.A.
January 22, 2010	Dr. Arsenio M Fialho Institute for Biotechnology and Bioengineering (IBB), Center for Biological and Chemical Engineering, Instituto Superior Tecnico, Portugal.
January 22, 2010	Dr. Barry R. Bloom Harvard School of Public Health, Boston, U.S.A.
January 22, 2010	Dr. Adrie JC Steyn Department of Microbiology, University of Alabama at Birmingham, U.S.A.
January 22, 2010	Prof. V. Nagaraja Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore, India.
January 22, 2010	Dr. Jaya Tyagi Dept. of Biotechnology, All India Institute of Medical Sciences, New Delhi, India.
January 22, 2010	Dr. V.K. Nandicoori Signal Transduction Lab, National Institute of Immunology, New Delhi, India.
January 22, 2010	Dr. Suman K. Dhar School of Molecular Medicine, Jawaharlal Nehru University, New Delhi, India.
January 22, 2010	Dr. Shobhona Sharma Tata Institute of Fundamental Research, Mumbai, India.
January 22, 2010	Dr. R. S. Gokhale Institute of Genomics and Integrative Biology, New Delhi, India.
January 22, 2010	Dr. Bhaskar Saha National Centre for Cell Science, Pune, India.
January 22, 2010	Dr. Seyed E. Hasnain University of Hyderabad, Hyderabad, India.
January 22, 2010	Dr. Kanury V.S. Rao International Centre for Genetic Engineering & Biotechnology, New Delhi, India.

January 22, 2010	Dr. Utpal Tatu Indian Institute of Science, Bangalore, India.
January 22, 2010	Dr. Amitabha Mukhopadhyay National Institute of Immunology, New Delhi, India.
January 22, 2010	Dr. Anil Tyagi University of Delhi South Campus, New Delhi, India.
January 22, 2010	Dr. Beenu Joshi National JALMA Institute for Leprosy and OMD, Agra, India.
January 22, 2010	Dr. Yogendra Singh Institute of Genomics and Integrative Biology, New Delhi, India.
January 22, 2010	Dr. Akash Ranjan Centre for DNA Fingerprinting & Diagnostics, Hyderabad, India.
January 22, 2010	Dr. Devinder Sehgal National Institute of Immunology, New Delhi, India.
January 22, 2010	Dr. V. M. Katoch Director General, Indian Council of Medical Research (ICMR), New Delhi, India.
January 22, 2010	Dr. Robin Mukhopadhyaya Tata Memorial Centre, Mumbai, India.
February 8, 2010	Dr. Takashi Itoh Japan Collection of Microorganisms, RIKEN Bio Resource Center, Saitama, Japan.
February 9, 2010	Dr. Hans-Jurgen Busse Institute of Bacteriology, Mycology and Hygiene, University of Veterinary Medicine, Veterinarplatz, Vienna.
February 10, 2010	Mr. Pat McFadden Member of Parliament, United Kingdom.
February 10, 2010	Dr. Aharon Oren, Department of Plant and Environmental Sciences, The Institute of Life Sciences, The Hebrew University of Jerusalem, Jeruslaem, Israel.
February 15, 2010	Dr. Gary Sayler, Director, Center for Environmental Biotechnology, Knoxville, TN, U.S.A.
May 10, 2010	Dr. Pankaj Soni Los Alamos National Laboratory, Bioscience Division, Los Alamos, NM, U.S.A.
June 23, 2010	Dr. U.S. Sudheeendra Hamilton College, N.Y., U.S.A.
August 2, 2010	Dr. Susmit Suvas Assistant Prof., Oakland University, Michigan, U.S.A.
August 23, 2010	Dr. Krishna Rao University of Texas Medical Branch, Galveston, TX, U.S.A.
September 1, 2010	Dr. Rupinder Kaur DNA Fingerprinting & Diagnostics, Hyderabad, India.
September 11, 2010	Dr. Rashi Iyer Los Alamos National Laboratory, Bioscience Division, Los Alamos, NM, U.S.A.
September 21, 2010	Prof. Hana Sychrova Department of Membrane Transport, Institute of Physiology, Academy of Sciences Videnska, Prague, Czech Republic.
October 7, 2010	Dr. S.N. Gupta I.I.S.E.R., Mohali, India.
October 19, 2010	Dr. Mayukh Sarkar University of Utah, Utah, U.S.A.
November 15, 2010	Prof. Nikolai Kolchanov, Russian Foundation for Basic Research (RFBR).
November 15, 2010	Dr. Yuri Matushkin Russian Foundation for Basic Research (RFBR).
November 15, 2010	Dr. Alexey Kochetov Russian Foundation for Basic Research (RFBR).
November 15, 2010	Dr. Vladimir.A. Ivanisenko Russian Foundation for Basic Research (RFBR).
November 15, 2010	Dr. Dmitry Afonnikov Russian Foundation for Basic Research (RFBR).
November 15, 2010	Dr. Alexander V. Efimov Russian Foundation for Basic Research (RFBR).
November 15, 2010	Prof. Alexis S. Ivanov Russian Foundation for Basic Research (RFBR).

November 15, 2010	Dr. Vseldov Makeev Russian Foundation for Basic Research (RFBR).
November 15, 2010	Dr. Alexey Kochetov Russian Foundation for Basic Research (RFBR).
November 15, 2010	Dr. Roman Efremov Russian Foundation for Basic Research (RFBR).
November 15, 2010	Prof. S.M. Deyev Russian Foundation for Basic Research (RFBR).
November 30, 2010	Dr. Saman Habib C.D.R.I., Lucknow, India.
December 12, 2010	Mrs. Dagmara Jankowska Bundesministerium für Bildung und Forschung, Bonn, Germany.
February 3, 2011	Dr. Vainav Patel NCI-Frederick, N.I.H., U.S.A.
February 13, 2011	Ms. Anke Trautwein, Bundesministerium für Bildung und Forschung, Bonn, Germany.
February 15, 2011	Dr. Pooja Arora Albert Einstein College of Medicine, Department of Microbiology and Immunology,Bronx, NY, U.S.A.
February 18, 2011	Dr. Deepak Sharma , Laboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, U.S.A.
March 8, 2011	Dr. Masahiro Takeo Dept of Material Science and Chemistry, University of Hyogo, Graduate School of Engineering, Himeji, Hyogo, Japan.
March 27, 2011	Dr. Hans-Peter Klenk DSMZ - German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7 B, Braunschweig, Germany.
March 27, 2011	Dr. Aravind L. Iyer NCBI, NLM, National Institutes of Health, Bethesda, MD, U.S.A.
March 27, 2011	Dr. Milind Mahajan, Yale Center for Genome Analysis, Yale University, Orange, CT, U.S.A.
March 27, 2011	Dr. George M. Garrity Biomedical & Physical Sciences Bldg, Michigan State University East Lansing, MI, U.S.A.
March 27, 2011	Prof. David S. Guttman Research Department of Cell & Systems Biology, University of Toronto, Toronto, ON, Canada.
March 27, 2011	Dan Knights University of Colorado, Colorado, U.S.A.
March 27, 2011	Dr. Mani Arumugam EMBL, Meyerhofstr, Heidelberg, Germany.

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conferences/seminars attended

Conferences/Seminars attended

Name	Date (from)	Date (to)	Name of Conference / Seminar/ Workshop	Venue, City, Country
Sekhar Majumdar	March 2, 2009	March 5, 2009	International symposium on DNA-Protein Transactions and 12th Transcription Assembly.	IMTECH, Chandigarh, India.
K. L. Dikshit	April 1, 2009	March 31, 2011	Gordon Research Conference on Microbial Stress Response.	Mount Holyoke, South Hadley, Massachettes, U.S.A.
Vijay Sonawane	April 4, 2009	April 16, 2009	Scientific Achievements of SC/ST Scientists and Technologists.	National Aerospace Laboratory, Bangalore, India.
Saumya Raychaudhuri	April 6, 2009	April 9, 2009	Emerging Infectious Diseases (EID) in the Pacific Rim Focused on Enteric Diseases.	Kolkata, India.
G.Rajamohan	April 9, 2009	April 9, 2009	8th Annual Research day.	Center for microbial interface biology, The Ohio State University, Columbus, U.S.A.
Javed N. Agrewala	April 27, 2009	May 2, 2009	Controlling Laboratory Biorisks Training Course.	Sandia National Laboratories, Albuquerque, U.S.A.
Harvinder Jassal	April 28, 2009	April 30, 2009	Transactional Analysis for Inter-Personal Skills Development.	Human Resource Development Centre, Ghaziabad, India.
G.Rajamohan	May 17, 2009	May 21, 2009	109th ASM General Meeting.	Philadelphia, Pennsylvania, U.S.A.
Garry Bedi	June 1, 2009	June 12, 2009	Technology Commercialization.	Administrative Staff College of India, Hyderabad, India.
Dibyendu Sarkar	July 26, 2009	July 31, 2009	Gordon Research Conference on Microbial Adhesion and Signal Transduction.	Salve Regina University, Newport.
Harvinder Jassal	July 31, 2009	July 31, 2009	Open Source Software	C-DAC, Mohali, India.

			Tools/Technologies Schedule.	
Girish Sahni	August 2, 2009	August 8, 2009	International Union of Biochemistry and Molecular Biology.	Shanghai, China.
Jagmohan Singh	September 1, 2009	September 5, 2009	Eukaryotic DNA Replication & Genome Manitenance.	Cold Spring Harbor Laboratory, New York, U.S.A.
K. Suresh	September 7, 2009	September 8, 2009	International Symposium of East Asian Young Scientists Follow-up Program.	Himeji-Shosha Campus, University of Hyogo, Japan.
Pawan Gupta	September 25, 2009	September 29, 2009	EMBO Conference on Nuclear Receptors.	Cavtat-Dubrovnik, Croatia.
Neeraj Khatri	October 1, 2009	October 1, 2009	Accreditation of Laboratory Animal Facilities, Quality Control and Quality Assurance Criteria.	National Institute for research in Reproductive Health, Mumbai, India.
Neeraj Khatri	October 1, 2009	October 3, 2009	Laboratory Animal Science: Applications in Biomedical Research.	National Institute for research in Reproductive Health, Mumbai, India.
Javed N. Agrewala	October 4, 2009	October 6, 2009	3rd Vaccine Congress.	Singapore.
Debendra K. Sahoo	November 4, 2009	October 6, 2009	EU-India S&T co-operation days	Jawaharlal Nehru University, New Delhi, India.
Neeraj Khatri	December 7, 2009	December 8, 2009	Vagyanik tatha Prashasnik Shabdawli.	IMTECH, Chandigarh, India.
Harvinder Jassal	December 11, 2009	December 12, 2009	Recent Trends in Patinformatics.	URDIP/NCL, Pune, India.
Debendra K. Sahoo	December 27, 2009	December 30, 2009	CHEMCON 2009 and 62nd Annual Session of Indian Institute of Chemical Engineers.	Andhra University, Vishakhapatnam, India.
Neeraj Khatri	January 15, 2010	January 15, 2010	National Conference of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).	Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.
Pawan Gupta	January 21, 2010	January 24, 2010	Understanding and	IMTECH, Chandigarh,

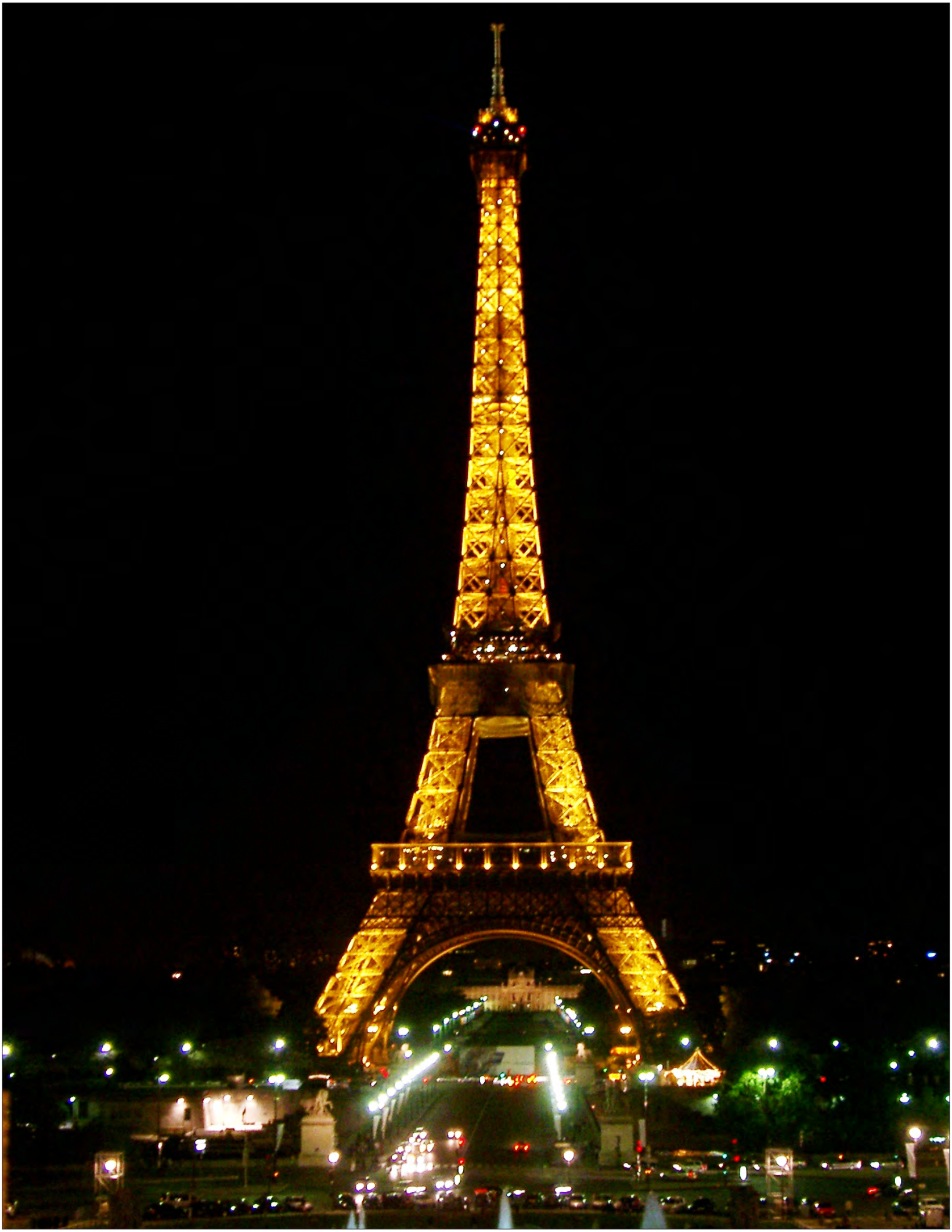
			Managing Pathogenic Microbes 2010.	India.
Alka Rao	January 22, 2010	January 24, 2010	Understanding and Managing Pathogenic Microbes.	IMTECH, Chandigarh, India.
Pushpa Agrawal	January 22, 2010	January 24, 2010	Understanding and Managing Pathogenic Microbes.	IMTECH, Chandigarh, India.
Charu Sharma	January 22, 2010	January 24, 2010	Understanding and Managing Pathogenic Microbes.	IMTECH, Chandigarh, India.
Sekhar Majumdar	January 22, 2010	January 24, 2010	Understanding and Managing Pathogenic Microbes.	IMTECH, Chandigarh, India.
K. L. Dikshit	January 22, 2010	January 24, 2010	Understanding and Managing Pathogenic Microbes.	IMTECH, Chandigarh, India.
Manoj Kumar	February 6, 2010	February 6, 2010	Intellectual Property Rights Awareness.	PHD Chamber of Commerce and Industries, Sector 31-A, Chandigarh.
K. L. Dikshit	February 6, 2010	February 6, 2010	Emerging Trends in Globin Research: need to imbibe new approaches and technologies.	Delhi University, South Campus, New Delhi, India.
K. Ganesan	February 7, 2010	February 7, 2010	Characterization of Microbial Communities Associated with Polluted Environments using Polyphasic Approach.	IMTECH, Chandigarh, India.
Charu Sharma	February 17, 2010	February 17, 2010	Symposium on Frontiers in Biotechnology.	Panjab University, Chandigarh, India.
Garry Bedi	February 22, 2010	February 23, 2010	IP Protection and Management Issues.	H.R.D.C., Ghaziabad, India.
Manoj Raje	April 10, 2010	April 10, 2010	Workshop on Application of the Transmission Electron Microscope in Advance Nanobiology and Life Sciences.	I.H.B.T., Palampur, India.
Dibyendu Sarkar	May 31, 2010	May 31, 2010	Jacques Monod Commemorative Mini symposium on Gene Expression and Signaling	Institut Pasteur, Paris, France.

			in bacteria.	
Pawan Gupta	June 13, 2010	June 18, 2010	FASEB Summer Research Conference on Retinoids.	Carefree, Arizona, U.S.A.
Harvinder Jassal	June 14, 2010	June 20, 2010	Cyber Laws, Information Security and Computers for Scientists and Technologists.	Indian Institute of Public Administration, New Delhi, India.
K. L. Dikshit	July 17, 2010	July 23, 2010	Gordon Research Conference on Microbial Stress Response.	Mount Holyoke College in South Hadley Massachusettes, U.S.A.
K. L. Dikshit	August 18, 2010	August 22, 2010	XVI International Conference on Oxygen Binding and Sensing Proteins.	University of Antwerp, Antwerp, Belgium.
Debendra K. Sahoo	September 14, 2010	September 18, 2010	14th International Biotechnology Symposium and Exhibition.	Palacongressi, Riminy, Italy.
Garry Bedi	September 27, 2010	September 28, 2010	Accelerated Commercialization of Technology and Innovation-Technology Transfer (ACTIV-TT).	Venture Center, NCL Innovation Park, Pune, India.
Ashish	September 30, 2010	October 1, 2010	Accelerated Commercialization of Technology and Innovation (ACTIV-SR).	Venture Center, NCL, Pune India.
Girish Sahni	September 30, 2010	October 1, 2010	Accelerated Commercialization of Technology and Innovation (ACTIV-SR).	Venture Center, NCL Innovation Park, Pune, India.
P. B. Patil	October 11, 2010	October 12, 2010	National Consultative Meet on Bioinformatics in Horticulture.	Indian Institute of Spice Research, Calicut, Kerala, India.
G. S. Prasad	October 12, 2010	October 16, 2010	International Symposium of Conservation and Sustainable Use of Asian Microbial Resources.	National Institute of Technology and Evaluation, Chiba, Japan.
S. Karthikeyan	October 25, 2010	October 27, 2010	39th National Seminar on Crystallography.	University of Jammu, Jammu, India.
Pradip K. Chakraborti	October 25, 2010	October 28, 2010	NDP Kinase/Nm23/awd Family-From Basic Science to Clinical	Heidelberg, Germany.

