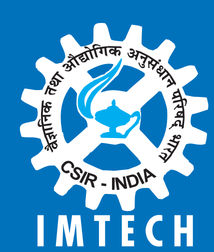
BIENNIUM REPORT
द्विवार्षिक प्रतिवेदन 2005-2007



INSTITUTE OF MICROBIAL TECHNOLOGY
सूक्ष्मजीव प्रौद्योगिकी संस्थान

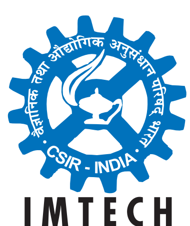
OBJECTIVES

- To provide integrated research, development and design base for microbial technology.
- To undertake basic and applied research and development programmes in established and newly emerging areas of relevant biotechnology including genetic engineering.
- To optimise the existing microbial processes 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

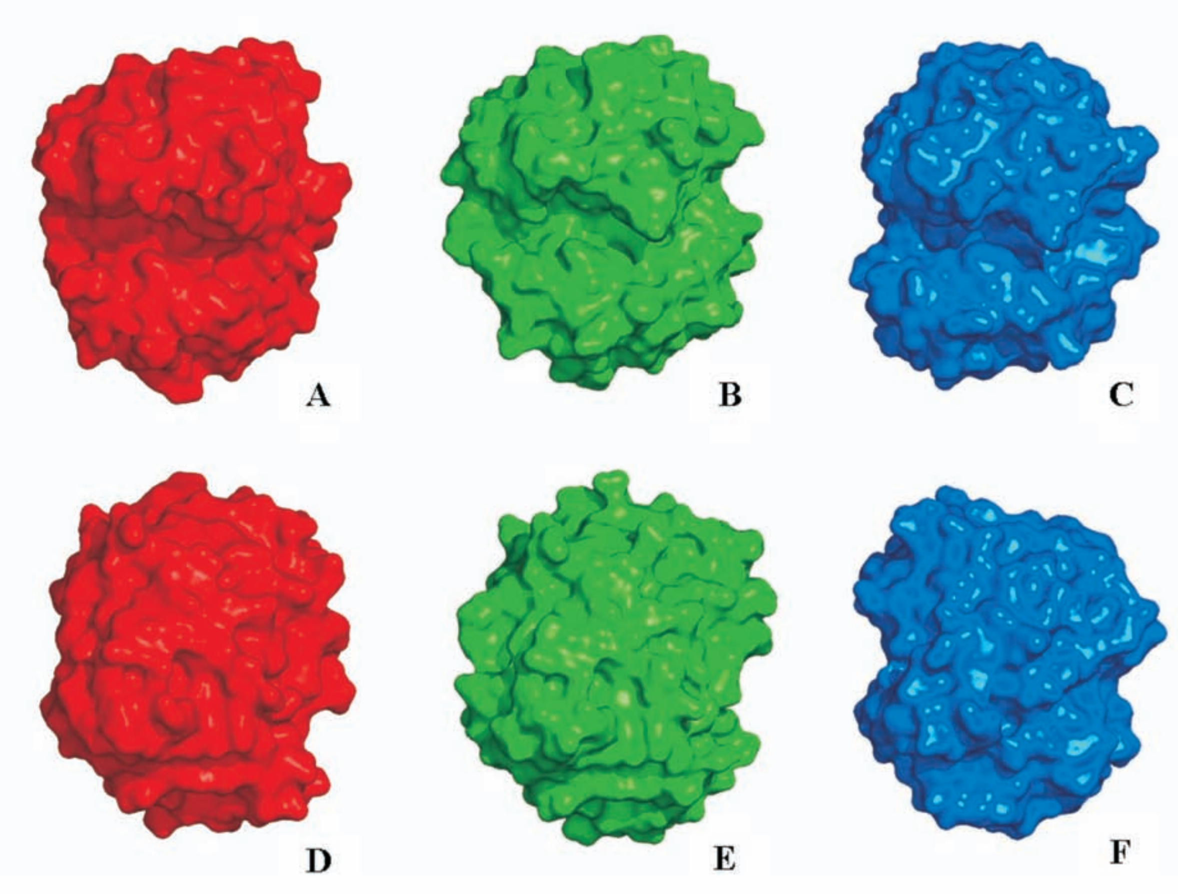
Dr. Girish Sahni
Director,
IMTECH

द्विवार्षिक प्रतिवेदन
BIENNIUM REPORT
2005 - 2007



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

सूक्ष्मजीव प्रौद्योगिकी संस्थान
सैक्टर 39-ए, चण्डीगढ़



The surface structural features of three cellulases/endoglucanses (Cel12A), including two 'parent' endoglucanases from (i) a thermophile bacterium, R. marinus (in red), and (ii) a mesophile fungus, T.reesei (in blue), as well as (iii) a 'baby' protein-engineered endoglucanase (in green) produced by combining features of the thermophile (red) and mesophile (blue) parent Cel12A enzymes. To produce the 'baby' enzyme shown in green, residues forming the substrate-binding groove of the parent enzyme shown in blue were identified and 'transplanted' onto the equivalent regions of the parent enzyme shown in red through ~36 non-contiguous mutations. Structure determination of the baby enzyme revealed that it derives its groove (panel B) from the mesophile parent (panel C), but the rest of its surface (panel E) including its bottom from the thermophile parent (panel D), as intended. Functional studies showed that the baby enzyme has the thermal stability of the thermophile parent and the functional temperature optimum of the mesophile parent. This work resulted from a collaboration between the groups of Guptasarma, Singh and Karthikeyan.

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Preamble

It is my heartfelt pleasure to report on the performance of our Institute during the two years gone by. This biennium report chronicles our achievements during the years 2005-2007. As we look back, we find that our institute made significant strides in its chosen path. It maintained its position among the top five CSIR laboratories in terms of the average impact factor per paper. Our research programmes, as a perusal of this report will make apparent, have lead to several positive outcomes; more than 187 papers were published in internationally peer reviewed journals and 14 patents were filed, with 11 being granted in India and abroad.

One of our scientist's was conferred the prestigious 'Dr. S.S. Bhatnagar Award' while two were conferred with 'Pitamber Pant National Environmental Fellowship Award' and 'AVRA Young Scientist Award'. Another two scientists bagged the 'National Bioscience Award for Career Development', and another was elected Fellow of the Association of Microbiologists of India.

It is established that modern biological research is heavily dependent on the infrastructural facilities available. Hence special efforts were made to upgrade many of our existing facilities and acquire new ones. Modernisation of our fermentation facilities and the procurement of several sophiscated equipments were the outcome of this. As a result, public-private mode participation and utilization of these facilities especially for fermentation and protein purification have increased significantly at IMTECH.

Often the results of basic academic research, no matter how significant, are usually still far from the point from where they can be taken to the market. To overcome this paradigm, commercial linkages were established with a number of companies/ organizations and a few of our technologies mentioned below were licensed to major companies.