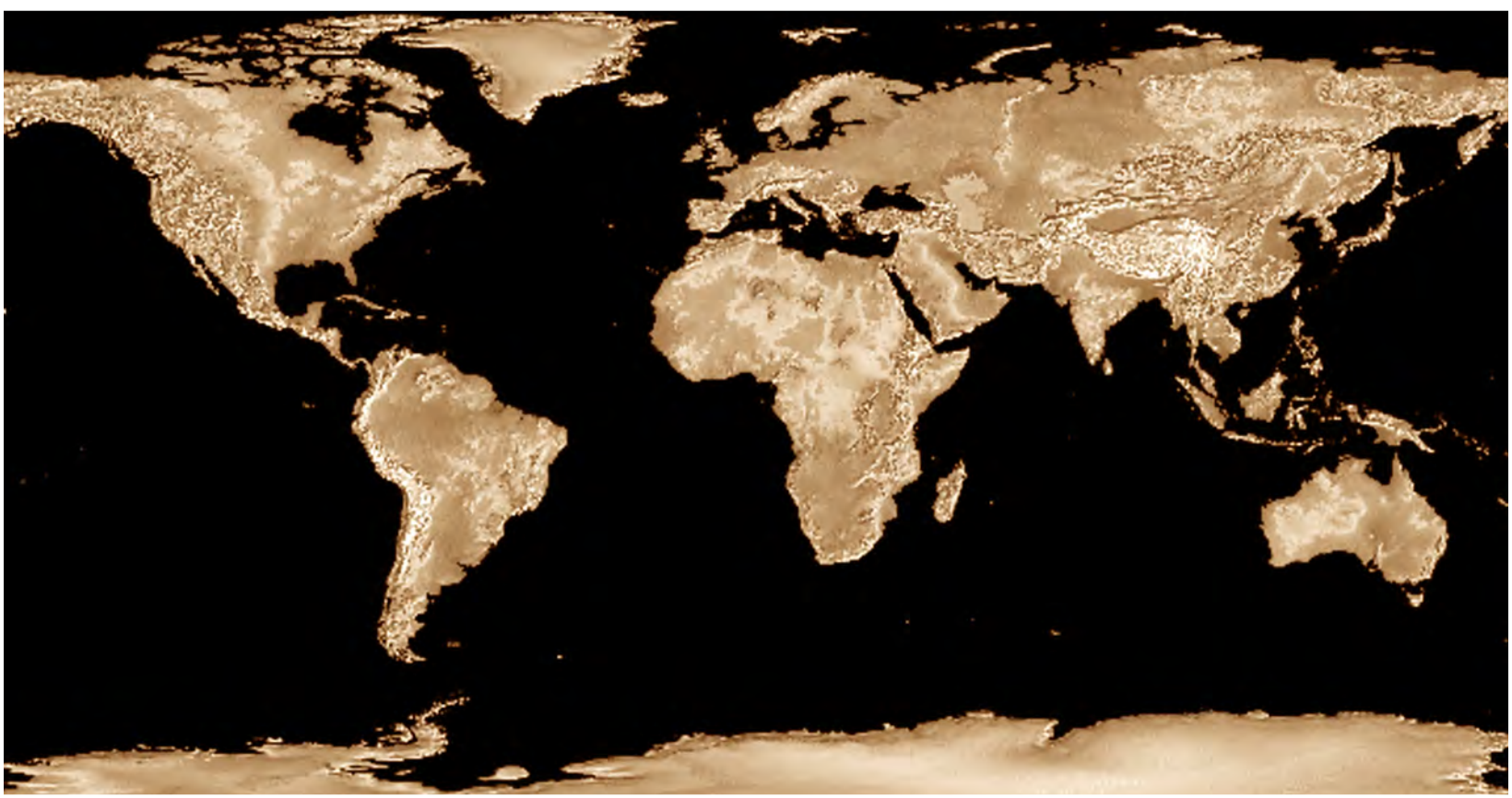
			Application.	
Pawan Gupta	November 3, 2010	November 7, 2010	American Society of Tropical Medicine and Hygiene.	Atlanta, U.S.A.
Pushpa Agrawal	November 12, 2010	November 14, 2010	Genomic Sciences 2010 and VII Convention of the Biotech Research Society.	Madurai Kamraj University, Madurai, India.
Srikrishna Subramanian	November 15, 2010	November 19, 2010	Indo-Russian workshop on Predictive Biology using Systems and Integrative analysis and methods.	IMTECH, Chandigarh, India.
Balvinder Singh	November 19, 2010	November 19, 2010	Indo-Russian workshop Predictive Biology using Systems and Integrative analysis and methods.	IMTECH, Chandigarh, India.
Charu Sharma	November 30, 2010	December 3, 2010	French-Indian Inter-Academic Symposium on Infectious Diseases.	National Institute of Immunology, New Delhi, India.
Jagmohan Singh	December 4, 2010	December 6, 2010	International Symposium on Chromosome/Chromatin Dynamics: Epigenetics and Disease.	Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India.
Shanmugam Mayilraj	December 8, 2010	December 10, 2010	Soil Metagenomics-Implications of Next-Generation Sequencing Technologies on Microbial Diversity Research and soil ecology.	Johann Heinrich von Thunen Institute (vTI), Institute for Biodiversity, Braunschweig, Germany.
Girish Sahni	December 8, 2010	December 10, 2010	Soi Metagenomics : Implications of next - generation sequencing technologies on microbial diversity research & soil ecology.	Brauschweig, Germany.
Alka Rao	December 16, 2010	December 17, 2010	International Symposium on Tuberculosis Diagnostics: Innovating to Make an Impact.	I.C.G.E.B., New Delhi, India.
Pushpa Agrawal	December 16, 2010	December 17, 2010	International Symposium on Tuberculosis Diagnostics: Innovating to make an Impact.	I.C.G.E.B., New Delhi, India.

Ashwani Kumar	December 16, 2010	December 17, 2010	International Symposium on Tuberculosis Diagnostics: Innovating to make an Impact.	I.C.G.E.B., New Delhi, India.
Charu Sharma	December 16, 2010	December 17, 2010	International Symposium on Tuberculosis Diagnostics: Innovating to make an Impact.	I.C.G.E.B., New Delhi, India.
Sekhar Majumdar	December 16, 2010	December 17, 2010	International Symposium on Tuberculosis Diagnostics: Innovating to make an Impact.	I.C.G.E.B., New Delhi, India.
Garry Bedi	December 20, 2010	December 20, 2010	CSIR Enterprise Transformation- ICT Intervention Project.	C.S.I.O., Sector 30, Chandigarh.
P. B. Patil	January 19, 2011	January 24, 2011	Hands on NGS (Next Generation Sequencing).	Genotypic Technology, Bangalore, Karnataka, India.
G. S. Prasad	January 24, 2011	January 26, 2011	International Multi-Stakeholder Expert Dialogue on Access and Benefit-Sharing for Genetic Resources (GR) for Food and Agriculture.	University of Catholique de Louvain, Brussels, Belgium.
Neeraj Khatri	January 28, 2011	January 29, 2011	New Paradigms in Laboratory Animal Science in an Era of Advanced Biomedical Research.	Indian Veterinary Research Insitute, Izatnagar, U. P., India.
Ashwani Kumar	January 30, 2011	February 1, 2011	Redox status and control in TB:From basic research to drug development.	C.D.F.D., Hyderabad, India.
K. L. Dikshit	January 30, 2011	February 1, 2011	Indo-Canadian workshop on Redox Status and control in tuberculosis.	C.D.F.D., Hyderabad, India.
Ashwani Kumar	February 5, 2011	February 5, 2011	Biotechnology: Expanding Horizons.	G.G.D.S.D. College, Chandigarh, India.
Javed N. Agrewala	February 7, 2011	February 10, 2011	3rd International Conference on Drug Discovery and Therapy.	Dubai. U.A.E.
Ashish	February 8, 2011	February 11, 2011	Harnessing Intellectual Property for Strategic Competitive and	Indian Institute of Management (IIM) Ahmedabad, India.

			Collaborative Advantage.	
Neeraj Khatri	February 15, 2011	February 16, 2011	Pre-Conference Workshop on Canine Echocardiography & Telemetry (Accredited by RACE, U.S.A.	P.D.D.U.P.C.V.V.E.G.A.S. Mathura, U. P., India.
Neeraj Khatri	February 17, 2011	February 18, 2011	IV th International Conference on "The Challenges Ahead".	P.D.D.U.P.C.V.V.E.G.A.S. Mathura, U. P., India.
Kumaran	February 21, 2011	February 24, 2011	Indo-US symposium on modern trends in molecular structure.	IITB Powai, Mumbai, India.
Krishan Gopal	February 21, 2011	February 25, 2011	Research methodology and statistical methods - Designing for breakthroughs.	H.R.D.C., Ghaziabad, India.
Debendra K. Sahoo	March 21, 2011	March 23, 2011	World Congress on Biotechnology.	Hyderabad, India.
Rajagopal	March 28, 2011	March 30, 2011	International workshop on Computational aspects of working with genomes.	IMTECH, Chandigarh, India.
Alka Rao	March 28, 2011	March 30, 2011	International workshop on Computational aspects of working with genomes .	IMTECH, Chandigarh, India .
Manoj Kumar	March 28, 2011	March 30, 2011	International workshop on Computational aspects of working with genomes.	IMTECH, Chandigarh, India.
E. Senthil Prasad	March 28, 2011	March 30, 2011	International workshop on Computational aspects of working with genomes.	IMTECH, Chandigarh, India.
Srikrishna Subramanian	March 28, 2011	March 30, 2011	International workshop on Computational aspects of working with genomes.	IMTECH, Chandigarh, India.
Ramya T.N.C.	March 28, 2011	March 30, 2011	International workshop on Computational aspects of working with genomes.	IMTECH, Chandigarh, India.



visits abroad



visits abroad

Visits Abroad

Name	Date (from)	Date (to)	Venue, City, Country
G. Rajamohan	September 6, 2007	September 5, 2009	The Ohio State University, Columbus, U.S.A.
J. N. Agrewala	April 27, 2009	May 2, 2009	Sandia National Laboratories, Albuquerque, U.S.A.
S. Karthikeyan	July 20, 2009	July 27, 2009	ESRF, Grenoble, France.
Dibyendu Sarkar	July 26, 2009	July 31, 2009	Salve Regina University, Newport, U.S.A.
Girish Sahni	August 2, 2009	August 13, 2009	Shanghai-Wuhan-Hefei, China.
Jagmohan Singh	August 22, 2009	September 7, 2009	Cold Spring Harbor Laboratory, New York, U.S.A.
S. Kumaran	August 30, 2009	September 4, 2009	Weizmann Institute of Science, Rehovot, Israel.
K. Suresh	September 5, 2009	September 10, 2009	University of Hyogo, Himeji, Japan.
Pawan Gupta	September 25, 2009	September 29, 2009	Cavtat-Dubrovnik, Croatia.
Girish Sahni	October 3, 2009	October 10, 2009	Nostrum Pharmaceuticals Inc. Edison, NJ, U.S.A.
J. N. Agrewala	October 4, 2009	October 6, 2009	3rd Vaccine Congress-2009, Singapore.
Ashish	March 15, 2010	March 23, 2010	Brookhaven National Lab, Upton, NY, U.S.A.
Girish Sahni	April 11, 2010	April 21, 2010	Nostrum Pharmaceuticals, New York-Chicago-New Jersey, U.S.A.
K. L. Dikshit	April 15, 2010	April 22, 2010	University of Barcelona, Barcelona, Spain.
C. R. Suri	April 26, 2010	May 5, 2010	Moscow, Russia.
Dibyendu Sarkar	May 30, 2010	June 1, 2010	Institut Pasteur, Paris, France.
Pawan Gupta	June 13, 2010	June 18, 2010	Carefree, Arizona, U.S.A.
Girish Sahni	July 1, 2010	July 7, 2010	Cairo, Egypt
K. L. Dikshit	July 18, 2010	July 23, 2010	Mount Holyoke, South Hadley, MA, U.S.A.

K. L. Dikshit	August 18, 2010	August 22, 2010	University of Antwerp, Antwerp, Belgium.
C. R. Suri	August 22, 2010	August 27, 2010	Moscow, Russia.
Srikrishna Subramanian	September 5, 2010	September 12, 2010	Sanford Burnham Medical Research Institute, La Jolla, CA, U.S.A.
Debendra K. Sahoo	September 12, 2010	September 20, 2010	14th International Biotechnology Symposium, Riminy, Italy.
Girish Sahni	September 18, 2010	September 22, 2010	Jakarta, Indonesia.
G. S.Prasad	October 12, 2010	October 16, 2010	National Institute of Technology and Evaluation (NITE), Chiba, Japan.
Pradip K. Chakraborti	October 25, 2010	October 28, 2010	Heidelberg, Germany.
Pawan Gupta	November 3, 2010	November 7, 2010	Atlanta, U.S.A.
Ashish	November 9, 2010	November 18, 2010	Brookhaven National Lab, Upton, NY , U.S.A.
Girish Sahni	December 4, 2010	December 12, 2010	Gatersleben-Braunschweig, Germany.
Shanmugam Mayilraj	December 6, 2010	December 7, 2010	German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany.
G. S. Prasad	January 24, 2011	January 26, 2011	University of Catholique de Louvain, Brussels, Belgium.
J. N. Agrewala	Feburary 7, 2011	Feburary 10, 2011	3rd International Conference on Drug Discovery and Therapy, Dubai. U.A.E.
Girish Sahni	February 26, 2011	March 9, 2011	Nostrum Pharmaceuticals, New Jersey, U.S.A.
Ashish	March 27, 2011	April 4, 2011	Brookhaven National Lab, Upton, NY, U.S.A.

Ph.D. degrees awarded

Ph.D. degrees awarded

Ph.D. Degrees Awarded

S.No.	Name of the Research Fellow	Year	Name of the Supervisor
1	Ms. Sukhwinder Kaur	8.4.09	Dr. Grish C. Varshney
2	Mr. Arshad Jawed	24.4.09	Dr. D. K. Sahoo
3	Mr. Ajit Tiwari	18.5.09	Dr. Grish C. Varshney
4	Ms. Jaspreet Kaur	22.6.09	Dr. Anand Bachhawat
5	Mr. Kumar Rajesh	16.7.09	Dr. C. R. Suri
6	Ms. Divya Kapoor	12.8.09	Dr. P. Guptasarma
7	Manzoor A Mir	22.10.09	Dr. Javed N. Agrewala
8	Loveena	30.10.09	Dr. Sekhar Majumdar
9	Sneh Lata	6.11.09	Dr. G. P. S. Raghava
10	Janmejay Pandey	18.12.09	Dr. R. K. Jain
11	Puja Saluja	22.12.09	Dr. G.S . Prasad
12	Kishore Kumar Joshi	4.1.10	Dr. Girish Sahni
13	Neha Kasturia	8.1.10	Dr. Anand K. Bachhawat
14	Shilpy Srivastava	25.1.10	Dr. Grish C. Varshney
15	Shekhar Kumar	10.2.10	Dr. Girish Sahni
16	Amin-ul-Mannan	10.2.10	Dr. K. Ganesan
17	Ashok Kumar	16.2.10	Dr. Jagmohan Singh
18	Netrapal Meena	15.3.10	Dr. Alok Mondal
19	Richa Bajpai	12.4.10	Dr. Pushpa Agrawal
20	Mitesh Dongre	7.5.10	Dr. Saumya Ray Chaudhuri
21	Ashu Shah	12.5.10	Dr. Grish C. Varshney
22	Amit Bhattacharya	9.7.10	Dr. Tapan Chakraborti
23	Arvind Anand	18.6.10	Dr. K. L. Dikshit
24	Lomary S. Moon	19.7.10	Dr. R. S. Jolly
25	Rachna Aneja	11.8.10	Dr. G. Sahni
26	Sushma Sharma	3.9.10	Dr. K. Ganesan
27	Manish Datt	12.10.10	Dr. Balvinder Singh
28	Akesh Sinha	25.10.10	Dr. Dibyendu Sarkar
29	Archana Chauhan	27.10.10	Dr. R. K. Jain
30	Swati Haldar	15.11.10	Dr. Jagmohan Singh
31	Haider H. Dar	29,11.10	Dr. Pradip K. Chakraborti
32	Pankaj Kumar Chauhan	7.12.10	Dr. S. Karthikeyan
33	Shweta Jain	24.12.10	Dr. Javed N. Agrewala
34	Vijender Singh	24.12.10	Dr. Javed N. Agrewala
35	Anuj Pathak	21.2.11	Dr. Dibyendu Sarkar
36	Firoz Ahmed	4.4.11	Dr. G. P. S. Raghava
37	Nigam Kumar	4.2.11	Dr. R. Kishore
38	Mamoon Rashid	7.4.11	Dr. G. P. S. Raghava
39	Nitish Kumar Mishra	3.3.11	Dr. G. P. S. Raghava
40	Pavitra Kanudia	29.4.11	Dr. Pradip K. Chakraborti

institute committees

institute committees

Research Council (10 May 2007- 31 March 2010)

Prof. G. Padmanaban Distinguished Biotechnologist, Department of Biochemistry, Indian Institute of Science, Bangalore-560012.	Chairman
Prof. A. Surolia Director, National Institute of Immunology, JNU Campus, Shaeed Jeet Singh Marg, New Delhi-110067.	External Member
Prof. P. Rama Rao Director, NIPER, Sector 67, Mohali, S.A.S. Nagar-160062.	External Member
Dr. J. Gowrishankar Director, Centre for DNA Finger Printing & Diagnostics, Nacharam ECIL Road, Nacharam, Hyderabad-500076.	External Member
Prof. Umesh Varshney Professor, Department of Microbiology & Cell Biology, Indian Institute of Science, Bangalore-560012.	External Member
Dr. V. S. Bisaria Professor, Department of Biochemical Engineering & Biotechnology, Indian Institute of Technology, Haus Khas, New Delhi-110016.	External Member
Dr. M.D. Nair Former Vice President, SPIC (Southern Petroleum Chemical Industries, Chennai) & Consultant to Pharmaceutical Industry, A-11, Sagarika, 15, 3rd Seaward Road, Valmaki Nagar, Thiruvanmiyar, Chennai-600041.	External Member
Nominee of Secretary Department of Biotechnology, CGO Complex, Lodi Road, New Delhi-110003.	Member (Agency Representative)
Dr. J. S. Yadav Director, Indian Institute of Chemical Technology, Uppal Road, Hyderabad- 50000.	Sister Lab
Dr. K. C. Gupta Director, IITR, Lucknow.	D.G.'s Nominee
Dr. Girish Sahni Director, Institute of Microbial Technology, Sector 39-A, Chandigarh-160036.	Director
Head or his representative R&D Planning Division, CSIR, Rafi Marg, New Delhi-110001.	Permanent Invitee

Research Council (17 May 2010 - 31 March 2013)

Prof. G. Padmanaban Distinguished Biotechnologist, Deptt. of Biochemistry, Indian Institute of Science, Bengaluru - 560012.	Chairman
Dr. N. K. Ganguly Advisor, Translational Health Science & Technology, National Institute of Immunology, Aruna Asaf Ali, New Delhi - 110067.	Member
Prof. A. Surolia Director, National Institute of Immunology, JNU Campus, Shaeed Jeet Singh Marg, New Delhi - 110067.	Member
Prof. T. P. Singh Professor, Dept of Biophysics, AIIMS, Ansari Nagar, New Delhi - 110029.	Member
Prof. V. Nagaraj Professor, Dept. of Microbiology, Indian Institute of Science, Bengaluru - 560012.	Member
Prof. A. M. Lali Professor, Dept of Chemical Engineering, Institute of Chemical Technology, Mumbai University, Mumbai.	Member
Prof. N. K. Mehra Head Dept. of Transplantation Immunology, & Immuno - Genetics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110029.	Member
Dr. R.R. Sinha Adviser, Department of Biotechnology, CGO Complex, Lodi Road, New Delhi - 110003.	Agency Representative
Dr. Rajesh Gokhale Director, Institute of Genomics of Integrative, Biology University Campus, Mail Road, Delhi - 110007.	DG Nominee
Dr. K.C. Gupta Director, Indian Institute of Toxicology Research, Mahatma Gandhi Marg, Post Box. No. 80, Lucknow - 226001.	Sister Laboratory
Prof. Siddartha Roy Director, Indian Institute of Chemical Biology, 4, Raja SC Mullick Road, Jadavpur, Kolkata - 700032.	Cluster Director
Dr. Girish Sahni Director, Institute of Microbial Technology, Sector 39-A, Chandigarh - 160036.	Director (Member Secy.)
Head or his Nominee (Shri R.P. Singh/Dr. Vandana Bisht), Council of Scienctific & Industrial Research, Anusandhan Bhawan, 2, Rafi Marg, New Delhi - 110001.	Permanent Invitee

Management Council

1 July, 2007 - 31 December, 2009

Dr. Girish Sahni Director, IMTECH, Chandigarh.	Chairman
Dr. P. S. Ahuja Director, IHBT, Palampur.	Member
Dr. R. S. Jolly Scientist, IMTECH, Chandigarh.	Member
Dr. D. K. Sahoo Scientist, IMTECH, Chandigarh.	Member
Dr. G. P. S. Raghava Scientist, IMTECH, Chandigarh.	Member
Dr. Balvinder Singh Scientist, IMTECH, Chandigarh.	Member
Sh. A. K. Goel Superintending Engineer, IMTECH, Chandigarh.	Member
Sh. Rajendra Soni Scientist & Head, PTM, IMTECH, Chandigarh.	Member
Sh. Brijesh Kumar Finance & Accounts Officer, IMTECH, Chandigarh.	Member (Ex - Officio)
Sh. S.K. Sadana Controller of Administration, IMTECH,Chandigarh.	Member Secretary (Ex - Officio)

1 January, 2010 - 31 December, 2011

Dr. Girish Sahni Director, IMTECH, Chandigarh.	Chairman
Dr. Pawan Kapur Director, CSIO, Chandigarh.	Member
Dr. R.S. Jolly Scientist - G, IMTECH, Chandigarh.	Member
Dr. Javed N Agrewala Scientist-EII, IMTECH, Chandigarh.	Member
Dr.Ashwani Kumar Scientist, IMTECH, Chandigarh.	Member
Dr. (Ms) Charu Sharma Scientist - EI, IMTECH, Chandigarh.	Member
Sh. A.K.Goel Sr. Supdt. Engineer, IMTECH, Chandigarh.	Member
Sh. Rajendra Soni Scientist - EII, IMTECH, Chandigarh.	Member
Sh. Brijesh Kumar Finance & Accounts Officer, IMTECH, Chandigarh.	Member (Ex - Officio)
Sh. Chhering Tobden Administrative Officer, IMTECH, Chandigarh.	Member (Secretary)

institute staff

institute staff

Scientific Staff

S.No.	Name	Designation
1	Dr. Girish Sahni	Director
2	Dr. R. S. Jolly	Chief Scientist
3	Dr. P. K. Chakraborti	Chief Scientist
4	Dr. K.L. Dikshit	Chief Scientist
5	Dr. Jagmohan Singh	Chief Scientist
6	Dr. Javed N. Agrewala	Chief Scientist
7	Mr. C. H. Premani	Senior Principal Scientist
8	Dr. G. C. Varshney	Senior Principal Scientist
9	Dr. C. R. Suri	Senior Principal Scientist
10	Dr. Pushpa Agrawal	Senior Principal Scientist
11	Dr. Manoj Raje	Senior Principal Scientist
12	Dr. D. K. Sahoo	Senior Principal Scientist
13	Dr. G. S. Prasad	Senior Principal Scientist
14	Dr. K. Ganesan	Senior Principal Scientist
15	Dr. Alok Mondal	Senior Principal Scientist
16	Dr. Swaranjit Singh	Senior Principal Scientist
17	Dr. G. P. S. Raghava	Senior Principal Scientist
18	Dr. Ananthapadmanaban	Senior Principal Scientist
19	Mr. Kuljit Singh Sodhi	Senior Principal Scientist
20	Dr. Kishore Raghuvansh	Senior Principal Scientist
21	Dr. Sekhar Majumdar	Senior Principal Scientist
22	Dr. V. C. Sonawane	Principal Scientist
23	Mr. Rajendra Soni	Principal Scientist
24	Dr. G. Rajamohan	Principal Scientist
25	Dr. S. Mayil Raj	Principal Scientist

26	Dr. S. Karthikeyan	Principal Scientist
27	Dr. K. Rajagopal	Principal Scientist
28	Dr. Saumya Ray Chaudhuri	Principal Scientist
29	Dr. Srikrishna Subramanian	Principal Scientist
30	Dr. Hemraj S. Nandanwar	Senior Scientist
31	Dr. Dibyendu Sarkar	Senior Scientist
32	Dr. Balvinder Singh	Senior Scientist
33	Dr. Charu Sharma	Senior Scientist
34	Dr. Pawan Gupta	Senior Scientist
35	Dr. S. Kumaran	Senior Scientist
36	Dr. Ashish	Senior Scientist
37	Mr. Anirban Roy Choudhury	Senior Scientist
38	Dr. Pradip Sen	Senior Scientist
39	Dr. Ashwani Kumar	Senior Scientist
40	Dr. Ramya T.N.C.	Senior Scientist
41	Dr. Neeraj Khatri	Scientist
42	Dr. Manoj Kumar	Scientist
43	Mr. Garry Bedi	Scientist
44	Dr. Belle Damodara Shenoy	Scientist
45	Dr. Suresh Korpole	Scientist
46	Mr. E. Senthil Prasad	Scientist
47	Dr. Alka Rao	Scientist
48	Dr. P. Anil Kumar	Scientist
49	Dr. Prabhu B. Patil	Scientist
50	Mr. Kailash T. Bhamare	Scientist
51	Dr. Krishan Gopal	Scientist
52	Dr. Mani Shankar Bhattacharyya	Scientist
53	Mr. Harvinder Jassal	Junior Scientist

Administrative Staff

Administrative Staff

Sr.No.	Name	Designation
Administrative Staff		
1	Mr. Chhering Tobden	Administrative Officer
2	Mr. Parag Saxena	SO (G)
3	Mr. S.D. Rishi	SO (G)
4	Ms. Navneet Anand	Hindi Officer
5	Mr. Dhiraj Goswami	Security Officer
6	Mr. Bhagirath	Assistant (G)Gr.I
7	Mr. Gopal Krishan	Assistant (G)Gr.I
8	Mr. Gurdeep Singh	Assistant (G)Gr.I
9	Mr. Raghu Nath	Assistant (G)Gr.I
10	Mr. Sanjeev K Yadav	Assistant (G)Gr.I
11	Mr. Virender Lamba	Assistant (G)Gr.I
12	Mr. Arun Kumar	Assistant (G)Gr.II
13	Mr. Balwinder Singh	Assistant (G)Gr.II
14	Ms. Kuldeep Kaur	Assistant (G)Gr.II
15	Mr. Rajinder Nautiyal	Assistant (G)Gr.II
Finance and Accounts		
16	Mr. Brijesh Kumar	FAO
17	Mr. Surinder K Narad	SO (F&A)
18	Ms. Bimla	Assistant (F&A)Gr.I
19	Ms. Sudesh Sharma	Assistant (F&A)Gr.I
20	Mr. Raju Bansal	Assistant (F&A)Gr.I
21	Mr. Byomkesh Pandey	Assistant (F&A)Gr.I
22	Ms. Rani Devi	Assistant(F&A)Gr.II
Store & Purchase		
23	Mr. K.K. Bharat	SPO
24	Mr. Rajinder Singh Puri	SO (SP)
25	Mr. Kamal Kumar	Assistant (S&P)Gr.I
26	Mr. Bir Singh	Assistant (S&P)Gr.II

27	Ms. Tajinder Kaur	Assistant (S&P) Gr.III
Stenographic Cadre		
28	Ms. Shashi Batra	Private Secretary
29	Mr. Avtar Singh	Private Secretary
30	Ms. Surinder Passi	Private Secretary
31	Ms. Satya Gupta	Private Secretary
32	Mr. Ashok Kumar Batta	Sr. Stenographer
33	Mr. Sushil Kumar	Sr. Stenographer(ACP)
34	Ms. Baljit Kaur	Jr. Stenographer(ACP)
35	Ms. Kavita Kumari	Jr. Stenographer(ACP)
36	Ms. Manoj Rani	Jr. Stenographer
37	Mr. Praveen Kumar	Jr. Stenographer
38	Mr. Ravinder Singh	Jr. Stenographer
39	Ms. Manju Saini	Jr. Stenographer
Guest House		
40	Mr. Madan Mohan Parmanik	Guest Room Attendant
Canteen		
41	Mr. Bhupal Singh	Tea/Coffee Maker (ACP)
Staff Car Driver		
42	Mr. Ram Pal Singh	Staff Car Driver
43	Mr. Jaswant Singh	Staff Car Driver
44	Mr. Dharminder Kumar	Staff Car Driver
Group D (NT)		
45	Mr. R.N. Manjhi	Peon
46	Mr. Ralla Ram Dogra	Peon
47	Mr. Gian Singh	Security Guard
48	Mr. Jitender Singh	Security Guard
49	Mr. Raj Pal	Safaiwala (ACP)
50	Mr. Suresh	Safaiwala (ACP)
51	Ms. Sheela Devi	Safaiwala

Technical Staff

S.No.	Name	Designation
1	Mr. A. K. Goel	Senior Superintending Engineer Group III (7)
2	Dr. (Mrs.) Lata Verma	Senior Technical Officer (2)
3	Mr. Rajendra Dass	Senior Technical Officer (2)
4	Mr. S. S. Bawa	Senior Technical Officer (2)
5	Mr. R. N. Bansal (Library Officer)	Senior Technical Officer (1)
6	Mr. H. B. K. Bhatti	Senior Technical Officer (1)
7	Dr. Raj Kumar Mehta	Senior Technical Officer (1)
8	Mr. Dhan Parkash	Senior Technical Officer (1)
9	Ms. Paramjit Kaur	Senior Technical Officer (1)
10	Mr. Samir K. Nath	Senior Technical Officer (1)
11	Mr. Dawinder Singh	Technical Officer
12	Mr. R. K. Kanojia	Technical Officer
13	Mr. Muthu Krishnan	Technical Officer
14	Dr. K.P.S. Sengar (Library Officer)	Technical Officer
15	Mr. Deepak Bhatt	Technical Assistant
16	Mr. Davinder Singh	Technical Assistant
17	Mr. Harminder Singh	Technical Assistant
18	Ms. Chetna	Technical Assistant
19	Mr. Surjeet Singh	Technical Assistant
20	Mr. Paramjit Lal	Technical Assistant
21	Mr. Sandeep Kumar-I	Technical Assistant
22	Mr. Sandeep Kumar-II	Technical Assistant
23	Mr. Anil Chaudhary	Technical Assistant
24	Mr. Pradeep Kumar Patel	Technical Assistant
25	Mr. Vineet Kumar	Technical Assistant

26	Mr. Asheesh Kumar Khare	Technical Assistant
27	Mr. Sumit Mittal	Technical Assistant
28	Mr. Hariom Kushwaha	Technical Assistant
29	Mr. Bhupinder Singh Chopra	Technical Assistant
30	Ms. Anjali Koundal	Technical Assistant
31	Ms. Abha Shukla	Technical Assistant
32	Dr. Subhash Pawar	Technical Assistant
33	Mr. G.Senthil Kumar	Technical Assistant
34	Mr. Ajay Kumar	J.E. Group III (2)
35	Mr. Nitin Sharma	J.E. Group III (1)
36	Mr. Bhupinder Kumar	Senior Technician (2)
37	Mr. Paramjeet	Senior Technician (2)
38	Mr. Desh Raj Agnihotri	Senior Technician (2)
39	Mr. Raj Kumar-1	Senior Technician (1)
40	Mr. Harendra Singh Bisht	Senior Technician (1)
41	Mr. Kewal Krishan (Plumber)	Senior Technician (1)
42	Mr. Anil Kumar Sharma (Cataloguer)	Senior Technician (1)
43	Mr. Anil Theophilus	Senior Technician (1)
44	Mr. Bhimi Ram (Library Assistant)	Senior Technician (1)
45	Mr. Rohtas Ranga (AC Operator)	Senior Technician (1)
46	Mr. Upendra Singh	Senior Technician (1)
47	Mr. Maman Chand	Senior Technician (1)
48	Mr. Surinder Singh	Senior Technician (1)
49	Mr. Malkit Singh	Senior Technician (1)
50	Mr. Selvan	Senior Technician (1)
51	Mr. Raj Kumar-II	Senior Technician (1)
52	Ms.Sharanjit Kaur	Senior Technician (1)
53	Mr. Md. Mustafa	Senior Technician (1)
54	Ms. Vir Kanta Sharma	Senior Technician (1)
55	Mr. Jankey Prasad	Senior Technician (1)

56	Mr. Jaideep Mehta	Senior Technician (1)
57	Mr. Ashok Kumar	Senior Technician (1)
58	Mr. Ramesh Chander Sharma	Senior Technician (1)
59	Mr. Rakesh Kumar Dhiman	Technician (2)
60	Ms. Girja Kumari Ditya	Technician (2)
61	Ms. Renu	Technician (1)
62	Mr. Chander Parkash Midha	Technician (1)
63	Mr. Dinesh Kumar	Technician (1)
64	Mr. Randeep Sharma	Technician (1)
65	Mr. Manoj Kumar	Technician (1)
66	Ms. Neha Rana	Technician (1)
67	Ms. Harjeet Kaur	Technician (1)
68	Mr. Ramesh Singh	Lab Assistant
69	Mr. Prem Chand	Lab Assistant
70	Mr. Md. Younus	Lab Assistant
71	Mr. Vinod Kumar	Lab Attendant (2)

Recently Joined Staff

Sr.No.	Name	Designation	Date of Joining
1.	Sh. Sumit Mittal	Technical Assistant	01.04.2009
2.	Sh. Dhiraj Goswami	Security Officer	21.08.2009
3.	Dr. (Mrs.) Alka Rao	Scientist	20.11.2009
4.	Dr. Pinnaka Anil Kumar	Scientist	04.12.2009
5.	Dr. Prabhu B Patil	Scientist	21.12.2009
6.	Dr. (Mrs.) Ankita S Jaiswal	Scientist	22.03.2010
7.	Ms. Anjali Koundal	Technical Assistant	07.04.2010
8.	Dr. Ashwani Kumar	Senior Scientist	04.06.2010
9.	Dr. Srikrishna Subramanaian	Principal Scientist	27.09.2010
10.	Dr. (Mrs.) Ramya T.N.C	Senior Scientist	27.09.2010
11.	Ms. Abha Shukla	Technical Assistant	20.04.2010
12.	Dr. Subhash Pawar	Technical Assistant	03.05.2010
13.	Sh. G.Senthil Kumar	Technical Assistant	05.05.2010
14.	Dr. Krishan Gopal	Scientist	14.01.2011
15.	Dr. Mani Shankar Bhattacharyya	Scientist	01.03.2011
16.	Sh. Ajay Kumar	Junior Engineer (Civil)	15.03.2010
17.	Sh. Harpreet Singh	Junior Engineer (Elec.)	01.04.2009
18.	Sh. Hari Om Kushwaha	Technical Assistant	28.04.2009
19.	Sh. Bhupinder Singh Chopra	Technical Assistant	19.06.2010
20.	Dr. J.N.Agrewala	Chief Scientist	17.09.2010

New Faculty Members

Krishan Gopal

krishang@imtech.res.in

Dr. Krishan Gopal obtained his Ph.D. (2009) from Molecular Biophysics Unit, Indian Institute of Science Bangalore. He worked as a Research Associate in the same lab before joining IMTECH in 2011.

Mani Shankar Bhattacharyya

manisb@imtech.res.in

Mani Shankar Bhattacharyya received his Ph.D. (2007) from National Institute of Pharmaceutical Education and Research, Mohali and carried out his post-doctoral research at the Department of Chemical Science, University of Cagliari, Italy, before joining IMTECH in 2011.

Staff Promoted

Sr.No.	Name	Next Higher Grade/Group	Date of Promotion
1.	Dr. G. Rajamohan	Principal Scientist	06.05.2007
2.	Dr. R. Kishore	Senior Principal Scientist	18.05.2008
3.	Dr. S. Karthikeyan	Principal Scientist	03.06.2008
4.	Sh. E. Senthil Prasad	Scientist	04.10.2008
5.	Dr. Purnanand Guptasarma	Senior Principal Scientist	01.01.2009
6.	Sh. Anirban Roy Choudhury	Senior Scientist	11.01.2009
7.	Sh. R. S. Puri	S.O (S&P)	15.04.2009
8.	Dr. K. Rajagopal	Principal Scientist	25.04.2009
9.	Dr. Javed N. Agrewala	Senior principal Scientist	07.06.2009
10.	Dr. Jagmohan Singh	Chief Scientist	29.07.2009
11.	Dr. Shekhar Majumdar	Senior Principal Scientist	08.09.2009
12.	Dr. Pradip Sen	Senior Scientist	27.09.2009
13.	Mrs. Satya Gupta	Private Secretary	22.12.2009
14.	Sh. Kailash T. Bhamre	Scientist	23.12.2009
15.	Dr. Saumya Ray Chaudhury	Principal Scientist	05.03.2010

Staff Left

Sr.No.	Name	Designation	Date of Leaving	Reason
1.	Sh. Manoj Kumar	Technician	24.05.2010	Resignation
2.	Sh. Harpreet Singh	Junior Engineer (Elec.)	12.05.2010	Resignation
3.	Sh. Vimal Kumar	Junior Engineer (Civil)	22.09.2009	Resignation
4.	Sh. S. D. Bhatt	Chief Scientist	30.04.2010	Retirement
5.	Dr. P. R. Patnaik	Chief Scientist	05.7.10(FN)	Voluntary Retirement
6.	Sh. S. K. Sadana	COA	28.02.2010	Retirement
7.	Dr. (Mrs.) Ankita S Jaiswal	Scientist	15.02.2011	Resignation
8.	Sh. Ravi Garg	Section Officer	08.03.2011	Transfer to CSIO
9.	Sh. Gurbachan Singh	Driver	30.04.2010	Retirement
10.	Ms. Harjeet Kaur	Technician	15.07.2010	Resignation
11.	Sh. Mukesh Kumar	Technician	01.04.2011	Resignation
12.	Sh. Mohinder Kumar	Lab Attendant	03.08.2009	Voluntary Retirement
13.	Sh. Nand Lal	Peon	26.02.2010	Transferred to IHBT
14.	Sh. C. Chandrasekhar	Technical Assistant	29.05.2009	Transferred to IICT
15.	Dr. R. K. Jain	Chief Scientist	23.03.2011	Expired
16.	Dr. P. Guptasarma	Sr. Principal Scientist	20.10.2010	Lien for two years
17.	Dr. A. K. Bachhawat	Chief Scientist	16.09.2010	Lien for two years

research associates/fellows


Rocking
Adidas

Research Associates/Fellows

Name	Supervisor
Batch: Februrary, 2009	
Anupam	Dr. Alok Mondal
Appu	Dr. S. Kumaran
Ghanshyam Singh Yadav	Dr. Pradip K. Chakraborti
Gunjan Sharma	Dr. B. D. Shenoy
Kalpana Pandey	Dr. Ashish
Manendra Pratap Singh	Dr. Pawan Gupta
Pradip Kumar Singh	Dr. Suresh Korpole
Prerna Sharma	Dr. P. Guptasarma
Rakesh Bhatia	Dr. Grish C. Varshney
Ranjeet Singh	Dr. Dibyendu Sarkar
Sathi Babu Chodisetti	Dr. Javed N. Agrewala
Savita Yadav	Dr. S. Karthikeyan
Shikha Singh	Dr. Ashish
Shrijita Banerjee	Dr. S. Kumaran
Anup Deshpande	Dr. Anand Bachhawat
Ashish Kumar Solanki	Dr. Ashish
H. Kitdorlang Dkhar	Dr. Pawan Gupta
Yogendra Singh Rathore	Dr. Ashish
Udita Upadhyay	Dr. Jagmohan Singh
Vijay Singh	Dr. S. Kumaran
Batch : August, 2009	
Harinder Singh	Dr. G. P. S. Raghava
Md. Alfatah	Dr. K. Ganesan & Dr. Alok Mondal
Garima Trivedi	Dr. Charu Sharma
Monica Mittal	Dr. S. Kumaran
Neeraj Maurya	Dr. Pradip Sen
Navdeep	Dr. Manoj Raje
Md. Rehan	Dr. G. Sahni
Sahil Mahajan	Dr. Pawan Gupta
Sangita Kachhap	Dr. Balwinder Singh
Shailesh Kumar	Dr. G. P. S. Raghava
Vemika Chandra	Dr. Pawan Gupta
Vinay Kumar Bari	Dr. K. Ganesan
Yamini Agrawal	Dr. B. D. Shenoy
Kshipra Pachauri	Dr. D. K. Sahoo
Nagesh Peddada	Dr. S. R. Chaudhury & Dr. Ashish
Sahil Ahmed	Dr. G. Varshney