- Successful completion of technology transfer of clot-specific streptokinase (engineered new-generation clot dissolver protein) to Nostrum Pharmaceuticals Inc., USA. Work on Phase II has started and primate studies were successfully completed recently.

- Successful completion of technology transfer of recombinant staphylokinase to Strides Arcolab Ltd., Bangalore, with work on Phase II being started involving scale-up and animal toxicology.

- Phase III (clinical trials on humans) of technology transfer of recombinant streptokinase to Shasun Chemicals & Drugs Ltd., Chennai were successful and product launch is expected by middle of the year 2009.

- Know-how for the production of alpha-amylase and alkaline protease was transferred to Celestial Labs, Hyderabad.

In addition, IMTECH scientists have discovered a new immunosuppressive agent. Its further development as a lead molecule is in progress and a commercial tie-up is expected soon.

A key component of national development is the generation of highly trained scientific Ph.D. programme and organizing various short and long term training courses. During the period under report 21 students working at IMTECH obtained their Ph.D. degrees, 45 new students were admitted to our Ph.D. Programme and 70 others from various places were given intensive training for a period ranging from 2 to 6 months. We also ran two successful CSIR Programme on Youth for Leadership in Science (CPYLS) Programmes organized centrally by the Human Resource Development Group of CSIR.

Overall, the Institute moved forward on its chosen path with confidence and in quiet realization that it has the potential to do even better.

In keeping with the spirit of Team CSIR, the Institute has fostered closer ties with sister laboratories and hopes to strengthen these bonds further in the years to come. Perhaps, it is not out of place to mention here that in our endeavour to attract funds from outside agencies/organizations, we were successful to an extent not only in attracting funds from private organizations and other Govt. agencies but also from abroad.

Thus, the institute has moved forward significantly in the direction laid down by my predecessors. This, does not, however, mean that we are complacent in our endeavours. Excellence has no absolute standards.

As always our Research Council provided valuable advice and guidance. Our heartfelt thanks are due to CSIR, DBT, DST, ICMR and ICAR for the unstinted support all along. Last but not the least I extend my sincere thanks to all my colleagues, associates, students and other members of the IMTECH family for their alround help and co-operation.

Girish Sahni

(Girish Sahni)

आमुख

विगत दो वर्षों के दौरान संस्थान में हुए कार्य की रिपोर्ट प्रस्तुत करते हुए मुझे अत्यंत हर्ष हो रहा है। यह द्विवार्षिकी प्रतिवेदन वर्ष 2005–2007 की हमारी उपलब्धियों का प्रलेख (आईना) है। पीछे मुड़कर देखने पर हम पाते हैं कि संस्थान ने अपने चुने हुए मार्ग में कई महत्वपूर्ण सोपान तय किए है। संस्थान ने औसत इम्पैक्ट फैक्टर प्रति शोधपत्र मे मामले में सीएसआईआर की पांच शीर्ष प्रयोगशालाओं में अपना स्थान बनाए रखा है। हमारे शोध कार्यक्रमों से कई सकारात्मक परिणाम सामने आए हैं जैसाकि इस रिपोर्ट को देखने से ही स्पष्ट हो रहा है, अंतररास्ट्रीय ख्याति प्राप्त जर्नलो में 180 से अधिक पेपर प्रकाशित हो चुके हैं तथा 14 पेटेंट फाइल किए जा चुके है जिसमें से भारत तथा विदेश में मिलाकर 11 प्रदान भी किए जा चुके हैं।

हमारे एक वैज्ञानिक को प्रतिष्ठित 'डॉ.एस.एस. भटनागर पुरस्कार' तथा दो अन्य को 'पीताम्बर पंत राष्ट्रीय पर्यावरण फैलोशिप आवार्ड' तथा 'एवीआरए युवा वैज्ञानिक पुरस्कार' प्रदान किया गया। दो वैज्ञानिकों ने कैरिअर विकास के लिए 'राष्ट्रीय जीवविज्ञान पुरस्कार' प्राप्त किया, तथा एक को एसोसिएशन ऑफ माइक्रोबायोलॉजिस्ट ऑफ इंडिया का फैलो चुना गया।

यह माना जाता है कि आधुनिक जीव विज्ञान में शोध मुख्य तौर पर उपलब्ध कराई गई अवसंरचनात्मक सुविधाओं पर आश्रित होती है। अतः वर्तमान सुविधाओं के उन्नयन के लिए तथा नई सुविधाओं प्राप्ति के लिए विशेष प्रयास किए गए। किण्वन सुविधाओं का आधुनिकीकरण तथा विभिन्न उच्च क्षमता युक्त उपस्करों की खरीद उसी का परिणाम है। इसके फलस्वरूप इमटैक में, विशेषतः किण्वन तथा प्रोटीन शुद्धिकरण के लिए, इन सुविधाओं उपयोग तथा सार्वजनिक–निजी तंत्र की सहभागिता सार्थक तौर पर बढ़ी है।

अधिकतर मूल शैक्षणिक शोध के परिणाम, चाहे कितने भी महत्वपूर्ण क्यों न हों सामान्यतः उस पड़ाव तक नहीं पहुंच पाते जहाँ से उन्हें बाज़ार में लाया जा सके। इस बात को समझते हुऐ बहुत सी कम्पनियों, संगठनों के साथ वाणिज्यिक संबंध स्थापित किए गए तथा हमारी निम्नांकित कुछेक प्रौद्योगिकियाँ बड़ी कम्पनियों को लाइसेंसिकृत की गई।

– नॉस्ट्रम फार्मास्यूटिकल इंक यू.एस.ए को (थक्का घोलक प्रोटीन की अभियांत्रित नई पीढ़ी) थक्का विशिष्ट स्ट्रेप्टोकाइनेस की प्रौद्योगिकी का स्थानान्तरण सफलतापूर्वक किया गया है। फेस–2 पर कार्य आरंभ किया गया तथा आरंभिक अध्ययन हाल ही में सफलता पूर्वक पूरे किए गए।

– स्ट्राइड्स एक्रोलैब लि0, बंगलौर को पुनर्योगज स्टैफिलोकाइनेस की प्रौद्योगिकी स्थानान्तरण का कार्य सफलतापूर्वक पूरा किया गया तथा फेज़ 2 पर कार्य आरंभ किया गया।

– शाशुन कैमिकल्स और ड्रग्स, लि0, चैने को रिकम्बिनेंट स्ट्रेप्टोकाइनेस के फेज़–3 (मनुष्यों पर चिकित्सीय परीक्षण) की प्रौद्योगिकी सफलता पूर्वक स्थानान्तरित की गई तथा वर्ष 2009 के मध्य तक उत्पाद लाँच होने की संभावना है।

– एल्फा एमिलेस तथा एल्कालाइन प्रोटीस के उत्पादन के संबध में तकनीकी जानकारी सेलेस्शियल लैब्स, हैदराबाद को स्थानान्तरित की गई।

इसके साथ ही, इमटैक के वैज्ञानिकों ने एक नवीन प्रतिरक्षा निरोधी कारक की खोज की है। शीर्ष अणु के तौर पर जिसके विकास का कार्य प्रगति पर है तथा एक वाणिज्यिक गठबंधन शीघ्र ही संभावित है।