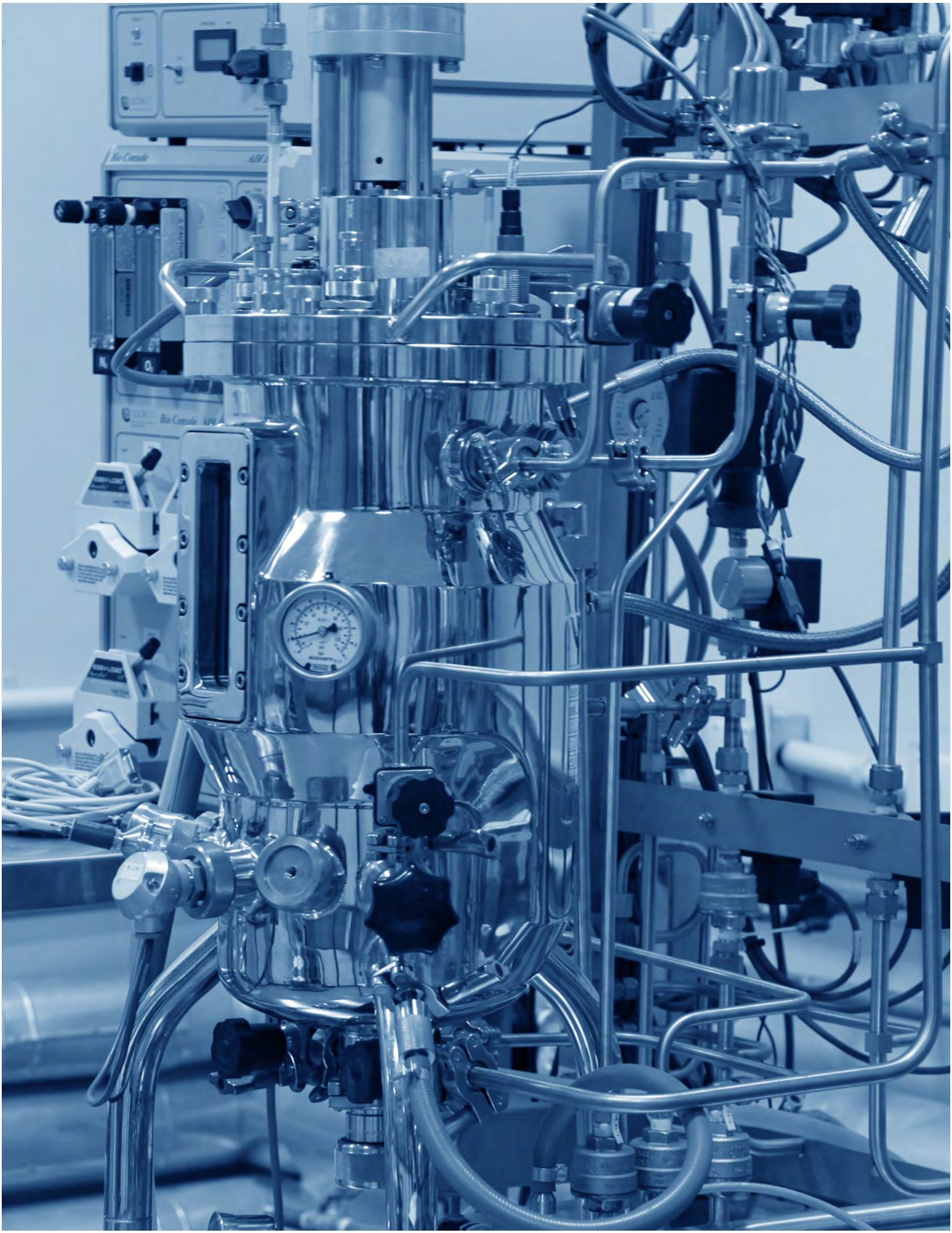
Sudhir Gupta	Dr. R.K. Jain
Vikas	Dr. S. R. Chaudhury

Batch: 2010

Aadil Hussain Bhat	Dr. Alka Rao
Amin Sagar	Dr. Ashish
Abid Qureshi	Dr. Manoj Kumar
Aurobind Vidyarthi	Dr. Javed Agrewale/Dr. Ankita
Giriraj Acharya	Dr. S.Karthikeyan/Dr. Srikrishna Subramanian
Gurdeep Singh	Dr. D. K. Sahoo
Kumardeep Chaudhary	Dr. G. P. S. Raghava
Lahari Das	Dr. Grish C. Varshney
Md. Ishaq Tantray	Dr. Sekhar Majumdar
Nisha Singh	Dr. Ashwani Kumar
Nupur	Dr. P. Anil Kumar
Pankaj Sharma	Dr. Ashish
Pradeep Kumar Rai	Dr. Javed N. Agrewala
Pradeep Mishra	Dr. R. S. Jolly
Praveen Sonkusre	Dr. Swaranjit Singh
Roma Garg	Dr. Girish Sahni
Shabir Ahmad Bhat	Dr. Ashwani Kumar
Shailza Sharma	Dr. S. Karthikeyan/Dr. T.N.C. Ramya
Shaminder Singh	Dr. Vijay C. Sonawane
Srishti Chawla	Dr. Alok Mondal
Weshely Kujur	Dr. Javed N. Agrewala
Suksham Pal	Dr. D. K. Sahoo
Swati Bijlani	Dr. G. Rajamohan
Vaidyanathan Vasanth	Dr. G. Rajamohan
Abhishek Trivedi	Dr. Ashwani Kumar
Adity Chopra	Dr. C. Raman Suri
Amar Nath Sharma	Dr. R. S. Jolly
Himanshu Malhotra	Dr. Manoj Raje
Anubhav Singh Nahar	Dr. K. Ganesan
Md. Amir	Dr. Javed N. Agrewala
Monika Sharma	Dr. Charu Sharma
Navinder Kumar	Dr. B. D. Shenoy
Nitin Kumar Singh	Dr. Mayilraj
Rahul Kumar	Dr. G. P. S. Raghava
Ravi Kanth Nanduri	Dr. Pawan Gupta
Reema Raghuvir Dhoke	Dr. Ashish
Richa Singh	Dr. Saumya R. Chaudhury
Ritika Bishnoi	Dr. S. Karthikeyan/Dr. T.N.C. Ramya
Gaurav Sharma	Dr. Sekhar Majumdar
Gurmeet Kaur	Dr. S. Karthikeyan/Dr. Srikrishna Subramanian
Ruchi Gautam	Dr. S. Karthikeyan
Sandeep Kumar	Dr. G. P. S. Raghava

support departments

support departments



Biochemical Engineering Research and Process Development Centre

Objective:

- To do research on the development of biochemical processes using fermentation technology and downstream processing.
- To undertake sponsored projects for the development of new products and/or new technologies.
- To evaluate technologies proposed to be imported or upgraded by either private or public sector.
- To collaborate with various industries and research centres in terms of scientific and technical information exchange and optimal use of different equipment.
- To train technical personnel from industries as well as universities and research institutions.
- To provide facilities to researchers from universities and other R&D organizations.

In house projects:

Process development for nanodelivery of protein therapeutics: The application of nanotechnology to drug delivery, such as delivery in the form of micrometer and nanometer sized polymer-protein drug conjugates, are reported to improve protein stability, pharmaceutical efficiency or dosing of protein pharmaceuticals. In most cases, the encapsulation of protein pharmaceuticals in microspheres/nanoparticles comprised of the bioerodible and biocompatible polyesters and PLGA has been widely investigated for sustained and controlled delivery. Since, the therapeutic activity of proteins is highly dependent on their conformational structure and the protein structure is flexible and sensitive to external conditions, while developing protein delivery systems, one must minimize the denaturation of protein drugs during manufacturing process. Using BSA as a model protein, investigating the molecular mechanism operating behind polyethylene glycol (PEG) mediated stabilization of protein (BSA), we have reported earlier that there was no significant changes in BSA conformation by the processes like lyophilization and sonication and PEG 8000 as a stabilizer at an optimum BSA:PEG molar ratio (1:0.75) stabilized BSA due to strong physical adsorption of PEG 8000 on buried hydrophobic core of BSA along with surface adsorption. Next, we studied the structural integrity of released bovine serum albumin (BSA) from polymer based microspheres prepared with different mixtures of surfactants, BSA and BSA coated with PEG (polyethylene glycol). The primary emulsion of lyophilized BSA and BSA coated with PEG was prepared using ethyl acetate and poly DL-lactide-co-glycolide (PLGA), and secondary emulsification was accomplished by using different ratios of surfactants. The morphology of prepared microspheres was studied using dynamic light scattering (DLS) and scanning electron microscopy (SEM). BSA released from lyophilized microspheres was evaluated for the structural, conformational and thermal stability by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), fluorescence spectroscopy, circular dichroism (CD)-spectroscopy, along with differential scanning calorimetry (DSC). The primary structural integrity of released protein was analyzed by SDS-PAGE. CD-spectroscopy showed greater increase in secondary structural content of BSA in sample containing PEG and surfactant mixture of CMC (carboxymethyl cellulose) and Tween 20 as compared to that containing mixture of CMC and Tween 80 or Tween 20 and Tween 80. The increase in the thermal stability of BSA, released from samples containing PEG, was indicated by the increase in peak area (ΔH)

and midpoint of denaturation (T_m). In addition, the protein showed no aggregation in size exclusion chromatography-high performance liquid chromatography (SEC-HPLC) analysis whereas the fluorescence spectra showed the compactness of BSA. These results suggested macromolecular crowding due to PEG, molecular confinement because of microspheres and interfacial increase in Gibbs free energy with strong electrostatic repulsion, the last phenomenon was due to chosen surfactants that were responsible for making the protein more compact and structurally integrated. Our investigations proposed molecular mechanism of improved structural integrity of protein in polymer based microspheres and resulted in a process for improved protein integrity in final formulation.

Screening and Process Development for Microbial Exopolysaccharides: The project started recently with the objective of developing a suitable process for production of microbial exopolysaccharides namely pullulan and microbial cellulose. In the initial stage several strains were screened and out of those an osmotolerant and non-pigmented isolate A. pullulans RBF-4A3 was found to be the best. This organism produced more than 66g/L pullulan with 15% (w/v) glucose in production media. Subsequently, five different agri-industrial wastes *viz.*, rice bran oil cake, soya bean oil cake,cotton seed oil cake, mustard seed oil cake and corn steep liquor (CSL) were used as nitrogen source along with 15% (w/v) glucose as carbon source for biosynthesis of pullulan. CSL supported production of 77.92 g/L pullulan under un-optimized conditions. Single point optimization technique used for optimization suggested that media containing 15% (w/v) glucose, 2% (w/v) CSL, 20°C incubation temperature, 300 rpm shaker speed, initial pH 4.5 and 5% (v/v) inoculum were optimum for pullulan production.

New bioactives from microbes: Hopes and remedies: A library of more than 6000 organic extracts from diverse microbes isolated from unexplored niches were generated. These extracts are exploited for various targets set up in the lab such as antimicrobials for broad spectrum bacteria, methicillin resistant Staphylococcus aureus (MRSA), vancomycin resistant Enterococcii (VRE), extended spectrum β-lactamase (ESBLase), α-glucosidase and azole resistant-Candida albicans. *In vitro* (micro-titre plate based) as well as agar-plate-based assays for β-lactamase and α-glucosidase inhibitor and also, in-vitro screening assays for tyrosinase and trypsin inhibitor were developed. Multi-drug resistance is serious problem in tuberculosis and other bacterial infectious disease. It is mostly due to cell-wall barrier, bio-film formation at the target and efflux of drugs from target. Efflux pump inhibitors stops the efflux of drugs and restores at the site of action to give complete therapeutic efficacy and so, an assay to screen efflux pump inhibitors has been set up. In terms of output some promising bioactive compounds against MRSA, azole-resistant C. albicans, α-glucosidase inhibitor, ESBLase inhibitor, bio-film inhibitor and bio-film enhancer, are under purification and characterization stage.

CSIR Network Projects:

Zero Emission Research Initiative - Screening, production, purification and characterization of alkaline proteases: In leather processing, the removal of hair from hides and skins is the first step in beam house. The traditional chemical process for un-hairing involves addition of sodium sulphide and lime to help cleaning the hide and aiding hair removal. Much of the hair is degraded into soluble organic material and the rest remains as insoluble fibres. The process generated large amount of effluent having high chemical oxygen demand and suspended solids content, and the presence of excess sulphides in effluent requires additional treatment before discharge to sewer. With increasing stringent environment requirements, it has become necessary to reduce

pollution load in wastewater as much as possible. The use of enzymes in leather processing provides an alternative with a number of advantages and their potential application in leather processing include soaking, bating, degreasing and treatment of chrome savings. The project was initiated with an objective to develop proteases for soaking, bating and enzyme assisted unhairing and hence, interest was focussed on the use of enzymes that achieve maximum effect in a highly alkaline medium. Also, since proper shavings of raw-material is essential for obtaining good quality leather, another objective of the project was to use proteolytic enzymes to degrade interfibrilar protein significantly facilitating water adsorption and shortening the soaking operations. After screening a large number of proteases, four were selected for further studies.

IMT Protease N01, produced by a Bacillus sp. and having an optimum pH of 10.5 and optimum temperature 50-55°C for its activity and stable in pH range of 7.5 – 11.0, temperature range of 20 – 55°C and in presence of detergent (SDS) and bleach components (sodium perborate) demonstrated the potential of this protease for application under conditions used in leather processing and its commercial exploitation. The media and process parameters were optimized for yielding maximum enzyme activity. The enzyme was recovered from fermentation broth using centrifugation/ microfiltration and concentrated by ultrafiltration followed by ammonium sulphate/ solvent precipitation. Another high alkali-stable protease, IMT Protease N02 produced by Bacillus pseudofirmus, with an optimum pH of 11.0 and optimum temperature of 50-55°C for its activity was found to be stable in pH range of 7.0 – 12.0, temperature range 20 – 55°C. Stability of this enzyme in presence of detergents (SDS and DBS, Triton X100 and Tween 80) and bleach component (sodium perborate) indicated its potential for leather processing and commercial exploitation. The media and process parameters for this protease production were optimized and the downstream processing conditions were standardized. Two other proteases, IMT protease N03 and IMT protease N04, from newly isolated organisms, Serratia sp. and Stenotrophomonas maltophilia, were purified and characterized. Serratia protease with pH and temperature optima at 9.0 and 60°C, respectively was stable at pH range 7.0-10 and in DMSO, ethyl acetate, hexane, benzene, SDS, sodium perborate, sodium benzene sulfonate, Tween 80 and triton X-100. Protease from Stenotrophomonas maltophilia was found to have pH and temperature optima at 7.5 – 8.0 and 55°C, respectively, stable at pH range 7.0-10.0 and in organic solvents (ethyl acetate, benzene, xylene, hexane, dimethyl sulphoxide, octanol *etc.*) and surfactants (SPB, DBS, Tween 80 and Triton X100) suggesting its industrial applications.

Work under microbial biodiversity programme (NWP-006): A total of 7 libraries have been procured. Out of these libraries 13,09,400 clones have been screened for cellulase. Two clones, one from Bayer Ind Library and another from Woodchip Library, showed cellulase activity. 50-100 putative positive clones were found, but when checked at molecular level these were found to be cellobiose phosphoylase, lichenase and glycosyl transferase. So these were related biotechnologically important genes, but not exactly cellulases.

Sponsored Projects:

Process know how/ technology for CSSK production from yeast at Pilot Scale (Sponsored by Symmetrix Biotech Private Limited, Mumbai):A process know how/ technology for CSSK production from yeast was developed at 500 L Pilot Scale fermenter and transferred to Symmetrix Biotech Private Limited, Mumbai.

NMITLI Project on "Biotechnology for Leather: towards cleaner processing Phase II" : This project was initiated with primary objectives to develop bioprocessing alternatives to currently employed chemical methods in leather manufacturing technologies. In this multi-laboratory NMITLI project, we studied the use of enzymes in leather processing, specifically selection of specific proteases, capable of replacing chemical inputs in dehairing, enzymatic opening of fibre bundles without osmotic swelling by lime and other alkalies and the

development of bioprocesses for selected enzymes. Following optimization of media and process parameters, the process was scaled up to 700 L scale. The extracellular alkaline protease was recovered using centrifugation and further concentrated by ammonium sulphate precipitation. The precipitated enzymes, applied for leather processing at Pilot Scale, was assessed for unhairing efficiency individually and in an integrated enzyme only process where it is used along with a lipase and an amylase. In integrated process, the waste water did not contain sulphide and there was significant reduction in COD. High BOD/COD ratio in enzyme based process (EBP) indicated better degradability of waste water from EBP as compared to CBP. The quality of leathers from EBP was smoother than that of CBP, where as the fullness, tightness and other physical properties of leathers were comparable in both cases.

NMITLI sponsored project on "Conversion of cellulose and hemi-cellulose into sugars and ethanol" Cellulase production using Penicillium funiculosum (NCL, Pune) was carried out. The fermentation medium consisted of cellulose powder (CP-123), wheat bran, urea, ammonium sulphate, peptone, trace elements, Tween-80 and other salts at shake flask level and the process was scaled up to 7 L fermenter scale. After investigating the effects of various parameters, a HPLC based method tailored for analysis of a mixture of sugar and sugar alcohols (*e.g.*, glucose, cellobiose, xylose, glycerol, xylitol and ethanol) expected from simultaneous saccharification and fermentation (SSF) process was developed using a single column and validated using cellulose hydrolysate fermentation broth. Enzymatic hydrolysis of cellulosic substrates was carried out using different bagasse substrates: plain crushed bagasse (PCB), steam exploded bagasse (SEB) and treated bagasse (TB) (all from NCL, Pune) and Avicel (as a standard cellulosic substrate) and a commercial cellulase enzyme. Among three different type of bagasse used, treated bagasse yielded maximum reducing sugar. The effect of surface-active additive such as Tween 80 on enzymatic hydrolysis of different cellulosic substrates was investigated.

Process development for production of bacteriorhodopsin from *Halobacterium* sp. (DST sponsored): Bacteriorhodopsin, a biological photochrome with exceptional properties, is produced by halophilic Archaea living in extreme salt concentrations of 4-5 M NaCl. Bacteriorhodopsin is a 26 kDa retinal protein present in the two-dimensional, crystalline lattice of purple membrane in conjunction with a lipid bilayer structure, and functions as a light driven proton pump. The effect of cultivation conditions on production of bacteriorhodopsin (bR) by mutant strain Halobacterium salinarium S9 and its quality was studied. The purified purple membrane obtained under static conditions after exponential growth phase showed good optical purity ($A_{280\ nm}/A_{570nm}$ = 2.08) and bacteriorhodopsin production (17.02 mg/ L). Altered growth conditions in the early exponential phase (1st day) and late exponential phase (5th day) indicated that initial growth phase played an important role in generating cell biomass followed by purple membrane synthesis in cells at late exponential phase. Two different forms of bR (26.5 and 25.5 k Da) was identified in SDS PAGE and further characterization by N-terminal sequencing showed the presence of additional amino acids near N-terminus region due to incompletely processed leader sequences. Decreased alpha helical content (obtained from CD values) of bR obtained under dark conditions speculates optically active chromophores contributed to differential light scattering.

Development of antimicrobial agents from soil microflora (DST sponsored): This project was carried out along with CDRI Lucknow, NIF Ahmedabad and Karnataka Antibiotics Limited on development of novel

antibiotics. NIF has a collection of actinomycetes showing activities against several pathogens. The major objectives of this work was characterization and taxonomic classification of the isolated strains, bulk production of the antimicrobial compounds (which is required to carry out further studies) and process development for production of selected antibiotics . The process will initially be developed in shake flask level and then it will be scaled up to fermenter scale. Out of extracts from 75 cultures sent to CDRI, 9 showed significant antimicrobial activity.

Up-gradation of facility: The centre is now augmented with a state of the art clean room facility with class areas for fermentation and downstream processing. The facility is equipped with latest fermentation, purification and analytical equipments with validation facility and is compatible for production of toxicological grade material through fermentation route. Efforts are on to make this facility cGMP certified.

Consultancy, Technology Demonstration and Licensing:

- Product development work of Symmetrix Biotech Private Limited, Mumbai was carried out using BERPDC bioincubator facilities including laboratory (5L – 20 L) and Pilot Scale (15 L- 150 L) fermenters.
- Product development work Bigtec, Bangalore was carried out using fermentation facility at 100 L scale.
- Fermentation of lysine at 5L fermenter scale was carried out for Bharat Starch Industries Limited, Yamunanagar
- Production of one microbial metabolite was carried out for Chemical Resources Private Limited using Fermentation and analysis facility of the centre.