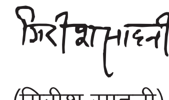
राष्ट्रीय विकास का एक मुख्य घटक है उच्च वैज्ञानिक प्रशिक्षण युक्त पीएचडी कार्यक्रम चलाना तथा विभिन्न लघु एवं दीर्घावधि प्रशिक्षण कोर्स आयोजित करना। रिपोर्ट की अवधि के दौरान इमटैक में कार्यरत 21 शोध छात्रों ने पीएचडी की डिग्री अर्जित की, 45 नए छात्रों ने हमारे पीएचडी कार्यक्रम में प्रवेश लिया तथा विभिन्न स्थानों से आए 70 अन्य छात्रों को 2 से 6 महीने की अवधि का गहन प्रशिक्षण प्रदान किया गया। हमने इस दौरान सीएसआईआर के एचआरडीजी द्वारा केन्द्रीय तौर पर आयोजित 'सीएसआईआर का विज्ञान में युवा नेतृत्व कार्यक्रम' दो बार चलाया।

समग्रत : संस्थान अपने इच्छित पथ पर पूरे आत्मविश्वास के साथ तथा इस संज्ञान के साथ आगे बढ़ रहा है कि उसमें इससे भी उत्तम कार्य करने की क्षमता है।

टीम सीएसआईआर की भावना को पूरा करते हुए संस्थान ने अपनी सहप्रयोगशालाओं के साथ घनिष्ठ संबंध बनाए हैं तथा आशावान है कि आने वाले वर्षों में यह और सुदृढ़ होंगे। तथापि यहाँ यह बताना अन्यथा नहीं होगा कि अन्य बाह्य एजेसियों, संगठनो से निधि प्राप्त करने के प्रयास में हम कुछ हद तक न केवल निजी संगठनों तथा अन्य सरकारी एजेसियों से निधि प्राप्त करने में सफल हुए है अपितु विदेशों से भी निधि प्राप्त कर पाए हैं।

समग्रतः संस्थान मेरे पूर्वाधिकारियों द्वारा दी गई दिशा में संस्थान ने महत्वपूर्ण उन्नति की है। तथापि इसका यह तात्पर्य नहीं है कि हम अपने प्रयासों से संतुष्ट हैं। श्रेष्ठता का कोई मानक नहीं होता।

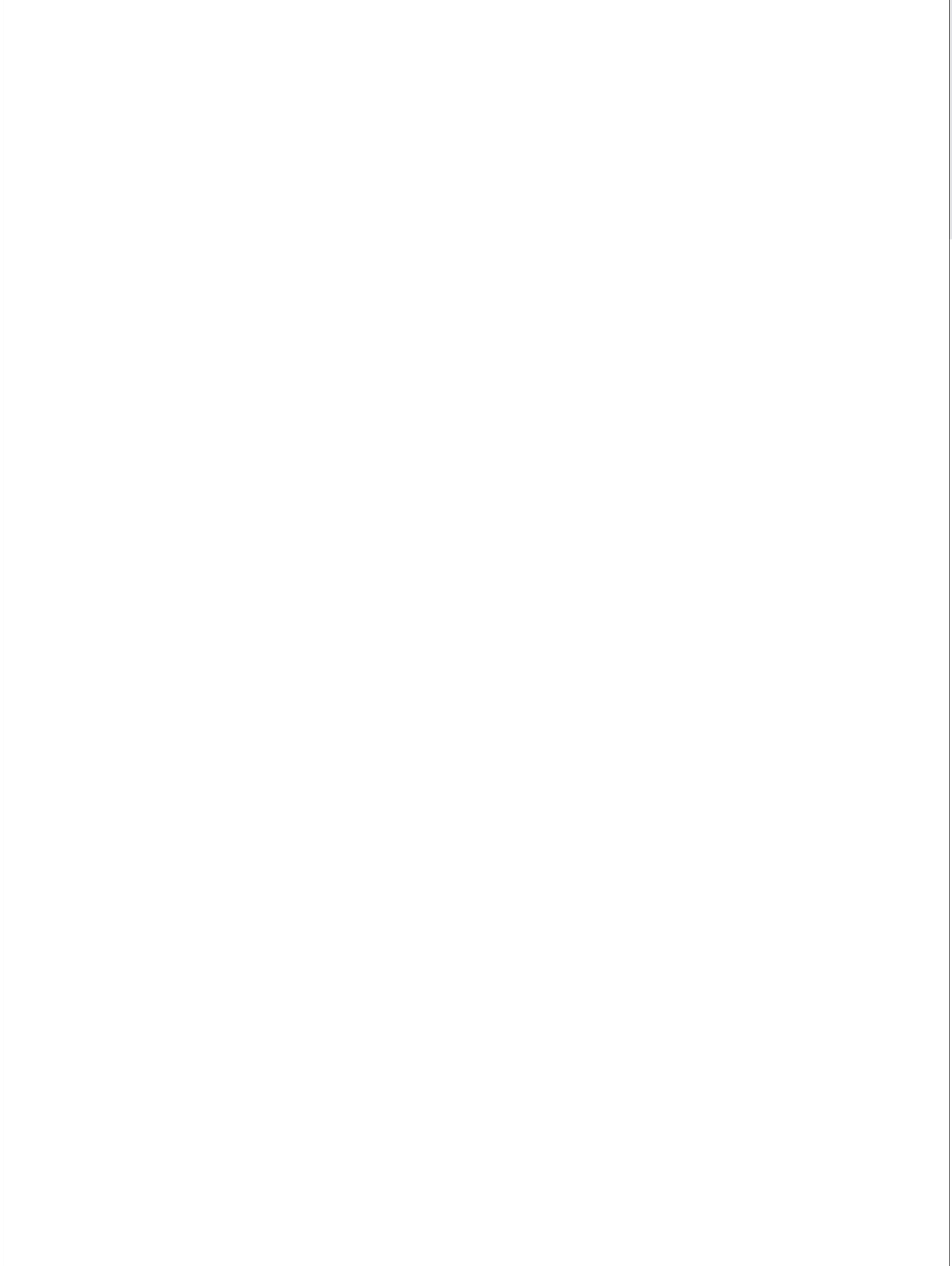
हमेशा की तरह हमारी अनुसंधान परिषद् की ओर से मूल्यवान परामर्श तथा मार्गदर्शन प्राप्त होता रहा है। सीएसआईआर, डीबीटी, डीएसटी, आईसीएमएआर तथा आईसीएआर, उनके खुले सहयोग के लिए हमारी ओर से हार्दिक धन्यवाद के पात्र हैं। अंततः मैं अपने सभी सहकर्मियों, सहायकों, शोध छात्रों तथा इमटैक परिवार के अन्य सभी सदस्यों को उनके सर्वांगीन सहयोग तथा मदद के लिए हार्दिक धन्यवाद देता हूँ।



(गिरीश साहनी)

R&D Programmes

R&D Programmes



Dibyendu Sarkar

dibyendu@imtech.res.in

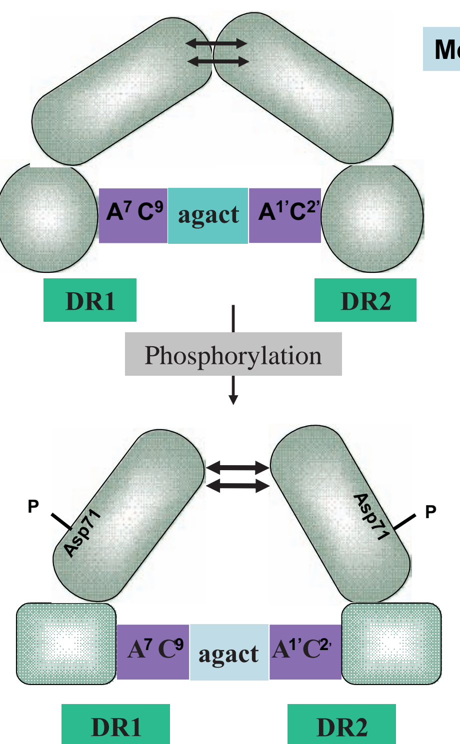
IDENTIFICATION AND CHARACTERIZATION OF THE REGULATORY COMPONENTS OF PHOSPHATE ASSIMILATION IN *Mycobacterium tuberculosis*

Project Leader: Dr. Dibyendu Sarkar

Other Participants: Mr. Sankalp Gupta, Mr. Akesh Sinha, Mr. Anuj Pathak, Mr. Arijit Das, Ms. Rajni Goel

The overall aim of the project is to identify and characterize regulatory molecules of *M. tuberculosis* that are involved in phosphate sensing.

M. tuberculosis PhoP regulates expression of unknown virulence determinants and controls biosynthesis of complex lipids. In the laboratory, for the first time, PhoP and



Mechanism of Action of *M. tuberculosis* PhoP

Phosphorylation of MtPhoP at the N-domain induces a conformational change in the C-domain of the protein, which in turn regulates DNA-dependent protein-protein interactions

Two MtPhoP protomers dimerize on the adjacent direct repeat motifs in a head-to-head orientation via N-terminal receiver domains

Conserved adenines, A1 of DR1 and A7 of DR2 appears to be critical for MtPhoP-DNA complex formation

truncated PhoR sensor proteins were shown to participate in phosphotransfer reactions using conserved residues characteristic of two-component signaling systems. β-Galactosidase activity originating from *phoP* promoter-*lacZ* construct was inhibited in the presence of PhoP, suggesting transcriptional auto-inhibition by the response regulator. High-resolution DNA-protein contact studies revealed a consensus recognition sequence within the *phoP* promoter that includes three 9-bp direct repeat units. Each repeat unit adjusts to the consensus $^{1}AC^{T}/_{G}{}^{T}/_{G}{}^{T}/_{G}P_{y}AP_{u}C^{9}$. Alterations in the sequence of the newly-identified direct repeat units relieved *phoP* transcriptional repression in presence of PhoP, suggesting that PhoP represses its own expression by sequence-specific interaction(s) with the repeat units. Together, these results identified hitherto unknown PhoP-regulated genetic determinants in the regulatory region of the *phoP* promoter which are central to understanding how PhoP possibly functions as a global regulator in *M. tuberculosis.*

Although much work remains to be done in identifying the genes regulated by PhoP, the present work, identifying a gene promoter regulated by the MTB PhoP and biochemically characterizing its binding interactions(s), represents a significant advancement in our understanding of the PhoPR two-component system. Elucidation of the specific signal sensed by the PhoR histidine kinase is the next challenge, i.e., to identify whether the signal is Mg^{+2} as in *Salmonella*, phosphate as in *Streptomyces,* acetate as in *E. coli,* or otherwise. We wish to understand the conditions and determinants of regulation of PhoP function. Meanwhile, identification of the target sites to which PhoP binds may help find PhoP-regulated promoters with a consensus PhoP-binding site, and allow future studies on regulatory interactions of the PhoP response regulator.

Dr. Dibyendu Sarkar (standing, fourth from left) and group

Pushpa Agrawal
pushpa@imtech.res.in

MOLECULAR CHARACTERIZATION OF *whiB*-LIKE GENES OF *Mycobacterium tuberculosis* H37Rv

Project Leader: Dr. Pushpa Agrawal
Other Participants: Mr. Saurabh K. Garg
Mr. Md Suhail Alam
Ms. Shikha Sharma

The objectives of this project were to decipher the function of a class of predicted *whiB*-like ORFs of *Mycobacterium tuberculosis* H37Rv. Initially, these small ORFs of 86-114 amino acids were implicated in regulation of sporulation in *Streptomycetes*. However, as genome sequences of different organisms belonging to actinomycetes made available, it became clear that these small ORFs are distributed throughout the group actinomycetes, although the number of ORFs of similar nature vary from organism to organism. The importance of these predicted ORFs (with no apparent functions) cannot be ignored, especially in mycobacteria, because even *M. leprae* which has undergone severe retrogressive evolution has retained four ORFs of this group/family. All the *whi*B-like ORFs have four conserved cysteine residues. The goal was to study the function of some of these ORFs in *M. tuberculosis* H37Rv, and the nature of the proteins encoded by them.

Iron-sulfur clusters are key to the regulatory function of at least three transcription factors SoxR, IscR and FNR whereas the function of OxyR, global regulator Spx, Hsp33, RsrA, Yap1 are regulated by thiol-disulfide. Unlike SoxR, IscR and FNR, in which an Fe-S cluster is needed for regulation, it is essential for aconitase to lose its Fe-S cluster before it can bind to RNA and perform its regulatory function. Recently, human mitochondrial glutaredoxin2 (Grx2) was also reported to have an EPR silent non-oxidizable $[2Fe\text{-}2S]^{2+}$ cluster that bridges two Grx2 molecules via two structural Cys residues to form dimeric holo Grx2. Similar to aconitase, human holo Grx2 with Fe-S cluster is enzymatically inactive and the loss of Fe-S cluster activates the Grx2. The Grx2 along with the Fe-S cluster also has a thioredoxin- like motif. So far, only two proteins in nature, i.e., human Grx2 and *Aquifex aeolicus* ferredoxin with a thioredoxin-like fold, are known to co-ordinate an Fe-S cluster. Thioredoxins and glutaredoxins are thiol-disulfide oxidoreductases that help in the maintenance of cellular redox homeostasis. They both share similar structure and overlapping functions and usually contain a Cys-X-X-Cys motif as an active site.