Participants: Girish Sahni, Debendra K. Sahoo, V. Sonawane, Hemraj Nandanwar, Anirban R. Choudhury, E.S. Prasad, Chetan Premani, Lata Verma

Experimental Animal Facility

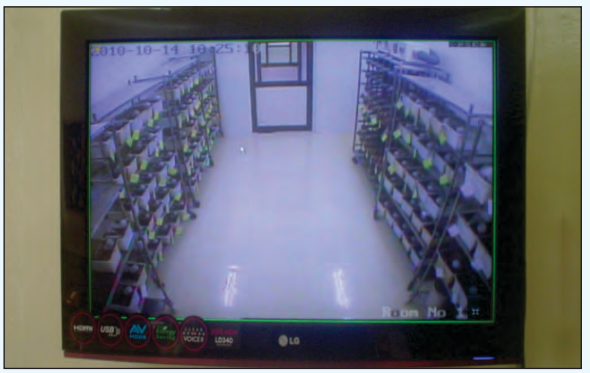
Experimental Animal Facility (EAF): EAF is a core service division of the Institute with an objective to procure, breed, supply and raise experimental animals of high quality and provide husbandry, veterinary care and technical/professional support to the scientific community of the Institute to facilitate research using animals. The facility maintains following species and strains of various laboratory animals for breeding as well as experimentation:

MICE	RATS	RABBITS	HAMSTERS	CHICKEN
BALB/c	Sprague Dawley	New Zealand White	Syrian	Egg laying hens only for experimentation
C57BL/6J				
DBA/1				
C3H/HeJ				

Our EAF with a floor space of 30,000 sq. ft. (distributed in three floors) houses all animals maintained at different rooms of fixed temperature, relative humidity and light – dark cycle, strictly follows CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines.

The facility is under electronic surveillance (CCTV system), and defined barrier practices are followed strictly with restricted access to authorized persons only.

Access control system



CCTV Camera (Room View)

Further, care is taken to eliminate/ minimize infection by restricting the dirty corridors to the outer perimeter of this facility. To prevent transmission of infection, the health quality procedures i.e.; handling of animals by gloved hands, disinfections of animal rooms regularly, use of sterilized cages, bedding, nesting material, drinking water and water bottles, are followed. Defined breeding protocols and management and husbandry procedures are followed to ensure the genetic purity of the pedigree of each mouse strain of foundation colony. The facility is extending technical support and advice to the scientists regarding ethical and humane care and use of animals in experiments under various Institutional Animal Ethics Committee (IAEC) approved projects.

The facility is providing following services as per requirement of the users: (a) collection of blood and other samples, (b) immunizations of animals for generation of polyclonal antibodies, (c) assistance in planning and writing of Animal study Protocols, (d) dissemination of information to update of the scientific community of the Institute about the rules and guidelines issued by the CPCSEA, Ministry of Environment and Forest, Government of India.

EAF is registered with the CPCSEA and is governed by its own Institutional Animal Ethics Committee (IAEC) constituted specifically to address scientific and ethical considerations of animal use for research. During this period, 4 meetings of IAEC and 3 meetings of subcommittee of IAEC were held in which 43 new projects were approved.

EAF regularly organizes workshops and trainings on various aspects of animal breeding, maintenance, handling and practical aspects of quality control techniques, on request, to scientific and technical personnel as well as PhD students of the Institute. During the reported period two certified trainings/workshops were organized. Besides this, new batches of Ph.D students under JNU-programme were also given lectures as well as lab orientation course under 'Tools and Techniques'.

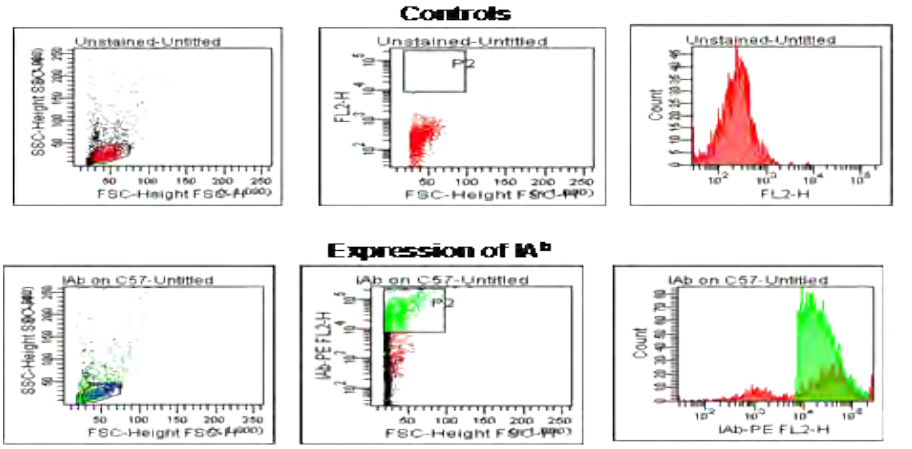
EAF is able to meet the needs of the animal users for various IAEC approved projects. During the reported period, Animal Facility has supplied (i) 5607 mice, (ii) 51 rabbits, (iii) 191 rats and (iv) 14 chickens to various IAEC-approved projects of the institute as well as outside CPCSEA registered institutes. To this effect, our EAF has generated Rs. 1, 74425/- through sale of animals to outside CPCSEA registered institutes as ECF.

Quality control:

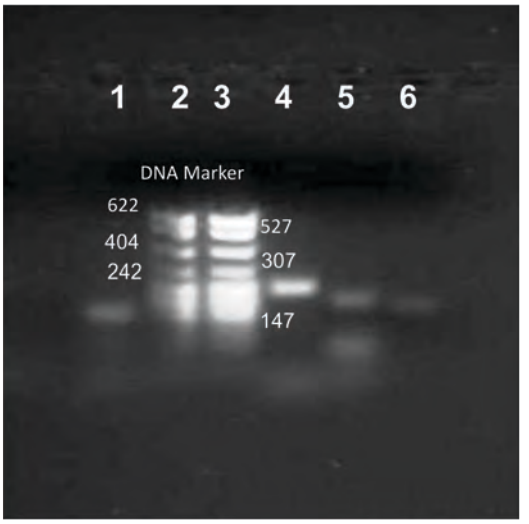
Monitoring of genetic background of mice is being done by FACS, PCR based DNA genotyping using different markers (D6Mit39, D10Mit35, D12Mit5 and D19Mit41).

- **Genetic Monitoring:**

1(a) IA^b expression using FACS: IA^b is a MHC class II haplotype specific for C57BL/6J mice. IA^b expression in splenocytes of C57BL/6J was confirmed using FACS. Splenocytes of C57BL/6 were stained with anti IA^b antibodies conjugated to phycoerythrin (PE). Cells were acquired on FACS caliber and data was analysed by DIVA software. Cells were gated on lymphocyte zone and expression of IA^b was monitored on FSC and IA^b positive population.



1(b) Genetic monitoring of mice using single sequence length polymorphism (SSLP) markers:



We have checked the purity of C57BL/6J mice using 4 different micro satellite markers viz. D6Mit39, D10Mit35, D12Mit5 and D19Mit41 and the results seem to be consistent with those described in Mouse Genome Informatics for these markers. Further, nucleotide sequencing of PCR product of D10Mit35 marker was done to ascertain the sequence. The BLAST analysis of this sequence exhibited similarity of 95% with Mus musculus strain C57BL/6J.

Lane 1: DNA sample of C57BL/6J mouse showing PCR product of 146 bp using D6Mit39 marker

Lane 2 & 3:.Standard molecular weight markers

Lane 4: DNA sample of C57BL/6J mouse showing PCR product of 228 bp using D10Mit35 marker

Lane 5: DNA sample of C57BL/6J mouse showing PCR product of 176 bp using D12Mit5 marker

Lane 6: DNA sample of C57BL/6J mouse showing PCR product of 160 bp using D19Mit41 marker

• **Health monitoring of animals:** For health monitoring of animals, the facility is using 13 ELISA based diagnostic kits (viral and bacterial diseases) to test the coded samples of mice monthly. In addition to this, mice are also examined for exposure to mycobacterium on the basis of presence of antibodies to purified protein derivative of mycobcateria (PPD). Further to assess the health status of mice, blood samples are checked for all hematological parameters monthly. Quality control of water (by PA Coliform kit and Hardness testing kit) as well as other material is being done routinely.

Participation in Institutional projects: Dr. Neeraj Khatri is participating in the following collaborative projects: a) Role of Cae A on skin allografts in mice: Skin allograft was done to evaluate immunosuppressive activity using different derivatives of Cae A in collaboration with Dr. J. N. Agrewala (technology licensed to Nostrum Pharmaceuticals, USA) b) Development of Technologies for Therapeutic proteins:Under this project few *in vivo* experiments have been done to evaluate *in vivo* survival of the recombinant streptokinase, staphylokinase over time.

Participants: Neeraj Khatri, S.S. Bawa, Bhupinder Singh Chopra, Vinod Kumar

Administration

Administration being a vital part of the Institute, is providing utmost support and assistance to scientific and other staff in managing day to day R&D affairs and diverse activities of the Institute so as to achieve desired scientific goals as envisioned in the institutional mandate. Broadly, duties and responsibilities of administration involve handling HRD related issues, service matters, implementation of personnel policies, and sundry general services *viz.* security transport, telecommunication *etc*.

Purchase

The Stores & Purchase section of IMTECH plays a vital role in procurement & keeping inventory of store items of regular use for the various ongoing projects in IMTECH. Most of the activities of Stores & Purchase dept. of IMTECH are now computerized for smooth functioning. During the currency of the past two years, the purchase section processed approximately 4000 indents and procured highly sophisticated equipments such as Laser Scanning Confocal Microscope, Whole Body Imaging System, Automated Liquid Handling System, Dynamic Laser Scattering System *etc.* and processed many other equipments for the newly constructed Protein Centre, IMTECH.

Finance & Accounts

Finance & Accounts Division plays a vital role in an Institution. Finance department is expected to perform not only to relay all the needed financial rules and ensure proper book entering but also to participate in making overall ethically sound decisions. The major responsibilities of finance & accounts division are also to actively associate with the planning of annual budget of the Institute, arranging all payments, adjustment of OB items, reconciliation of bank statement with main cash book, various type of remittances, reply of audit paras, investment of surplus funds and also recording, classifying, summarizing, and interpreting the financial transactions and communicating the results thereof, in the shape of final accounts, to CSIR.

Instrumentation

Library

The Knowledge Resource Centre (Library) was established in 1984, almost simultaneously with the institute, with a mission to serve the education and information needs of its staff and students. The KRC (Library) is equipped with modern facilities and resources in the form of books, printed journals, e-journals, microfilms/microfiche, online-databases, general magazines, theses and audio-visual materials.

The centre also provides services like literature search, document delivery, resource sharing, user awareness programs, photocopying, reference and referral services, printing, binding, transactions of information resources etc. It has a collection of 26,122 volumes including books and journals and continues to subscribe to 178 national and international periodicals (including online versions) on microbiology/microbial genetics, molecular biology, cell biology/immunology, protein science/engineering, applied microbiology, fermentation technology, and bioinformatics. It also enables users to access an additional 4000 full-text e-journals publishers of national and international repute viz. Science Direct, Springer, Nature Publishing Group, American Chemical Society, John Wiley, Oxford University Press, Science, IndianJournals.com NISCAIR Publications, etc. made available through CSIR-DST e-Journals Consortium. Additionally, patent and citation databases such as Web of Science, DII and Delphion and other database have been made available through CSIR-DST e-Journals Consortium.

The functions and services of the KRC are fully automated and run on a library management software “e-Granthalaya”, which was developed by NIC with unlimited access to Web OPAC. The OPAC is available at: http://172.141.122.14/.

Digital Institutional Repository Service (DIRS)@IMTECH (available at: http://172.141.121.232:8080/dspace/) is in place to host full text of research publications of scientists of the Institute using DSpace, an open source digital library software developed by MIT-HP which supports the Open Archives Initiative's Protocol for Metadata Harvesting (OAI-PMH).

Engineering Services Division

Engineering Services Division provides vital Engineering and Utility services' support across the wide spectrum of institutional R&D activities and national facilities of IMTECH. This Division is composed of several expertise *viz*, Civil Engineering, Electrical Engineering, Mechanical Engineering, Air Conditioning, and Refrigeration together meet the specific requirements of R&D activities of IMTECH.

Services Offered:

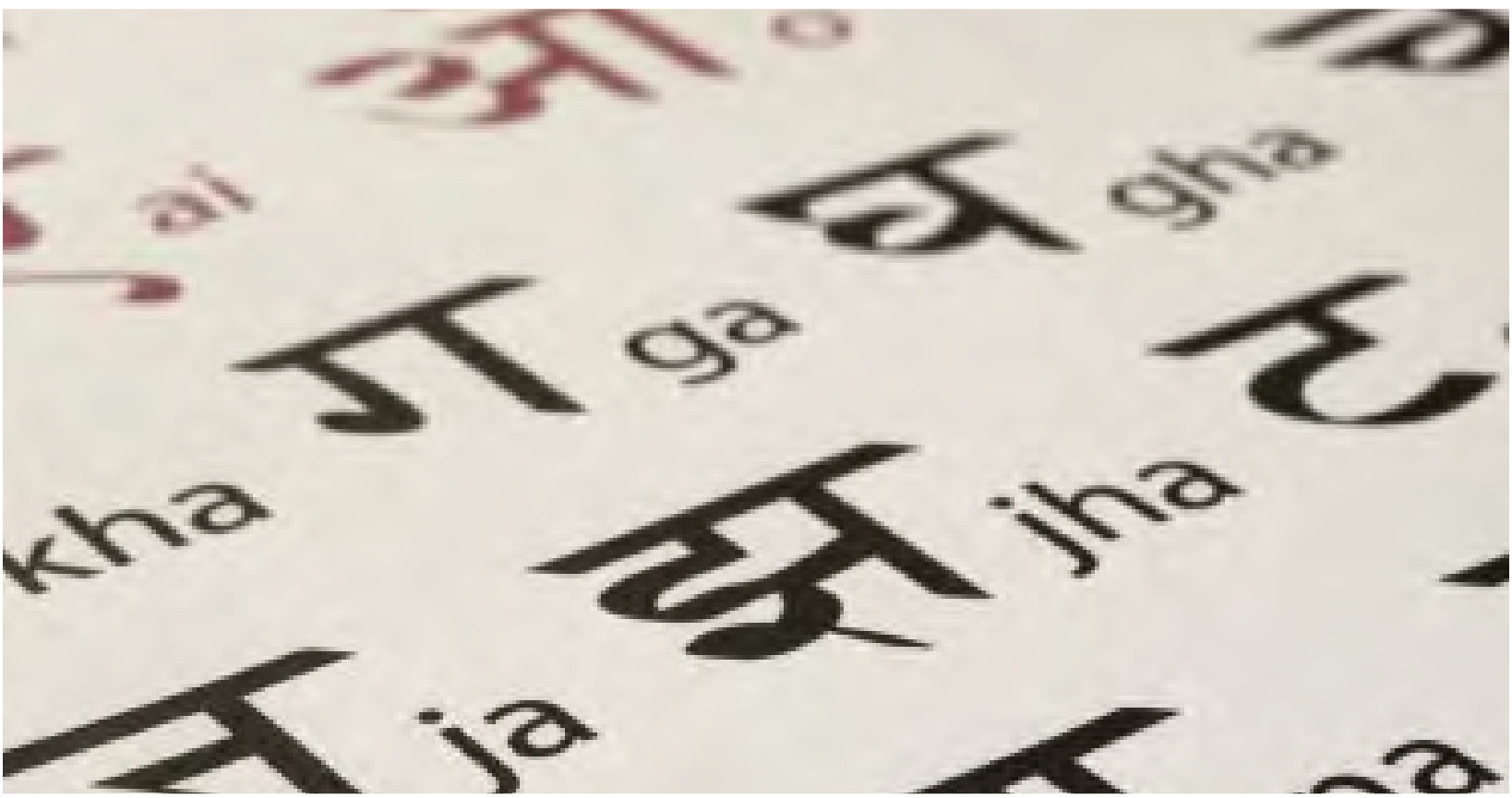
The division is equipped with basic facilities required for metal joining, metal cutting, wood-working, plumbing, electrical, air conditioning and refrigeration maintenance. It also looks after dry saturated & superheated stream requirements of the institute through operation of oil fired and electrical boilers. It performs specific roles in the following fields.

- Planning, management and execution of building design, lay out, construction and maintenance.
- Operation and maintenance of electrical services in the institute and housing.
- Operation and maintenance of utility services like air-compressor, cooling tower and boilers.
- Maintenance of utility supply lines.
- Maintenance of elevators.

Projects handled:

- Creation of a facility with certifiable Bio-safety Level-III.
- Construction of Protein, Information, Science Engineering and Technology building.
- Construction of a hostel with 100 rooms for Ph.D. scholars.
- Creation of the state of the art facility equipped with clean air for bio-pharmaceuticals production and research.

राजभाषा गतिविधियाँ



राजभाषा गतिविधियाँ

संस्थान के पुस्तकालय में उपलब्ध

हिन्दी पुस्तकों की प्रदर्शनी

सूक्ष्मजीव प्रौद्योगिकी संस्थान (इमटैक), चण्डीगढ़

राजभाषा गतिविधियाँ

संस्थान में राजभाषा कार्यान्वयन के विशेष प्रयास किए जाते हैं जिससे संस्थान में राजभाषा के प्रयोग के लिए अनुकूल वातावरण बने तथा संस्थान के सदस्यों का रुझान इस ओर स्वेच्छा से बढ़े । विगत वर्षों में इस क्षेत्र में निरन्तर प्रगति हुई है व इसके लिए संस्थान में वर्षभर विभिन्न गतिविधियाँ आयोजित की जातीं हैं ।

स्वागत कक्ष में बोर्ड पर हिन्दी – अंग्रेज़ी शब्द – स्वागत कक्ष में इलेक्ट्रॉनिक बोर्ड पर हिन्दी के तकनीकी शब्दों के अंग्रेज़ी पर्याय लगाए जाते हैं । वर्षभर में प्रदर्शित शब्दों की सूची बनाकर परिचालित की जाती है व इसके आधार पर 'हिन्दी शब्दज्ञान प्रतियोगिता' आयोजित की जाती है ।

विज्ञान पर हिन्दी में सेमिनार – 'विज्ञान हिन्दी में संभव नहीं' इस भ्रांति को दूर करने के लिए निरन्तर प्रयास किए जा रहे हैं इसका सशक्त प्रमाण इमटैक में विगत 15 वर्षों से वैज्ञानिक एवं तकनीकी स्टाफ द्वारा मास के अंतिम शुक्रवार को किन्ही वैज्ञानिक एवं तकनीकी जनरुचि के विषयों पर हिन्दी में सेमिनार दिए जाते हैं । वर्ष के दौरान ''लाइम रोग में साल्प – 15 की भूमिका , मधुमेह रोग – समस्याएँ एवं सावधनियाँ, गुर्दे के रोग और उपचार '', ''विषाणु जनित मानव व्याधियाँ'', ''पर्यावरणीय समस्याएँ – खतरे की घंटी'' आदि विषयों पर सेमिनार दिए गए । इन सेमिनारों का निदेशक द्वारा गठित निर्णायक मंडल के पैनल द्वारा मूल्यांकन किया जाता है तथा सर्वश्रेष्ठ तीन सेमिनारों को हिन्दी दिवस समारोह में पुरस्कृत किया जाता है ।

तकनीकी पत्र प्रस्तुति – श्री योगेन्द्र सिंह ठाकुर, क. शोधकर्ता द्वारा हिम तथा अवधाव अध्ययन संस्थान, चण्डीगढ में दिनांक 9 अप्रैल, 2009 को आयोजित वैज्ञानिक / तकनीकी संगोष्ठी में "लाइम रोग: स्व-अस्तित्व बनाम प्रतिरक्षा दमन" विषय पर वैज्ञानिक वार्ता प्रस्तुत की ।
श्री भुपिंदर सिंह चोपड़ा, तकनीकी सहायक द्वारा हिम तथा अवधाव अध्ययन संस्थान, चण्डीगढ में दिनांक 14 फरवरी, 2011 को आयोजित वैज्ञानिक / तकनीकी संगोष्ठी में "मधुमेह रोग : शिक्षा और विारण" विषय पर वैज्ञानिक वार्ता प्रस्तुत की।