Thioredoxins are small ubiquitous proteins, which mediates dithiol-disulphide exchanges in all living cells. These proteins function through the formation or reduction of a disulphide bond in their target protein. They also share a common

structure known as the Trx motif which consists of four - helices and five - sheets and a pair of redox active cysteines present in the form of a Cys-X-X-Cys motif. The actual role of each of thioredoxin/thioredoxin-like proteins in the cell is determined by : (a) the redox potential of the protein, and (b) the direction of the electron transport pathway it participates in. Thus, both kinetic and thermodynamic functions are important. Thioredoxins or thiol-specific redox systems are important in the protection of cells against toxic oxygen species, maintenance of the intracellular thiol-disulfide balance as well as in providing reducing power to key reductive enzymes, for e.g., ribonucleotide reductase. Both thioredoxin and glutaredoxin are maintained in a reduced form by their cognate thioredoxin reductase or glutathione /glutathione reductase at the expense of NADPH.

Biochemical analysis of WhiB1 and WhiB4 proteins of M. tuberculosis H37Rv. In depth analyses of the amino acid sequences of WhiB-like proteins of mycobacteria showed that they all have four conserved cysteines, with a Cys–X-X-Cys motif. The Cys–X-X-Cys motif is well characterized and known to be redox sensitive, which means that the cysteines in the motif respond to oxidative stress by forming disulfide bond. Both *whiB1* and *whiB4* genes were cloned and proteins were expressed and purified. Thus, the group tested the status of disulfide bond formation in both WhiB1 (Rv3219, 252bp/84aa) and WhiB4 (Rv3681c, 357bp/118aa) using MALDI-TOF-TOF. The results clearly showed the presence of two intramolecular disulfide bonds in both WhiB1 and WhiB4 proteins where one of the bonds, at least in WhiB4, occurs between two cysteines of the Cys–X-X-Cys motif and the other disulfide bond is formed by the two outside cysteines. The reducing/oxidizing capacity of a protein depends upon the sequence and structural context of the Cys–X-X-Cys motif. Similar to other proteins with Cys–X-X-Cys motif, WhiB1 also can be reduced by reduced glutathione. Therefore, the redox potential of WhiB1 was calculated by using the ratio of GSH/GSSG as standard; however an identical method could not be used for WhiB4. Attempts are being made to calculate the redox potential of WhiB4 using thioredoxin reductase and NADP+/NADPH ratio.

Both WhiB1 and WhiB4 are thioredoxin-like proteins as judged by their thiol transfer capacity to insulin, showing that both WhiB1 and WhiB4 are protein disulfide reductases. So far these results suggest that both WhiB1 and WhiB4 are likely to work by the oxidation and reduction of disulfide bonds. The concentrated and purified WhiB1 and WhiB4 proteins were brownish in colour, surprisingly the scanning of purified proteins produced a spectrum typical of Fe-S cluster containing proteins. Cysteine and histidine residues have the potential to co-ordinate Fe-S clusters in proteins. Mutational analysis of WhiB1 and WhiB4, where either individual or multiple cysteine residues were mutated to serine, showed that the Fe-S cluster is coordinated by all four-cysteine residues. The result was further confirmed using a cysteine modifier. The oxygen sensitivity of the WhiB1 and WhiB4 Fe-S clusters was studied by exposing the purified proteins to different concentrations of H_2O_2 and then scanning spectra. The Fe-S cluster of WhiB4 (data were similar for WhiB1) protein is oxygen sensitive.

The data clearly showed that WhiB like proteins are stress responsive, and that they function by oxidation/reduction of cysteines. Thus theoretically, after the Fe-S cluster is lost and under favourable conditions, i.e., when redox state of the cell is in normal condition, the protein should have capability to reconstitute the Fe-S cluster. In several other Fe-S cluster coordinating proteins, some other proteins mediate the reconstitution of the Fe-S cluster. Thus the purified WhiB1 protein was allowed to air oxidize which disassembled the Fe-S cluster and then10 mm DTT was added in the same preparation. The reaction was allowed to continue at room temperature for 6 hrs, and then absorbance at 420 nm (a characteristics of Fe containing proteins) was measured. The increased absorbance at 420 nm clearly showed that both WhiB1 and WhiB4 proteins reconstituted the Fe-S cluster under reducing condition without the help of any accessory protein. The result suggests that both WhiB1 and WhiB4 of *M. tuberculosis* are likely to be sensor proteins. Thioredoxins are regulatory proteins and their regulation property is governed by reversible oxidation/reduction of disulfide bonds. To study the nature of Fe-S cluster both WhiB1 and WhiB4 were subjected to electro-paramagnetic resonance (EPR) analysis. EPR spectra clearly showed that each molecule of WhiB1 and WhiB4 proteins co-ordinates a [4Fe-4S] cluster. The spectra further showed that there is continuous oscillation of $Fe3+$ and $Fe2+$ state between the Cys-X-X-Cys motif suggesting that WhiB1 can sense the stress by using its Fe-S cluster. Cysteine mutagenesis and alkylation studies showed that all the four cysteine residues are involved in the [4Fe-4S] cluster coordination. The removal of [4Fe-4S] cluster leads to conformation changes in both proteins. It was demonstrated that both WhiB1 and WhiB4 are protein disulfide reductases, with WhiB4 more active as a disulfide reductase than WhiB1. Mutational analysis showed that both the disulfide of WhiB4 contributes towards the disulfide reductase. Deletion of all the four cysteines abolished the [4fe-4S] cluster as well as disulfide reductase function. Further the holo-proteins were enzymatically inactive.

Dr. Pushpa Agarwal (standing, centre) and group

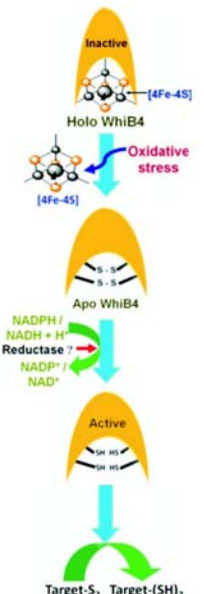
The work carried out under this project, as published, is as below:

Saurabh K. Garg Md. Suhail Alam, K.V. Radha kishan and Pushpa Agrawal. Expression and characterization of -(1,4) glucan branching enzyme Rv1326c of *Mycobacterium tuberculosis* H37Rv. **Protein Exp. Purif., 51**, 198-208, 2007.

Saurabh K. Garg, Md.Suhail Alam, Vishal Soni, K. V. Radhakishan and Pushpa Agrawal. Characterization of *Mycobacterium tuberculosis* WhiB1/Rv 3219 as a protein disulfide reductase. **Protein Exp. Purif., 52**, 422-432, 2007.

Md. Suhail Alam, Saurabh K. Garg and Pushpa Agrawal. Molecular function of WhiB4/Rv3286c of *Mycobacterium tuberculosis*H37Rv: a [4Fe-4S] coordinating protein disulfide reductase. **Mol. Microbiol**., **63**, 1414-1431, 2007.

The Research Council of IMTECH in its 31st meeting held on July 3-4, 2006 reviewed the work done under this project and declared the project as successfully completed.



Functional model of WhiB4 of M. *turberculosis.* WhiB4 remains as a holo protein under normal growth conditions but once *Mtb* invades the host, it encounters oxidative and nitrosative stress in the form of ROIs and RNIs. These agents lead to oxidation of Fe^{2-} to Fe^{3+} followed by disassembly of [4Fe-4S] cluster, formation of two intra-molecular disulfide bonds and generation of apo WhiB4. The process also brings conformational change in the protein. Apo WhiB4 accepts electrons either from a reductase (not yet identified) or directly from a reducing agent (not know as yet) and gets activated. The active form of protein then transfer the signal to target protein(s) as a disulfide reductase.

IS DDE MOTIF ESSENTIAL FOR TRANSPOSASES? FUNCTIONAL ANALYSIS OF PIS 136 INTEGRASE GENE : A LIKELY TRANSPOSASE

Project Leader: Dr. Pushpa Agrawal
Other Participant: Ms. Richa Bajpai

The PIS 136 integrase is an arginine-rich DNA binding protein. The amino acid sequence of the protein has all conserved regions and residues of a site specific integrase. However, studies in the lab show that though the protein appears to be a site-specific tyrosine recombinase, *in vitro* the protein functions as a transposase as well as a resolvase. Sequence analysis of *in vitro* recombination products showed that every recombination site has CA di-nucleotides. The CA di-nucleotide is a characteristic of Mu and HIV transposase.

Temperate bacteriophages use site-specific recombination to stably integrate to the host chromosome and generate stable lysogens. The recombination is between *attB* and *attP* regions and in general tRNAs are the targets. The most common site-specific recombinase is tyrosine recombinase or serine recombinase. Tyrosine recombinases have been reported not just from bacteriophages but also from transposons e.g., Tn916, Tn1545 and CTn86 and CTn9343. Brom and colleagues described a novel feature of tyrosine recombinase which can generate a co-integrate between two plasmids of *Rhizobium elti* CNF42. However, this case is conditional because it happens when re-iterated sequences are present in the plasmid which acts as α recombination site. Co-integrates are resolved by serine recombinases, but exceptions have been reported where tyrosine recombinases are also used for resolution e.g., Tn4430 and Tn4651. However, genome shuffling by deletions or

duplication seem to be *rec* independent and plasmid or phage encoded recombination system is reported to play an important role.

Serine recombinase family of proteins which have both invertase and resolvase functions are mechanistically different from tyrosine recombinase. Tyrosine recombinases first create a staggered nick at identical positions in two opposite strands by generating a 3'-phosphotyrosyl bond and a 5' hydroxyl group. Each nicked strand swivels around its partner strand to form a Holliday junction which then is resolved into reciprocal recombinants by breakage and exchange of the two parental strands at the other end of the spacer which involves the migration of the junction across the spacer. The joining reaction is mediated by nucleophile attack of 5'-OH group from a cleaved strand of one substrate on the 3' phosphotyrosyl bond formed on the cleaved strand of its partner. In case of serine recombinases, the protein makes concerted double strand breaks in the two recombination sites before starting the exchange or any re-sealing of DNA strand can occur. Serine recombinases prefer orientation of recombination sites such as direct or inverted repeats.

Integrases require accessory host factors to perform their reactions along with specific *attP* and *attB* sequences, while transposases normally work by a two-step process and do not require host factor. In the first step, the donor DNA molecule is fused to a target molecule in a process that involves DNA replication, which requires the function of transposase and makes co-integrate. Resolution takes place by a conservative site-specific recombination event at two directly repeated recombination sites known as *res* sites into donor and target molecules. In this case resolvase is transposon-encoded. All these reports show that whatever may be the pathway, recombination to resolution of recombinants require two separate enzymes.

The integrase gene of PIS 136 was cloned and expressed in the pET22b vector at 30 C. The protein was purified under denaturing condition and was refolded. The refolded protein had DNA binding property and also performed the first reaction in the recombination process that was DNA cleavage. Sequence analysis and comparison with other intergrases indicated that PIS 136 integrase might be a tyrosine recombinase. Therefore, the predicted tyrosine residue was mutated to phenylalanine by site directed mutagenesis followed by overlap PCR. The PCR product was also cloned and expressed in pET22b vector at 30 C. The protein was purified, as with the wild type, and refolded and its DNA binding properties were compared with the wild type protein. Surprisingly, the tyrosine mutant protein failed to bind to DNA but maintained the DNA cleaving property. PIS 136 integrase protein has a serine residue at the 9th position, indicating that the protein may also function as a serine recombinase, suggesting that it is a chimera. If that indeed is the case then it is not surprising that the protein retains the DNA cleaving property but some how cannot complete the recombination reaction. The cleaved products are being analysed to find the DNA sequence at which the protein works. The integrase protein showed gel shift of the linear DNA but when supercoiled DNA was used as a substrate.

Javed N. Agrewala
javed@imtech.res.in

COSTIMULATORY MOLECULES-MEDIATED REGULATION OF THE ACTIVATION AND DIFFERENTIATION OF ANTIGEN PRESENTING CELLS

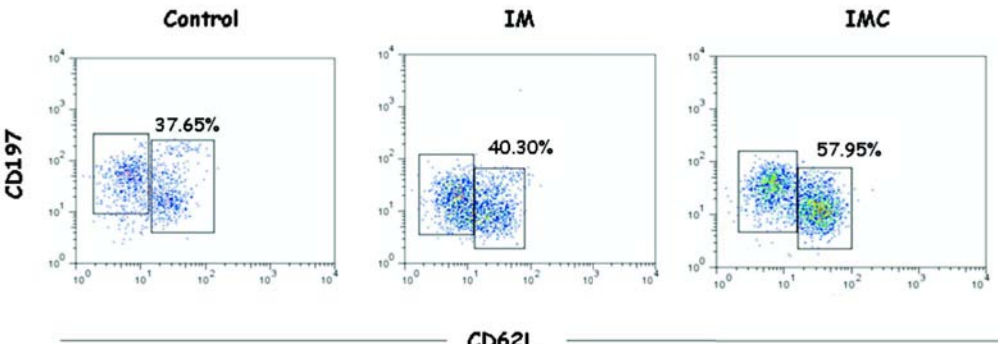
Project Leader: Dr. Javed N. Agrewala
Other Participants: Mr. Manzoor A. Mir
Dr. Vijender Singh
Ms. Shweta Jain
Mr. U. Gowthaman
Mr. Rajender Das

Evidence from a variety of studies suggests that a B cell's contact with T helper cell is crucial for its optimal activation and antibody secretion. Contact between B cell and T cell can be mediated by antigen presentation, as well as antigen-independent cell-cell interactions using molecules known as adhesion (LFA-1, LFA-3, ICAM-1, etc.) and costimulatory molecules (CD80, CD86, CD40, etc.). Signals from T cells induce two opposite fates in B cells: clonal expansion and clonal deletion. The role of costimulatory molecules is very well established in the activation of T cells, but nothing has been definitively determined about how these molecules operate in the activation and differentiation of B cells.