इमटैक में पृथ्वी दिवस समारोह का आयोजन – दिनांक 22 अप्रैल, 2009

पृथ्वी विज्ञान मंत्रालय, भारत सरकार के दिनांक 12.03.09 के अर्धशा. पत्रांक एमओइएस/ए एण्ड पी/2009/पीसी-वी के अनुसरण में पृथ्वी दिवस के उपलक्ष्य में ड्राइंग व पेंटिग प्रतियोगिता के लिए आयोजन संस्थान के निकट सैक्टर 40 व सैक्टर 39 के राजकीय, उच्चतर माध्यमिक विद्यालयों से सम्पर्क किया गया जिसमें से सैक्टर 40 के स्कूल की प्राचार्या सुश्री राजिंदर कोर ने अपने स्कूल में दिनांक 21 अप्रैल, 2009 को उक्त प्रतियोगिता के आयोजन के लिए सहमति दी ।

दिनांक 21 अप्रैल, 2009 को सैक्टर 40 के स्कूल के परिसर में मंत्रालय द्वारा प्रदान किए गए विषयों पर तीनों कक्षा वर्गों के लिए पेंटिग व ड्राइंग की प्रतियोगिता आयोजित की गई । प्रतियोगिता में तीनों आयु वर्गों के कुल मिलाकर 49 विद्यार्थियों ने पूरे

उत्साह से भाग लिया । स्कूल की प्रधानाचार्या द्वारा दिए गए सुझाव के अनुसार आयोजन समिति द्वारा निर्णय लिया गया कि चूंकि राजकीय विद्यालय में छात्रों को मिड डे मील योजना के तहत भोजन प्रदान किया जाता है अत: छात्रों को 30/-रु. प्रति छात्र की दर से जलपान देने के स्थान पर इसी राशि के रंग आदि प्रदान किए जाएँ ।

पत्र में दिए गए निदेशों के अनुसार दिनांक **22 अप्रैल, 2009** को 3.30 बजे संस्थान के सम्मेलन कक्ष में मुख्य समारोह आयोजित किया गया । जिसमें डॉ.पी. गुप्तासरमा, वैज्ञानिक ने पृथ्वी की सतह पर हो रहे परिवर्तनों को ब्रह्माण्ड के परिपेक्ष्य में चित्रित किया तथा बहुत सी रोचक व उपयोगी जानकारी दी । उन्होंने एक नए पहलू के विषय में जानकारी दी कि खगोलीय शोध

के अनुसार पृथ्वी के वातावरण हो रहे परिवर्तन ब्रह्माण्ड में अन्य स्थलों पर भी देखे गए हैं अर्थात् संभवत: आने वाले समय में शोध यह स्पष्ट हो जाए कि यह सब परिवर्तन धरती पर मनुष्य द्वारा किए जा रहे कार्यों की वजह से नहीं अपितु सम्पूर्ण ब्रह्माण्ड में प्राकृतिक तौर पर हो रहें हैं । प्रशासन नियंत्रक श्री स्वतन्त्र कु. सदाना व अन्य वरिष्ठ वैज्ञानिक एवं अधिकारीगण भी इस अवसर पर उपस्थित थे ।

तत्पश्चात् निदेशक, इमटैक डॉ.गिरीश साहनी ने विजेता छात्रों को पुरस्कार वितरित किए । डॉ.गिरीश साहनी ने अपने सम्बोधन में विजेता छात्रों को बधाई दी तथा पृथ्वी के पर्यावरण के विकास में आने वाली पीढ़ी के योगदान पर बल दिया । उन्होंने इस अवसर पर छात्रों को संस्थान के प्रबुध वैज्ञानिक डॉ.पी गुप्तासरमा के सम्भाषण से प्रेरणा ग्रहण करने का आह्वान किया । इस अवसर पर सैक्टर 40 जीएमएसएस स्कूल की कला अध्यापिका भी उपस्थित थीं । प्रतियोगिता के विजेताओं के नाम श्रेणीवार इस प्रकार है:-

श्रेणी - कक्षा दसवीं से बारहवीं

विषय - Violent Earth

1) अवि -XII B - प्रथम पुरस्कार
2) विजय कुमारी -XI - द्वितीय पुरस्कार
3) तजिंदर सिंह -X A - तृतीय पुरस्कार

श्रेणी - कक्षा VI **से** IX

विषय - Global Warming in your eyes

1) अंकित - VIII B - प्रथम पुरस्कार
2) सुमित - VIII B - द्वितीय पुरस्कार
3) कोमल कुमारी - VIII A - तृतीय पुरस्कार

<u>श्रेणी - कक्षा V से तथा उससे नीचे</u>

विषय - Environment Around you

1) सिमरन -VIII B - प्रथम पुरस्कार
2) कमलप्रीत कौर -VIII B - द्वितीय पुरस्कार
3) नैन्सी -VIII A - तृतीय पुरस्कार



उक्त कार्यक्रम के आयोजन के लिए आयोजन समिति/निर्णायक मण्डल के तौर पर डॉ.ए.के.बच्छावत, वैज्ञानिक, डॉ. मनोज राजे, वैज्ञानिक डॉ.पी गुप्तासरमा, वैज्ञानिक तथा सुश्री नवनीत आनंद, हिन्दी अधिकारी ने अपनी सेवाएँ दी । कार्यक्रम की समस्त परिकल्पना/ आयोजन राजभाषा अनुभाग व सामान्य अनुभाग के संयुक्त प्रयासों से किया गया । श्री स्वतन्त्र कु.सदाना, प्रशासन नियंत्रक ने समय-समय पर अमूल्य मार्गदर्शन दिया । रखरखाव अनुभाग, कैंटीन तथा पी.टी.एम. से श्री गैरी बेदी, वैज्ञानिक ने भी अपना सहयोग दिया।

हिन्दी पखवाड़े का आयोजन – वर्ष 2009

सूक्ष्मजीव प्रौद्योगिकी संस्थान, चण्डीगढ़ में दिनांक 01.09.09 से 14.09.09 तक हिन्दी पखवाड़े का आयोजन किया गया जिसके दौरान संस्थान में राजभाषा कार्यान्वयन के लिए अनुकूल वातावरण बनाने के उद्देश्य से ऐसे आयोजन किए गए जिससे संस्थान के सदस्यों को मनोरंजक गतिविधियों के माध्यम से हिन्दी प्रयोग में अपनी प्रतिभा दिखाने के लिए मंच प्रदान किया जाए।

संस्थान के पुस्तकालय में उपलब्ध हिन्दी पुस्तकों की प्रदर्शनी

प्रत्येक वर्ष संस्थान के पुस्तकालय के लिए उच्चकोटि के हिन्दी साहित्य व वैज्ञानिक तथा तकनीकी विषयों की पुस्तकें खरीदी जातीं हैं जिसकी सूची जारी की जाती है । पखवाड़े के दौरान दिनांक 08.09.09 को पुस्तकालय हाल में पुस्तकालय में उपलब्ध हिन्दी पुस्तकों की प्रदर्शनी लगाई गई तथा निदेशक, इमटैक द्वारा इसका उद्घाटन किया गया । उन्होंने आह्वान किया अच्छी पुस्तकें पढ़ने से स्वस्थ मानसिकता का विकास होता है तथा ऐसे आयोजनों से संस्थान के सदस्यों में परस्पर सद्भाव पनपता है । प्रदर्शनी दिनांक 14.09.09 तक प्रदर्शित की गई ।

दिनांक 14.09.09 को संस्थान के सभागार में मुख्य समारोह का आयोजन किया गया । संस्थान के निदेशक डॉ.गिरीश साहनी व्यस्तता के कारण समारोह में भाग नहीं ले सके । समारोह की अध्यक्षता श्री स्वतन्त्र कु. सदाना, प्रशासन नियंत्रक ने की तथा डॉ. निर्मल मित्तल, पूर्व हिन्दी प्रवक्ता, राजकीय महाविद्यालय, चण्डीगढ़ इस अवसर पर मुख्य अतिथि थीं । सर्वप्रथम इस समारोह का मुख्य आकर्षण 'हिन्दी वाद विवाद प्रतियोगिता' आयोजित की गई जिसका विषय था – ''वर्तमान शिक्षा प्रणाली में परिवर्तन जरूरी है'' जिसमें संस्थान के सभी वर्गों के सदस्य पूरे उत्साह से भाग लेते हैं । पक्ष व विपक्ष के दस

प्रतियोगियों ने विषय पर अर्थपूर्ण व सारगर्भित विचार रखे तथा अंत में पक्ष का पलड़ा भारी रहा ।

वाद विवाद प्रतियोगिता के आयोजन के पश्चात् पर श्री स्वतन्त्र कु. सदाना ने संस्थान के सदस्यों हिन्दी में अधिक कार्य करने को प्रेरित करते हुए निदेशक, इमटैक की ओर से संदेश दिया तथा तत्पश्चात् हिन्दी अधिकारी ने मुख्य अतिथि का परिचय दिया व उन्हें व्याख्यान हेतु आमंत्रित किया । डॉ. निर्मल मित्तल ने अंग्रेज़ी भाषा में हिन्दी शब्दों को लिखने की ऐसी प्रक्रिया विकसित की है जिससे उनका उच्चारण न बदले । उन्होंने इस विषय पर एक पुस्तक भी लिखी है । संस्थान के सदस्यों ने इसमें पूरी रुचि दिखाई । इसके पश्चात् मुख्य अतिथि डॉ. निर्मल मित्तल ने पखवाड़े दौरान आयोजित विभिन्न प्रतियोगिताओं के विजेताओं को पुरस्कार प्रदान किए । इसके अतिरिक्त ''सीएसआईआर के विज्ञान में युवा नेतृत्व कार्यक्रम'' की तरह आरंभ की गई स्कूली छात्रों के लिए हिन्दी में विशेष योग्यता प्राप्त करने पर नकद पुरस्कार प्रदान करने की प्रोत्साहन योजनास के तहत भी इमटैक सदस्यों के बच्चों को भी इस अवसरप पर पुरस्कृत किया गया । कार्यक्रम का संचालन सुश्री नवनीत आनंद, हिन्दी अधिकारी ने किया।

विजेताओं के नाम इस प्रकार हैं –

हिन्दी पत्र/आवेदन लेखन प्रतियोगिता

प्रथम पुरस्कार – श्री निगम कुमार, वरिष्ठ शोधकर्ता
द्वितीय पुरस्कार – श्री एस.डी.ऋषि, अनुभाग अधिकारी
तृतीय पुरस्कार – श्री टीकम चंद, कनिष्ठ शोधकर्ता
प्रोत्साहन पुरस्कार – श्री वीरेन्द्र लाम्बा, सहायक

कम्प्यूटर पर हिन्दी टंकण प्रतियोगिता

प्रथम पुरस्कार – श्री राजेन्द्र नौटियाल, सहायक
द्वितीय पुरस्कार – श्री अरूण खुराना, सहायक
तृतीय पुरस्कार – सुश्री तेजिंदर कौर, सहायक
प्रोत्साहन पुरस्कार –श्री भूपाल सिंह रावत, चाय एवं कॉफी बनाने वाला
प्रोत्साहन पुरस्कार –सुश्री मंजू सैनी, कनिष्ठ आशुलिपिक

हिन्दी शब्दज्ञान प्रतियोगिता

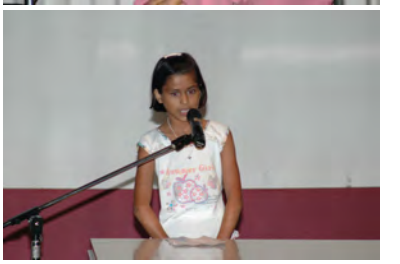
प्रथम पुरस्कार	–	श्री ब्योमकेश पाण्डेय, सहायक
द्वितीय पुरस्कार	–	श्री संजीव कुमार यादव, सहायक
तृतीय पुरस्कार	–	श्री एस.डी.ऋषि, अनुभाग अधिकारी
प्रोत्साहन पुरस्कार	–	श्री हेमंत कुमार वर्मा, वरिष्ठ शोधकर्ता

हिन्दी सुलेख प्रतियोगिता

प्रथम पुरस्कार	–	श्री हरप्रीत सिंह, कनिष्ठ अभियंता
द्वितीय पुरस्कार	–	सुश्री पायल मित्तल, वरिष्ठ शोधकर्ता
तृतीय पुरस्कार	–	श्री सत्यप्रकाश, वरिष्ठ शोधकर्ता
प्रोत्साहन पुरस्कार	–	सुश्री मीनाक्षी, परियोजना सहायक

हिन्दी कविता पाठ प्रतियोगिता

प्रथम पुरस्कार	–	सुश्री क्षिप्रा पचौरी, कनिष्ठ शोधकर्ता
द्वितीय पुरस्कार	–	श्री पंकज कुमार अरोड़ा, वरिष्ठ शोधकर्ता
तृतीय पुरस्कार	–	सुश्री प्रतिष्ठा द्विवेदी, कनिष्ठ शोधकर्ता
प्रोत्साहन पुरस्कार	–	श्री सत्यप्रकाश, वरिष्ठ शोधकर्ता



हिन्दी कविता पाठ प्रतियोगिता – स्टाफ के सदस्यों के बच्चे

प्रथम पुरस्कार	–	कुमारी सुर्पणा, सुपुत्री श्री मदन मोहन
द्वितीय पुरस्कार	–	कुमारी पल्लवी नारद, सुपुत्री श्री एस.के. नारगग
तृतीय पुरस्कार	–	सुश्री शिवानी, सुपुत्री श्री राकेश कुमार धीमान
प्रोत्साहन पुरस्कार	–	सुश्री ममता, सुपुत्री श्री सुरेन्द्र सिंह

हिन्दी में हस्ताक्षर प्रतियोगिता

प्रथम पुरस्कार	–	श्री अतुल कुमार गोयल, अधीक्षक अभियंता
द्वितीय पुरस्कार	–	श्री टीकम चंद, व.शोधकर्ता
तृतीय पुरस्कार	–	डॉ. केश्वेन्द्र प्रताप सिंह सेंगर, पुस्त. सहा.
प्रोत्साहन पुरस्कार	–	श्री मुहम्मद मुस्तफा, तकनीशियन

विज्ञान पर हिन्दी में सेमिनार प्रतियोगिता

प्रथम पुरस्कार	–	श्री असलम खान, कनिष्ठ शोधकर्ता
द्वितीय पुरस्कार	–	डॉ. आशीष गांगुली, वैज्ञानिक
तृतीय पुरस्कार	–	श्री संदीप, कनिष्ठ शोधकर्ता

हिन्दी में वाद विवाद प्रतियोगिता

प्रथम पुरस्कार	–	सुश्री क्षिप्रा पचौरी, क.शोधकर्ता
द्वितीय पुरस्कार	–	श्री पंकज कुमार अरोड़ा,व.शो.
तृतीय पुरस्कार	–	श्री यशपाल, व.शोधकर्ता
प्रोत्साहन पुरस्कार	–	सुश्री अनमोल दीप रंधावा,क.शो.

इमटैक स्टाफ के सदस्यों के बच्चें द्वारा हिन्दी में विशेष योगयता प्राप्त करने पर प्रोत्साहन

छात्र का नाम	**इमटैक सदस्य कस नाम**	**प्रतिशत**
अदिती	सुपुत्री श्री राजकुमार-।।	89.5%
कक्षा IX	तकनीशियन	
कोनिका शर्मा	पुत्री श्री देशराज अग्निहोत्री	87%
कक्षा IX	टूल मैकेनिक	
अभिषेक मेहता	डॉ0 राज कुमार मेहता	88.5%
कक्षा VIII	तकनीकी अधिकारी	

हिन्दी में मूल कामकाज की प्रोत्साहन पुरस्कार योजना – कैलेंडर वर्ष 2008

हिन्दी में मूल कामकाज के लिए चलाई जा रही प्रोत्साहन योजना में भाग लेने वाले सदस्यों के कार्य के मूल्यांकन के लिए गठित मूल्यांकन समिति की संस्तुति के आधार पर निदेशक, इमटैक ने संस्थान के कार्य की प्रकृति के अनुरूप उक्त प्रोत्साहन योजना को संस्थान स्तर पर निम्नलिखित प्रकार से लागू किया है जिससे संस्थान में हिन्दी में कामकाज की मात्रा बढ़े तथा अधिक से अधिक सदस्य प्रोत्साहन योजना के अंतर्गत हिन्दी में किए गए अपने कार्य का मूल्यांकन करवाएँ:-
कार्य की प्रकृति/मात्रा के अनुरूप निम्नलिखित पुरस्कार प्रदान किए जाते है –

नेटिंग कार्य

प्रथम पुरस्कार	–	श्री राजिंदर नौटियाल, सहायक
द्वतीय पुरस्कार	–	श्री संजीव कुमार, सहायक
तृतीय पुस्कार	–	श्री भागीरथ, सहायक
प्रोत्साहन पुरस्कार	–	श्री अरुण खुराना, सहायक

एंट्री का कार्य

प्रथम पुरस्कार	–	श्री हरिंदर सिंह बिष्ट, तकनीशियन
द्वितीय पुरस्कार	–	श्री मु. मुस्तफा, तकनीशियन
तृतीय पुरस्कार	–	श्री गोपाल, सहायक
प्रोत्साहन पुरस्कार	–	सुश्री बिमला, सहायक
प्रोत्साहन पुरस्कार	–	श्री मुहम्मद युनूस, प्रयोग.परिचारक
प्रोत्साहन पुरस्कार	–	सुश्री मनोज रानी, क.आशु.

इसके अतिरिक्त निम्नलिखित सदस्य जिन्होंने वर्ष 2008 के दौरान अपना अधिकतर कार्य हिन्दी में किया उन्हें भी सान्त्वना पुरस्कार प्रदान किया गया –

1. श्री भूपाल सिंह रावत, च./कॉ. बनाने वाला
2. श्री देशराज अग्निहोत्री, टूल मैकेनिक
3. श्री दविंदर सिंह, प्रयोगशाला पर्यवेक्षक
4. सुश्री सुदेश शर्मा, सहायक
5. सुश्री रानी, सहायक
6. श्री जसवंत सिंह, चालक
7. श्री अतुल कुमार गोयल, अधीक्षक अभियंता
8. श्री रविंदर कन्नौजिया, तक. अधि.