An array of costimulatory molecules, *e.g.* CD80, CD86, CD40, CD27, CD278, OX40L, etc., are expressed on the surfaces of antigen presenting cells. Their role has gained considerable significance in the activation of T cells, but very little is known in connection with the activation of antigen presenting cells. The exact mechanism of sequence of signals provided by different costimulatory molecules in the stimulation and inhibition of B cells, macrophages and dendritic cells is largely unknown.

The group has demonstrated for the first time that the signaling through costimulatory molecules CD80 and CD86 can modulate the activity of B cells and B cell lymphomas. Costimulation through CD80 induces apoptosis in B cell lymphoma by up-regulating the expression of pro-apoptotic molecules. In contrast, costimulation through CD86 augmented the level of anti-apoptotic molecules. Thus, it may be inferred from these findings that engagement of CD80/CD86 on antigen presenting cells with CD28/CD152 on T cells not only influences T cells but also B cells. The kinetics of rapid and high level of expression of CD86 in early immune responses and the delayed expression of CD80 on antigen presenting cells can be viewed as necessary activation and inhibitory signals, respectively, delivered by these molecules during the immune response. After the group's report, the role of CD80/CD86 in bi-directional costimulation has been internationally followed up by

Enhanced Central Memory Pool by Pro-inflammatory Cytokines



Pro-inflammatory cytokines IL-1, IL-6 and TNF-a enhance *M. tuberculosis* specific long-term central memory phenotype (CD197^{lo}/CD62L^{hi}). Mice were injected either with *M. tuberculosis* infected macrophages (IM) or in combination with IL-1β+IL-6TNF-α (IMC). After 240 days, peripheral blood was drawn and expression of central memory markers (CD197 and CD62L) were evaluated on CD4+T cells by 3 colour staining. Control group comprised of mice injected with uninfected macrophages.

many groups. The work has opened a new concept in costimulation and many groups have shown that signaling through CD80/CD86 can also control the function of B cells, dendritic cells and T cells. Recently, Logue and Sha 2004, [Nature Immunology: News and Views] have indicated that CD28-CD80 bi-directional signaling is a two-way street to activation. Further, this strategy is being currently utilized by Biogene for the treatment of relapsed and refractory follicular lymphoma patients.

Dr. Javed Agrewala (standing, fourth from right) and group

The group has also demonstrated that IL-4- and IFN- -induced secretion of IgG1 and IgG2a, respectively, could be inhibited by signaling B cells through CD80 molecule. IL-4 and IFN- acted synergistically with CD86 costimulation and augmented the production of IgG1 and IgG2a, respectively. Interestingly, IL-4 could inhibit the secretion of IgG2a induced by CD86 signaling but IFN- failed to obstruct the secretion of IgG1, even though the latter has antagonistic property for IL-4.

Currently, the group is studying the influence of signaling through costimulatory molecules on the survival of intracellular pathogens viz. *M. tuberculosis* and *S. typhimurium* in infected macrophages. Further, how costimulation of infected macrophages affects the secretion of cytokines and expression of costimulatory molecules and its impact on the activation *vs* anergy of T cells is also being studied.

Saumya Raychaudhuri
saumya@imtech.res.in

MOLECULAR BASIS OF THE MECHANISM OF ASSOCIATION OF *Vibrio cholerae* AND *Entamoebae histolytica*

Project Leader: Dr. Saumya Raychaudhuri
Other participants: Mr. Vibhu Jain
Mr. Mitesh Dongre
Ms. Chetna

In India, like in other developing countries, diarrhoea is a major problem. Despite considerable advancement in the understanding of diarrhoeal diseases as a whole, much remains to be learnt. Both acute watery diarrhoea and dysentery are prevalent in some regions of our country, particularly the coastal regions; therefore, in these areas, an individual might become a victim of a mixed infection by different diarrhoeal disease-causing pathogens. It has been observed that the interaction between two pathogens alters their pathogenic and survival potential. For example, it has been seen that interactions of *Mycobacterium avium* and *Legionella pneumophila* with water borne amoeba *Acanthamoeba castellani* enhance their virulence and *vice versa*. Therefore, interactions among different organisms could alter their virulence potential.

The objective of this project was to understand the mechanism of cross talk or interaction between diarrhea-causing bacteria and a protozoan namely *Vibrio cholerae* and *Entamoeba histolytica* and its subsequent influence in disease *per se*.

Vibrio cholerae is the causative agent of cholera, a form of diarrhoea, which continues to ravage and remains a major public health problem in the developing world. The organism has the capacity to survive in diverse estuarine environments as well as in human host. Recent studies suggested that the interaction with a fresh water amoeba, *Acanthameoba castellanii* could be one of the possible modes of its survival in the aquatic environment. It was also shown that *V. cholerae* could replicate intracellularly in *Acanthamoeba castellanii*, which further prompted studies of its interaction with a parasitic amoeba, *Entameoba histolytica*.

Entamoeba histolytica, the causative agent of amebic colitis and amebic liver abscess is the second leading cause of death from parasitic disease worldwide. In their natural environment, trophozoites of *E. histolytica* live in the colon region of the human intestine together with resident microflora, which under normal condition are usually composed of a complex mixture of mostly anaerobic or microaerophilic bacteria. In order to examine, the interaction of *Vibrio cholerae* by the trophozoites of *E. histolytica*, a gentamicin assay was employed as described elsewhere. In this assay, *E. histolytica* HM-1:IMSS trophozoites (a gift from Prof. Sudha

Bhattacharyya, JawaharLal Nehru University, New Delhi) were suspended in serum free TYI-S-33 medium at a concentration of 10^5 amoebae/ml. Trophozoites were incubated in triplicate in 24-well tissue culture plates (Falcon). Subsequently, *V. cholerae* O139, strain SG24 (gift from Dr. Ranjan Nandy, National Institute of Cholera and Enteric Research, Calcutta) was added to a final concentration of 10^7 cells/ml. The samples were incubated at 36^0C for 1 hour followed by addition of 200g/ml gentamicin for 2 hours to kill primarily extracellular bacteria. After gentamicin treatment, the trophozoites were washed three times with PBS by centrifuging at 280g for 7 min. After washing, trophozoites were counted with trypan blue to check viability and lysed by syringe passage in presence of 0.01% Triton X-100. Dilutions were plated on LB agar plates for colony enumeration, which was **$5.53 \pm 0.18 \times 10^3$**. The data suggested the presence of intracellular bacteria within the trophozoites of *E. histolytica*. In other way, trophozoites provided a protective barrier for *Vibrio cholerae* against the killing effect of gentamicin.

To further investigate the intracellular localization of the bacteria, the group performed a transmission electron microscopy of infected trophozoites. Microscopic pictures of 1 hr post-infected trophozoites revealed that *V. cholerae* O139 cells were localized intracellularly in the vacuoles.