राजभाषा निरीक्षण समिति द्वारा हिन्दी पखवाड़े के दौरान संस्थान के अनुभागों का राजभाषा संबंधी निरीक्षण

निदेशक, इमटैक द्वारा संस्थान की ओर से संसदीय समिति की दूसरी उपसमिति को दिए गए आश्वासनों की पूर्ति की दिशा में हो रही प्रगति की मॉनिटरिंग हेतु गठित निरीक्षण समिति निम्नलिखित समिति द्वारा हिन्दी पखवाड़े के दौरान दिनांक 02.09.09 को संस्थान के अनुभागों का निरीक्षण किया गया –

1. श्री एस.डी.भट्ट, वैज्ञानिक एवं राजभाषा अधिकारी
2. श्री छेरिंग तोबदन, प्रशासनिक अधिकारी
3. डॉ. देबेन्दु सरकार, वैज्ञानिक
4. सुश्री नवनीत आनंद, हिन्दी अधिकारी
5. डॉ. सुरेन्द्र कुमार शर्मा, सहायक निदेशक–बाहरी आमंत्रित सदस्य(रा.भा.), एवं सदस्य–सचिव, नराकास समिति, चण्डीगढ़

प्रत्येक निरीक्षित प्रयोगशाला/अनुभाग में राजभाषा कार्यान्वयन की सुचारुता सुनिश्चित करने के उद्देश्य से एक नोडल सदस्य नियत किया गया है –

प्रयोगशाला/अनुभाग	**नोडल सदस्य**
डॉ. अनिर्बान राय चौधरी–	श्री जयदीप मेहता, तकनीशियन
डॉ.पी.गुप्तासरमा–	सुश्री शरणजीत कौर, तकनीशियन
मॉड्यूलर अभियांत्रिकी–	श्री एच.बी.के.भट्टी, तकनीकी अधिकारी
डॉ.डी.के.साहू–	श्री मुकेश, तकनीशियन
डॉ.पी.आर.पटनायक–	श्री अशोक बत्ता, व.आशु.
डॉ.आर.एस.जौली–	श्री प्रदीप कुमार, तकनीशियन
डॉ.लता वर्मा–	श्री दिनेश कुमार, तकनीशियन
भण्डार–	श्री बीर सिंह, सहायक
क्रय–	श्री प्रवीण कुमार, क.आशु.
ई.एस.डी.–	श्री मुहम्मद मुस्तफा, तकनीशियन
पीटीएम–	सुश्री बलजीत कौर, क.आशु.
निदेशक सचिवालय–	श्री रघुनाथ, सहायक
प्र. नि. कार्यालय –	श्री अवतार सिंह, निजी सचिव
प्रशा. अधि. कार्यालय–	सुश्री मंजू सैनी, क.आशु.
वि. एवं लेखा अनुभाग–	सुश्री मनोज रानी, क.आशु.
स्था.।। अनुभाग–	श्री राजेन्द्र नौटियाल, सहायक
स्था.। अनुभाग–	श्री संजीव कुमार, सहायक
स्वागत कक्ष–	सुश्री वीरकांता, तकनीशियन

निरीक्षित प्रयोगशालाओं/अनुभागों में से कुछेक में जहाँ भी नई निदेशक पट्टिकाओं में धारा 3(3) के अनुपालन में कमी पाई गई इसे सुनिश्चित करने की ओर ध्यान दिलाया गया ।

डॉ.आर.एस.जौली, वैज्ञानिक, डॉ.के.एल.दीक्षित, वैज्ञानिक डॉ.नीरज खत्री, वैज्ञानिक पथसथथ श्री छेरिंग तोबदन, प्रशासनिक अधिकारी ने निर्णायक मंडल के तौर पर तथा आयोजन की अन्य परिकल्पना में राजभाषा अनुभाग को अपना पूरा सहयोग दिया । इसके अतिरिक्त श्री स्वतंत्र कु. सदाना, प्रशासन नियंत्रक ने समय-समय पर अमूल्य मार्गदर्शन एवं सहयोग दिया । श्री एस.डी.ऋषि, अनुभाग अधिकारी, श्री राजेन्द्र नौटियाल, सहायक (हिन्दी) एवं श्री टीकमचंद, व.शोधकर्ता ने भी आयोजन में अपना पूरा सहयोग दिया । इस अवधि के दौरान इस बात पर विशेष ध्यान दिया गया कि राजभाषा के प्रयोग के लिए संस्थान में अनुकूल वातावरण बने और हिन्दी के प्रयोग की ओर सदस्यों का रुझान बढ़े ।

हिन्दी पखवाड़े का आयोजन – वर्ष 2010

सूक्ष्मजीव प्रौद्योगिकी संस्थान, चण्डीगढ़ में दिनांक 01.09.10 से 14.09.10 तक हिन्दी पखवाड़े का आयोजन किया गया जिसके दौरान संस्थान में राजभाषा कार्यान्वयन के लिए अनुकूल वातावरण बनाने के उद्देश्य से ऐसे आयोजन किए गए जिससे संस्थान के सदस्यों को मनोरंजक गतिविधियों के माध्यम से हिन्दी प्रयोग में अपनी प्रतिभा दिखाने के लिए मंच प्रदान किया जाए ।

दिनांक 14.09.10 को संस्थान के सभागार में मुख्य समारोह का आयोजन किया गया । संस्थान के निदेशक डॉ.गिरीश साहनी व्यस्तता के कारण समारोह में भाग नहीं ले सके । समारोह की अध्यक्षता डॉ.कनक लता दीक्षित, वैज्ञानिक एवं राजभाषा अधिकारी ने की तथा श्री छेरिंग तोबदन, प्रशासनिक अधिकारी तथा राजभाषा कार्यान्वयन समिति के सदस्य भी इस अवसर पर उपस्थित थे । सर्वप्रथम इस समारोह का मुख्य आकर्षण 'हिन्दी वाद विवाद प्रतियोगिता' आयोजित की गई जिसका विषय था – ''पाश्चात्य संस्कृति छात्रों के चारित्रिक निर्माण में सहायक है'' जिसमें संस्थान के सभी वर्गों के सदस्य पूरे उत्साह से भाग लेते है । पक्ष व विपक्ष के बारह प्रतियोगियों ने विषय पर अर्थपूर्ण व सारगर्भित विचार रखे तथा अंत में पक्ष का पलड़ा भारी रहा ।

वाद विवाद प्रतियोगिता के आयोजन ठथम्क क्ष्थपैथथपथख पर डॉ.कनक लता दीक्षित ने संस्थान के सदस्यों हिन्दी में अधिक कार्य करने को प्रेरित करते हुए निदेशक, इमटैक की ओर से संदेश दिया तथा हिन्दी-अंग्रेज़ी टेलीफोन निदेर्शिका जारी की । संस्थान में टेलिफोन डायरैक्ट्री ई.मेल द्वारा जारी की जाती है तथा इसे यूनीकोड में जारी किया गया । डॉ.दीक्षित ने पखवाड़े दौरान आयोजित विभिन्न प्रतियोगिताओं के विजेताओं को पुरस्कार प्रदान किए । इस दौरान हिन्दी की विभिन्न प्रतियोगिताओं का आयोजन किया जाता है जिसमें शामिल थी - हिन्दी निबंध प्रतियोगिता, शब्द ज्ञान प्रतियोगिता, पत्र/नोटिंग/आवेदन लेखन प्रतियोगिता, हिन्दी में हस्ताक्षर प्रतियोगिता, हिन्दी कविता पाठ प्रतियोगिता एवं वाद-विवाद प्रतियोगिता आदि । इस बार एक नई प्रतियोगिता - हिन्दी में वैज्ञानिक पोस्टर बनाने की प्रतियोगिता आरंभ की गई ।

इसके अतिरिक्त ''सीएसआईआर के विज्ञान में युवा नेतृत्व कार्यक्रम'' की तरह स्कूली छात्रों के लिए हिन्दी में विशेष योग्यता प्राप्त करने पर नकद पुरस्कार प्रदान करने की प्रोत्साहन योजना के तहत इमटैक सदस्यों के बच्चों को भी इस अवसर पर पुरस्कृत किया गया । कार्यक्रम का संचालन सुश्री नवनीत आनंद, हिन्दी अधिकारी ने किया ।

विजेताओं के नाम इस प्रकार हैं -

हिन्दी पत्र/आवेदन लेखन प्रतियोगिता

प्रथम पुरस्कार - श्री संजीव कुमार यादव, सहायक
द्वितीय पुरस्कार - सुश्री सोनिका, परियोजना सहायक
तृतीय पुरस्कार - सुश्री रेणु, तकनीशियन
प्रोत्साहन पुरस्कार - श्री मदन मोहन प्रमाणिक, अतिथि गृह परिचारक

कम्प्यूटर पर हिन्दी टंकण प्रतियोगिता

प्रथम पुरस्कार - श्री राजेन्द्र नौटियाल, सहायक
द्वितीय पुरस्कार - श्री अरुण खुराना, सहायक
तृतीय पुरस्कार - श्री रविंद्र सिंह, क.आशुलिपिक
प्रोत्साहन पुरस्कार - सुश्री मनोज रानी, क.आशुलिपिक

हिन्दी शब्दज्ञान प्रतियोगिता

प्रथम पुरस्कार - श्री हेमंत कुमार वर्मा, वरिष्ठ शोधकर्ता
द्वितीय पुरस्कार - श्री संजीव कुमार यादव, सहायक
द्वितीय पुरस्कार - श्री अनुज पाठक, वरिष्ठ शोधकर्ता
तृतीय पुरस्कार - सुश्री प्रेरणा शर्मा, कनिष्ठ शोधकर्ता
तृतीय पुरस्कार - श्री ब्योमकेश पाण्डेय, सहायक
तृतीय पुरस्कार - श्री राजेन्द्र नौटियाल, सहायक
प्रोत्साहन पुरस्कार - श्री विरेन्द्र लाम्बा, सहायक

निबंध प्रतियोगिता

प्रथम पुरस्कार – श्री टीकम चंद, वरिष्ठ शोधकर्ता
द्वितीय पुरस्कार – श्री सत्यप्रकाश, वरिष्ठ शोधकर्ता
तृतीय पुरस्कार – सुश्री प्रेरणा शर्मा, कनिष्ठ शोधकर्ता
प्रोत्साहन पुरस्कार – सुश्री रेणु, तकनीशियन

हिन्दी कविता पाठ प्रतियोगिता

प्रथम पुरस्कार – सुश्री क्षिप्रा पचौरी, कनिष्ठ शोधकर्ता
द्वितीय पुरस्कार – श्री पंकज कुमार अरोड़ा, वरिष्ठ शोधकर्ता
तृतीय पुरस्कार – सुश्री सोनिका, परियोजना सहायक
प्रोत्साहन पुरस्कार – श्री अखिलेश कुमार, वरिष्ठ शोधकर्ता

हिन्दी कविता पाठ प्रतियोगिता – स्टाफ के सदस्यों के बच्चे

प्रथम पुरस्कार – कु. शिवानी धीमान, सुपुत्री श्री राकेश कु. धीमान
द्वितीय पुरस्कार – कुमारी सृष्टि सुपुत्री श्री धीरज गोस्वामी
तृतीय पुरस्कार – पुरु मेहता सुपुत्र श्री जयदीप मेहता
प्रोत्साहन पुरस्कार – कुमारी नव्या सुपुत्री डॉ. नीरज खत्री
प्रोत्साहन पुरस्कार – कु. अनीषा सुपुत्री डॉ. दिब्येन्दु सरकार
प्रोत्साहन पुरस्कार – कु. सरा सुपुत्री श्री मोहम्मद मुस्तफा

हिंदी में हस्ताक्षर प्रतियोगिता

प्रथम पुरस्कार – श्री हेमंत कुमार वर्मा, वरिष्ठ शोधकर्ता
द्वितीय पुरस्कार – श्री वैभव कुमार पंड्या, व.शोधकर्ता
तृतीय पुरस्कार – श्री जानकी प्रसाद, तकनीशियन
प्रोत्साहन पुरस्कार – श्री संजीव यादव, सहायक

हिंदी में पोस्टर प्रतियोगिता

प्रथम पुरस्कार – श्री वैभव कुमार पंड्या, व. शोधकर्ता
द्वितीय पुरस्कार – श्री भूपिदंर सिंह चोपड़ा, तक. सहायक
तृतीय पुरस्कार – सुश्री सोनिका, परियोजना सहायक
प्रोत्साहन पुरस्कार – सुश्री क्षिप्रा पचौरी, कनिष्ठ शोधकर्ता

इसके अतिरिक्त वर्ष के दौरान आयोजित

विज्ञान पर हिन्दी में सेमिनार प्रतियोगिता

प्रथम पुरस्कार – श्री योगेन्द्र सिंह राठौर, क. शोधकर्ता
द्वितीय पुरस्कार – सुश्री प्रतिष्ठा द्विवेदी, शोधकर्ता
तृतीय पुरस्कार – श्री भूपिंदर सिंह चोपडा, तक.सहायक
प्रोत्साहन पुरस्कार – सुश्री लीना मलिक, शोधकर्ता

इमटैक सदस्यों के बच्चें द्वारा हिन्दी में विशेष योग्यता प्राप्त करने पर प्रोत्साहन

छात्र का नाम	**इमटैक सदस्य कस नाम**	**प्रतिशत**
अदिती, कक्षा X	सुपुत्री श्री राजकुमार-।।,तक.	91%
कोनिका शर्मा, कक्षा X	सुपुत्री श्री देशराज अग्निहोत्री, टूल मैकेनिक	85.5%
शिराग बत्ता, कक्षा X	सुपुत्र श्री अशोक कुमार बत्ता, व. आशु	85.5%
हरमनप्रीत कौर, कक्षा VIII	सुपुत्री श्री गुरदीप सिंह, सहायक	90%

हिन्दी में वाद विवाद प्रतियोगिता

दिनांक 20.04.10 को **''महिलाओं के लिए राजनीति में आरक्षण एक प्रगतिशील नीति है''** विषय पर आयोजित प्रतियोगिता का घोषित किया गया परिणाम इस प्रकार है -

प्रथम पुरस्कार - श्री पंकज अरोड़ा, व.शोधकर्ता
द्वितीय पुरस्कार - सुश्री पूनम, क.शोधकर्ता
तृतीय पुरस्कार - सुश्री रचना, व.शोधकर्ता
तृतीय पुरस्कार - श्री अखिलेश कुमार, व.शोधकर्ता
विशेष पुरस्कार - श्री चेतन प्रेमाणी, वैज्ञानिक

हिन्दी में वाद विवाद प्रतियोगिता

दिनांक 14.09.10 को **''पाश्चात्य संस्कृति छात्रों के चारित्रिक निर्माण में सहायक है''** विषय पर आयोजित प्रतियोगिता का घोषित किया गया परिणाम इस प्रकार है -

प्रथम पुरस्कार - श्री पंकज अरोड़ा, व.शोधकर्ता
द्वितीय पुरस्कार - श्री जयदीप मेहता, तकनीशियन
तृतीय पुरस्कार - श्री अनुज पाठक, व.शोधकर्ता
सांत्वना पुरस्कार - सुश्री क्षिप्रा पचौरी, व. शोधकर्ता
सांत्वना पुरस्कार - श्री यशपाल, व. शोधकर्ता

हिन्दी में मूल कामकाज की प्रोत्साहन पुरस्कार योजना - कैलेंडर वर्ष 2009

हिन्दी में मूल कामकाज के लिए चलाई जा रही प्रोत्साहन योजना में भाग लेने वाले सदस्यों के कार्य के मूल्यांकन के लिए गठित मूल्यांकन समिति की संस्तुति के आधार पर निदेशक, इमटैक ने संस्थान के कार्य की प्रकृति के अनुरूप उक्त प्रोत्साहन योजना को संस्थान स्तर पर निम्नलिखित प्रकार से लागू किया है जिससे संस्थान में हिन्दी में कामकाज की मात्रा बढ़े तथा अधिक से अधिक सदस्य प्रोत्साहन योजना के अंतर्गत हिन्दी में किए गए अपने कार्य का मूल्यांकन करवाएँ:-

कार्य की प्रकृति/मात्रा के अनुरूप निम्नलिखित पुरस्कार प्रदान किए जाते है -

नोटिंग कार्य

प्रथम पुरस्कार - श्री राजिंदर नौटियाल, सहायक
द्वितीय पुरस्कार - श्री संजीव कुमार, सहायक
तृतीय पुस्कार - श्री नारद, सहायक

प्रविष्टियों का कार्य

प्रथम पुरस्कार	–	श्री मो. मुस्तफा, तकनीशियन
द्वितीय पुरस्कार	–	सुश्री बिमला, सहायक
द्वितीय पुरस्कार	–	श्री गोपाल, सहायक
तृतीय पुरस्कार	–	सुश्री मनोज रानी, क.आशु.
तृतीय पुरस्कार	–	श्री हरिंदर सिंह बिष्ट, तकनीशियन
तृतीय पुरस्कार	–	श्री दविंदर सिंह, प्रयोगशाला पर्यवेक्षक
प्रोत्साहन पुरस्कार	–	सुश्री सुदेश शर्मा, सहायक
प्रोत्साहन पुरस्कार	–	श्री देसराज अग्निहोत्री, सहायक
प्रोत्साहन पुरस्कार	–	श्री मुहम्मद युनूस, प्रयोगशाला परिचारक
प्रोत्साहन पुरस्कार	–	श्री भूपाल सिंह रावत, च/कॉ बनानेवाला

इसके अतिरिक्त निम्नलिखित सदस्य जिन्होंने वर्ष 2009 के दौरान अपना अधिकतर कार्य हिन्दी में किया उन्हें भी सान्त्वना पुरस्कार प्रदान किया गया –

1. श्री अरुण खुराना, सहायक
2. सुश्री रानी, सहायक
3. सुश्री वीरकांता शर्मा, तकनीशियन
4. श्री ब्योमकेश पांडेय, सहायक
5. सुश्री कुलदीप कौर, सहायक
6. श्री अनिल शर्मा, सूचीकार
7. श्री राकेश कुमार धीमान, तक
8. श्री रविंदर कन्नौजिया, तक. अधि.
9. श्री चन्द्रप्रकाश, तकनीशियन
10. श्री जसवंत सिंह, चालक

हिन्दी पखवाड़े के दौरान संस्थान के सदस्यों के लिए हिन्दी कार्यशाला (टेबल वर्कशॉप) का आयोजन

राजभाषा नियमों के अनुसार हिन्दी का ज्ञान रखने वाले सदस्यों को वर्ष में एक बार कार्यशाला में भाग लेना/प्रशिक्षण लेना अनिवार्य है । संस्थान में एक कार्य प्रकृति के स्टाफ की संख्या सीमित होने के कारण हिन्दी में कार्य करने के अभ्यास के लिए सामूहिक कार्यशाला के स्थान पर टेबल वर्कशाप का आयोजन अनुभाग स्तर पर किया जाता है । इसमें नियमितता लाने के उद्देश्य से प्रत्येक तिमाही में एक वर्ग प्रकार की कार्यशाला व्यक्ति स्तर पर आयोजित की जाती है तथा इसी कड़ी में हिन्दी पखवाड़े के दौरान दिनांक 02 सितम्बर, 2010 को टेबल वर्कशाप का आयोजन किया गया जिसमें निम्नलिखित के साथ-साथ विगत तिमाहियों में आयोजित की गई टेबल वर्कशाप में शेष सदस्यों की टेबल वर्कशाप आयोजित की गई जिसमें राजभाषा नियमों के साथ-साथ सदस्यों को यूनीकोड का प्रयोग करते हुए कम्प्यूटर पर अंग्रेज़ी की-बोर्ड के माध्यम से हिन्दी टाइपिंग की भी जानकारी दी गई ।

तकनीशियन

क्रम सं.	**नाम**	**क्रम सं.**	**नाम**
1.	श्री भूपेन्द्र कुमार	17.	श्री रमेश चन्द शर्मा
2.	श्री हरिन्दर सिंह बिष्ट	18.	श्री जानकी प्रसाद
3.	श्री राजकुमार (I)	19.	सुश्री गिरिजा दित्या
4.	श्री परमजीत	20.	श्री देसराज अग्निहोत्री
5.	श्री अनिल थियोफिलिस	21.	श्री राकेश कुमार धीमान

6.	श्री रोहतास रंगा	22.	सुश्री रेनु
7.	श्री मामनचंद	23.	श्री चन्द्र प्रकाश मिढ्ढा
8.	श्री उपेन्द्र सिंह	24.	श्री मुकेश कुमार
9.	श्री सुरेन्द्र सिंह	25.	श्री दिनेश कुमार
10.	श्री मलकीत सिंह	26.	श्री रणदीप शर्मा
11.	सुश्री शरणजीत कौर	27.	श्री हरमिंदर सिंह
12.	श्री जयदीप मेहता	28.	श्री मनोज कुमार
13.	श्री सेलवन	29.	सुश्री नेहा राणा
14.	श्री मोहम्मद मुस्तफा	30.	श्री केवल कृष्ण
15.	श्री राजकुमार (II)	31.	श्री अनिल शर्मा
16.	सुश्री वीरकांता	32.	श्री भीमी राम

पत्राचार पाठयक्रम द्वारा निजी प्रयत्नों से हिन्दी टाइपलेखन परीक्षा निजी प्रयत्नों से हिन्दी टाइपलेखन परीक्षा पास करने पर तथा विशेष योग्यता प्राप्त करने पर प्रोत्साहन प्रदान किए गए ।

सुश्री तजिंदर कौर, सहायक
सुश्री मंजू सैनी, क.आशु.
सुश्री मंजू सैनी, क.आशु. ने अखिल भारतीय स्तर पाँचवा स्थान प्राप्त किया ।

डॉ. स्वर्णजीत सिंह, वै., डॉ चारू शर्मा, वै., डॉ. पवन गुप्ता, वैज्ञानिक, श्री छेरिंग तोपदन, प्रशासनिक अधिकारी तथा सुश्री नवनीत आनंद, हिन्दी अधिकारी ने निर्णायक मंडल के तौर पर अपनी सेवाएँ दीं तथा आयोजन की अन्य परिकल्पना में राजभाषा अनुभाग को अपना पूरा सहयोग दिया । श्री एस.डी.ऋषि, अनुभाग अधिकारी, श्री राजेन्द्र नौटियाल, सहायक (हिन्दी) ने भी आयोजन में अपना पूरा सहयोग दिया । इस अवधि के दौरान इस बात पर विशेष ध्यान दिया गया कि राजभाषा के प्रयोग के लिए संस्थान में अनुकूल वातावरण बने और हिन्दी के प्रयोग की ओर सदस्यों का रुझान बढ़े ।

अनुवाद कार्यशाला – दिनांक 04-05.12.09.09 को नराकास के सहयोग से नगर स्तर पर केन्द्रीय कार्यालयों के अनुवादकों के लिए अनुवाद कार्यशाला आयोजित की गई । जिसमें चण्डीगढ़ स्थित केन्द्रीय कार्यालयों के 36 अनुवादकों ने भाग लिया तथा डॉ.गिरीश साहनी, निदेशक इमटैक द्वारा उद्घाटन किया गया ।
समापन सत्र में श्री स्वतंत्र कु.सदाना, प्रशासन नियंत्रक ने समापन सत्र की अध्यक्षता की तथा प्रतिभागियों को प्रमाण पत्र वितरित किए ।
शब्दावली कार्यशाला – (दिनांक 07.12.09 से 08.12.09 तक)

संस्थान में राजभाषा नियमों के सुचारु कार्यान्वयन की दृष्टि से समय - समय पर विभिन्न गतिविधियाँ आयोजित की जातीं हैं । इसी कड़ी में संस्थान में विगत वर्षो में नए भर्ती व हिन्दी का कार्यसाधक ज्ञान प्राप्त सदस्यों को राजभाषा हिन्दी में कार्य करने के लिए जागरूक करने, नियमों से अवगत कराने व राजभाषा में कार्य करने के लिए सहयोग देने के उद्देश्य से वैज्ञानिक एवं तकनीकी शब्दावली आयोग, नई दिल्ली के सहयोग से **दिनांक 7 व 8 दिसम्बर, 2009** को दो दिवसीय शब्दावली कार्यशाला का आयोजन किया गया ।

कार्यशाला का उद्घाटन निदेशक, इमटैक की ओर से संस्थान के राजभाषा अधिकारी श्री एस.डी.भट्ट, वैज्ञानिक ने किया तथा श्री छेरिंग तोबदन, प्रशासनिक अधिकारी, इमटैक भी इस अवसर पर उपस्थित है । उद्घाटन कथन के दौरान श्री एस.डी.भट्ट, वैज्ञानिक ने इस बात पर विशेष बल दिया कि लोकप्रिय विज्ञान के क्षेत्र में हिन्दी में लेखन की व्यापक उपयोगिता एवं संभावनाएँ हैं जिससे वैज्ञानिक उपलब्धियों को आम जनता तक पहुँचाया जा सकता है परन्तु वैज्ञानिक शोध के क्षेत्र में हमारे देश में हिन्दी की ऐसी

उच्चस्तरीय शोध पत्रिकाओं का अभाव है जिनका उच्च इमपैक्ट फैक्टर हो तथा उच्चस्तरीय वैज्ञानिक शोध को प्रकाशित किया जा सके । वैज्ञानिक एवं तकनीकी शब्दावली आयोग, नई दिल्ली के अध्यक्ष प्रो.के.विजय कुमार कार्यक्रम-परिवर्तन के कारण उद्घाटन सत्र में भाग नहीं ले सके तथा डॉ.धर्मेन्द्र कुमार, सहायक निदेशक ने आयोग का प्रतिनिधित्व किया तथा आयोग के कार्य व उपलब्धियों की जानकारी दी । उन्होंने आश्वासन दिया कि हिन्दी की ऐसी उच्चस्तरीय शोध पत्रिकाओं का अभाव काफी देर से महसूस किया जा रहा है तथा वे इसे आयोग के ध्यान में लाएँगे तथा विशेष प्रयास करेगे कि इस संबंध कोई ठोस कदम उठाया जाए।

अन्य विभूतियों में केन्द्रीय हिन्दी प्रशिक्षण संस्थान, नई दिल्ली से सेवानिवृत प्रो.क.के. गोस्वामी व डॉ. ठाकुरदास, भाषाविद् तथा डॉ.प्रदीप शर्मा,

वैज्ञानिक एफ एवं संपादक, विज्ञान प्रगति, सीएसआईआर, नई दिल्ली ने उद्घाटन सत्र में भाग लिया । डॉ.प्रदीप शर्मा जोकि वैज्ञानिक शोध से विज्ञान लेखन की ओर मुड़े हैं, इमटैक के वैज्ञानिकों से अपने अनुभव साझा किए कि किस प्रकार विज्ञान प्रगति पत्रिका के माध्यम से छात्रों व आम जनता की विज्ञान में अभिरुचि बढ़ रही है । दो दिवसीय कार्यशाला में केन्द्रीय हिन्दी प्रशिक्षण संस्थान से प्रो.क.के.गोस्वामी व डॉ.ठाकुरदास डॉ.निमिष कपूर, वैज्ञानिक, विज्ञान प्रसार, डी.एस.टी.नोएडा, डॉ.निखिल कपूर, वैज्ञानिक एफ, एनबीआरआई, लखनऊ ने विभिन्न विषयों पर सारगर्भित एवं रोचक व्याख्यान दिए ।

कार्यशाला में भाग लेने हेतु विगत वर्षों में नए भर्ती लगभग 60 सदस्य नामित किए गए जिसमें वैज्ञानिक, तकनीकी स्टाफ तथा प्रशासन के सदस्य व अधिकारी शामिल थे ।

समापन समारोह की अध्यक्षता निदेशक, इमटैक की ओर से श्री एस.डी.भट्ट, वैज्ञानिक जी एवं राजभाषा अधिकारी ने की तथा प्रो. क.के.गोस्वामी, सेवानिवृत केन्द्रीय हिन्दी प्रशिक्षण संस्थान ने प्रतिभागियों को प्रमाण पत्र वितरित किए । कार्यशाला का संचालन डॉ. धर्मेन्द्र कुमार, सहायक निदेशक एवं सुश्री नवनीत आनंद, हिन्दी अधिकारी, इमटैक ने किया ।

राजभाषा अधिकारी सम्मेलन – सुश्री नवनीत आनंद, हिन्दी अधिकारी ने दिनांक 16 व 17.07.09 को क्षेत्रीय प्रबंधन संस्थान द्वारा संयुक्त रूप से आयोजित एक-दिवसीय राजभाषा अधिकारी सम्मेलन में भाग लिया व संस्थान में हो रही राजभाषा गतिविधियों की प्रस्तुति दी ।

इमटैक को राजभाषा पुरस्कार – वर्ष 2008-09 में राजभाषा प्रयोग के सराहनीय प्रयासों के लिए संस्थान को नगर राजभाषा कार्यान्वयन समिति, चण्डीगढ़ द्वारा 'द्वितीय पुरस्कार' प्रदान किया गया । यह पुरस्कार टैगोर थियेटर, में दिनांक 18.02.2010 को नराकास, चं. के वार्षिक राजभाषा समारोह में श्री पी.के.चोपड़ा, मुख्य आयकर आयुक्त द्वारा प्रदान किया गया । इसमें निदेशक डॉ. गिरीश साहनी के साथ-साथ श्री एस.डी.भट्ट, वै. एवं राजभाषा अधिकारी के साथ-साथ सुश्री नवनीत आनंद, हिन्दी अधिकारी को भी सम्मानित किया गया ।

नराकास, चण्डीगढ़ द्वारा नगर स्तर पर आयोजित स्व-रचित काव्य पाठ प्रतियोगिता-इमटैक को प्रथम स्थान

नराकास, चण्डीगढ़ द्वारा आयोजित स्व-रचित काव्य पाठ प्रतियोंगिता के दूसरे व अंतिम चरण का आयोजन दिनांक 09.09.10 को दूरदर्शन केन्द्र, चण्डीगढ़ में किया गया जिसमें भाग लेने के लिए संस्थान की सुश्री क्षिप्रा पचौरी, वरिष्ठ शोधकर्ता को चुना गया जिसकी रिकार्डिंग दूर दर्शन से प्रसारित की गई तथा इसका प्रसारण हिन्दी पखवाड़े के दौरान संस्थान के सभागार में किया जाता है।

इमटैक, चण्डीगढ़ में सतर्कता जागरूकता सप्ताह का आयोजन

भारत सरकार के केन्द्रीय सर्तकता आयोग से प्राप्त अनुदेशो के अनुसरण मे सूक्ष्मजीव प्रौद्योगिकी संस्थान, चण्डीगढ़ इमटैकद्ध में दिनांक 25.10.10 को सतर्कता जागरूकता सप्ताह का आरंभ शपथ ग्रहण समारोह से किया गया । संस्थान के सेमिनार हाल मे डॉ. गिरीश साहर्नी, निदेशक, इमटैक ने संस्थान के सदस्यो को कर्तव्य पालन व सत्यनिष्ठा की शपथ दिलाई । संस्थान के सभी वरिष्ठ वैज्ञानिक एवं वरिष्ठ अधिकारी इसमे उपस्थित थे ।

इसके अतिरिक्त संस्थान के सदस्यों मे सतर्कता के प्रति जागरूकता उत्पन्न करने के उद्देश्य से सप्ताह के दौरान इस विषय पर हिन्दी मे निबंध लेखन व स्लोगन लेखन प्रतियोगिताएँ आयोजित की गईं जिनके विजेता इस प्रकार हैं :

स्लोगन लेखन प्रतियोगिता

श्री भूपिंदर सिंह चोपड़ाए तकनीकी सहायक - प्रथम पुरस्कार

सबके हित की, सबके हक की व्यवस्था होगी जनमत की,
यदि भ्रष्ट प्रणाली हो खत्म, दिशा होगी प्रगति पथ की ।

श्री राजेन्द्र नौटियाल, सहायक - द्वितीय पुरस्कार
सतर्कता की उपलक्ष्य में, आओ जपैं एक नया मंत्र,
ईमानदारी की रोटी, गर्व की साँस, चैन की नींद और
तंदुरस्त, लोकतंत्र । जय हो ।

श्री दविन्द्र सिंह, प्रयोगशाला पर्यवेक्षक . तृतीय पुरस्कार
सर्तक जागरूकता है वो अभियान ।
सुदृढ़ देश की बने पहचान ।।
श्री भूपाल सिंह, . तृतीय पुरस्कार

अखण्डता व पारदर्शिता लाये हम, संस्थान के कार्य को स्वच्छ बनाये हम ।।

हिन्दी निबंध लेखन

प्रस्तावित विषय –

1. गैर इरादतन भ्रष्टाचार से बचने के उपाय/सुझाव/Measures to avoid unintentional corruption
2. सरकारी खर्च में मितव्ययिता के लिए सरकारी नीतियों में बदलाव/Changes required in Govt. Policies to accommodate austerity measures

विजेताओं के नाम

हथथभ जानकी प्रसाद, तकनीशियन – प्रथम पुरस्कार
हथथभ पंकज कुमार अरोड़ा, शोधकर्ता – द्वितीय पुरस्कार
श्री चन्द्रप्रकाश मिढ़ढा, तकनीशियन – तृतीय पुरस्कार

दिनांक 29.10.10 को समापन दिवस पर एक वार्ता आयोजित की गई थी । इस आयोजन का मुख्य उद्देश्य संस्थान के सदस्यों को सतर्कता के सकारात्मक स्वरूप से परिचित कराना तथा संस्थान की कार्यप्रणाली को और अधिक प्रभावशाली बनाना था जिससे कार्यक्षमता बढ़े । डॉ. अरमान दीप, पुलिस अधीक्षक, सीबीआई, चं. को **'निवारक सतर्कता'** विषय पर अभिभाषण देने हेतु विशेष रूप से आमंत्रित किया गया था । इस अवसर पर संस्थान के निदेशक डॉ. गिरीश साहनी, श्री छेरिंग तोबदन, प्रशासनिक अधिकारी व बहुत से वरिष्ठ वैज्ञानिक उपस्थित थे । डॉ. गिरीश साहनी ने मुख्य वक्ता का स्वागत करते हुए कहा कि हमें समेकित तौर पर देश व समाज के चरित्र निर्माण के बारे में अपने योगदान का आकलन करना है तथा इसके लिए उदात्त भाव से अपनी अंतर आत्मा की आवाज़ को सुनना है जो हमें कभी पथ भ्रष्ट नहीं होने देगी । मुख्य वक्ता ने भ्रष्टाचार निवारण में हमारे योगदान के लिए व्यावहारिक उदाहरण देकर रोचक प्रस्तुति की । उन्होंने इस वार्ता के माध्यम से देश की सुरक्षा व्यवस्था, पुलिस व प्रशासन तंत्र के लिए समाज को सकारात्मक सोच विकसित करने के लिए प्रेरित किया तथा याद दिलाया कि सरकार की ओर से भी इस दिशा में आम जनता की ओर कदम बढ़ाए गए हैं जिसका स्पष्ट उदाहरण एस.एम.एस है जोकि सीबीआई की ओर से आम जनता को भेजा जा रहा है कि यदि आप किसी प्रकार के भ्रष्टाचार से पीड़ित हैं तो अंकित फोन नं. पर सूचित करें । कक्ष में उपस्थित सदस्यों ने खुलकर उनसे वार्ता की व अपने संशय व्यक्त किए । इसके पश्चात् सप्ताह के दौरान आयोजित प्रतियोगिताओं के विजेताओं को अतिथि वक्ता द्वारा पुरस्कार प्रदान किए गए। कार्यक्रम का संचालन सुश्री नवनीत आनंद, हिन्दी अधिकारी द्वारा किया गया ।

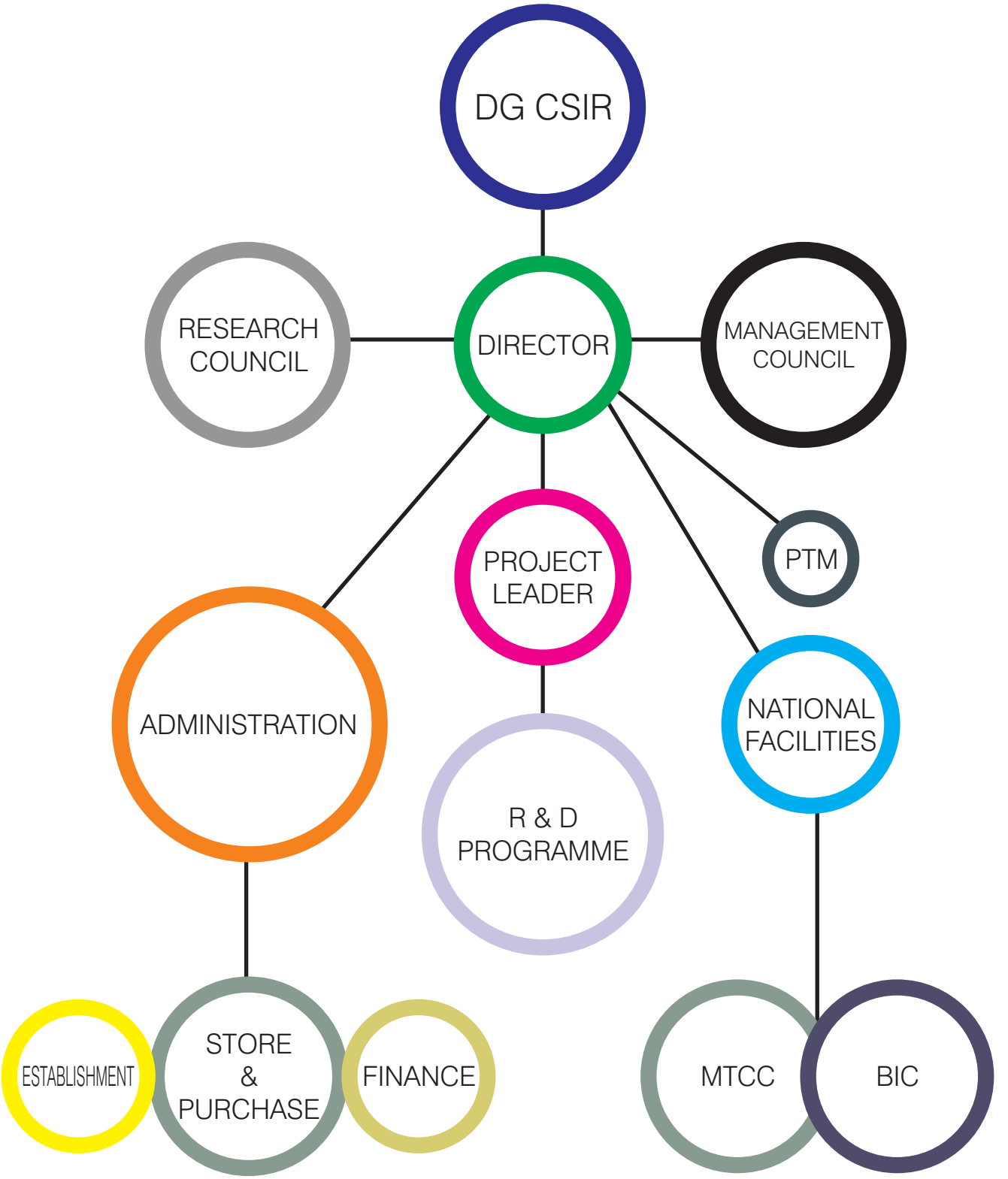
सतर्कता जागरूकता सप्ताह का आयोजन राजभाषा अनुभाग के सहयोग से किया गया । डॉ. पवन गुप्ता, वै., श्री छेरिंग तोबदन, प्रशासनिक अधिकारी तथा सुश्री नवनीत आनंद, हिन्दी अधिकारी ने निर्णायक मंडल के तौर पर अपनी सेवाएँ दीं । सतर्कता अनुभाग से श्री संजीव कुमार, सहायक व राजभाषा अनुभाग से सुश्री नवनीत आनंद, हिन्दी अधिकारी ने अपना योगदान दिया तथा श्री छेरिंग तोबदन, प्र.अ. एवं श्री पराग सक्सेना, अनुभाग अधिकारी ने समय-समय पर मार्गदर्शन दिया ।

टेबल वर्कशाप – वर्ष के दौरान 8 नए भर्ती सदस्यों का नाम राजभाषा रोस्टर में शामिल किया गया व टेबल वर्कशाप आयोजित करके राजभाषा नियमों की जानकारी दी गई तथा वर्ष 20110-11 के दौरान प्रत्येक तिमाही में विभिन्न वर्गों की टेबल वर्कशाप आयोजित की गई । इस वर्ष चारों तिमाहियों में क्रमश: तकनीकी सहायकों, तकनीशियनों, वैज्ञानिकों और प्रशासन के सदस्यों की टेबल वर्कशाप आयोजित की गई जिसके दौरान सदस्यों के कार्यस्थल पर जाकर

उन्हें हिन्दी में कार्य करने का अभ्यास कराया जाता है तथा उनकी कठिनाइयों का निवारण किया जाता है । सदस्यों को पुन: राजभाषा नीति से अवगत कराया जाता है व नियमों की जानकारी दी जाती है ।

वार्षिक गोपनीय रिपोर्टों में श्अन्य उपलब्धियोंश के कॉलम में राजभाषा हिन्दी में अच्छा कार्य करने संबंधी उल्लेख - सीएसआईआर, नई दिल्ली के परिपत्र संख्या 15-3/1/71-ओ एण्ड एम-।।(अ) दिनांक 8 जनवरी, 2003 के माध्यम से राजभाषा विभाग, गृह मंत्रालय, नई के अ.शा. प. सं. 1/14013/03/94-रा.भा. (नीति-1) दिनांक 8 नवम्बर, 2002 के अधीन प्राप्त माननीय प्रधानमंत्री के निदेशों के अनुसार सभी अधिकारियों/कर्मचारियें की वार्षिक गोपनीय रिपोर्टों में 'अन्य उपलब्धियों' के कॉलम में राजभाषा हिन्दी में अच्छा कार्य करने संबंधी उल्लेख करने हेतु श्राजभाषा हिन्दी में किया गया सराहनीय कार्य की स्टैम्प बनाकर लगाई गई है ।

Organisational Chart



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