Taken together these findings suggest that *V. cholerae* O139 cells were recognized by the trophozoites of *E. histolytica* and localized primarily in the vacuoles. Earlier various surface molecules of *V. cholerae* were shown to be involved in diverse functions. For example, the extensively studied type 4 pilus, the toxin-coregulated pilus (TCP), is necessary for colonization of the mammalian intestine. Similarly, a mannose-sensitive hemagglutinin (MSHA) was found to promote the adherence of *V. cholerae* to zooplankton. Besides promoting adherence to biotic surface, MSHA

Dr. Saumya Raychaudhuri (standing, second from left) and group

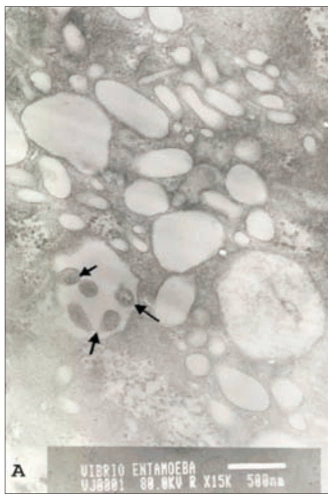
is also important in developing biofilm formation on abiotic (borosilicate glass) surface. At this stage, the cell surface molecules of *V. cholerae* that mediate its interaction with the trophozoites of *E. histolytica* are not known. This warrants further investigation. Further, association of *Vibrio cholerae* with free-living as well as parasitic amoeba may serve as a model to explore the biology of the bacterium. This is the first report that describes the interaction of *V. cholerae* with human intestinal parasitic protozoa, *Entamoeba histolytica*.

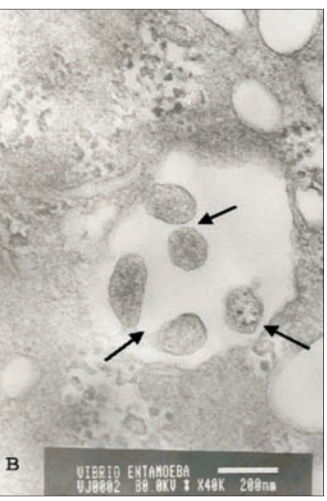
The work carried out under this project, as published, is as below:

Vibhu Jain, Mitesh Dongre and Saumya Raychaudhuri. Interaction of *Vibrio cholerae* O139 with an intestinal parasite *Entameoba histolytica*. **J. Med. Microbiol.**, **55**, 1755-1756, 2006.

Raychaudhuri, S.; Jain, V. and Dongre, M. Identification of a constitutively active variant of LuxO that affects production of HA/protease and biofilm development in a non-O1, non- O139 Vibrio cholerae O110. **Gene, 369**,126-33, 2006.

The Research Council of IMTECH in its 31st meeting held on July 3-4, 2006 reviewed the work done under this project and declared the project as successfully completed.





Electron micrograph of thin section of (A) Entamoeba histolytica trophozoites incubated for one hour at 36°C with Vibrio cholerae SG24 (B) Vacuole magnification. Thin sections were prepared after epoxy resin embedding. Bacteria were found inside vacuoles. Arrow indicates bacteria.

EXPLORATION OF TYPE III SECRETORY SYSTEM IN NON-O1, NON-O139 STRAINS OF *V. Cholerae*: ROLE OF QUORUM SENSING IN THE REGULATION OF TTS SYSTEM

Project Leader: Dr. Saumya Raychaudhuri
Other Participants: Mr. Vibhu Jain
Mr. Mitesh Dongre
Ms. Ranjana Tripathy
Ms. Chetna

The objectives of this project are :

1. To detect the presence of various TTSS related genes among all the available clinical and environmental non-O1, non-O139 strains. For this, TTSS associated genes found in the reference sequenced strain AM-19226 belonging to serogroup O39 will serve as a template for designing primers and probes.

2. Study of the role of quorum sensing phenomena in coordinating the function of type III secretion system of *V. cholerae* non-O1, non-O139 strains.

Type III secretion systems play a crucial role in the virulence of many Gram negative pathogens by directly mediating the secretion and translocation of bacterial effector proteins into the cytosol of eukaryotic cells. So far, more than 20 proteins have been found to be associated with the formation of TTSS macromolecular complex. The apparatus proteins of TTSS are highly conserved among bacteria that possess the system. In contrast, the effector proteins secreted via this system are not conserved among those bacteria and thus evoke a wide range of biological activity in target cells.

It has now become evident that quorum sensing plays an important role in the regulation of type III secretion system. In *V. harveyi*, quorum sensing has been shown to regulate inversely the expression of genes encoding TTS system. At high cell density, in presence of auto inducers the TTS system of *V. harveyi* switches to non-operational mode. In contrast to *V. harveyi*, quorum sensing positively controls the expression of type III gene and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*.

Recent studies suggest that the strains of *V. cholerae* belonging to the diverse non-O1, non-O139 serogroup could emerge as a potential threat having capacity to cause sporadic diarrhoea and outbreaks. Although majority of these diarrhoea-causing non-O1, non-O139 strains are devoid of cholera toxin and toxin co-regulated pili, sequencing data reveals the presence of TTSS gene clusters in strains belong to this diverse serogroup. It is, therefore, conceivable that the TTSS gene products as well as proteins that are secreted through this system could contribute to the virulence of *V. cholerae* strains belong to these diverse serogroups. The organization as well as the involvement of the quorum sensing phenomena in the regulations of type III systems in non-O1, non-O139 serogroups of *V. cholerae* strains are not completely understood, which motivated us to embark on this project.

Recently Mekalanos and co-workers identified eleven type III associated genes in a non-O1, non-O139 *V. cholerae* strain AM-19226. To examine the distribution of those genes in the various non-O1, non-O139 clinical isolates of Indian origin, a total of 54 strains were subjected to Southern hybridization by using radiolabeled probe corresponds to *vspD* gene. Out of 54, 14 strains proved to be positive. VspD is a secreted protein having 80% homology with EspD protein of *Vibrio parahemolyticus*. These 14 strains positive for *vspD* were further subjected to another round of Southern hybridization with two more genes namely *vcsQ2* and *vcsC2*. All strains but one proved to be positive for both genes.

In addition to the above mentioned projects, the group also focuses on mechanisms of quorum sensing by using *V. cholerae* as a model organism to study cell-cell communication phenomena and various properties controlled by related signal transduction processes. Towards this end, the group recently characterized a variant of central regulator of the quorum sensing circuit of *V. cholerae* conferring a phenotypic alteration in this strain.

Anand K. Bachhawat

anand@imtech.res.in

STUDIES ON THE ROLE OF - GLUTAMYL TRANSPEPTIDASE IN GLUTATHIONE HOMEOSTASIS IN YEAST

Project Leader: Dr. Anand K. Bachhawat

Other Participants: Mr. Chitranshu Kumar
Mr. C.V. Srikanth
Mr. Dwaipayan Ganguli
Ms. Jaspreet Kaur
Ms. Neha Kasturia
Ms. Hardeep Kaur
Mr. Akhilesh Kumar

The main objectives of the project are i) to determine the role of -GT in the utilization of glutathione as a sulphur/nitrogen source (exogenous and endogenous) and ii) to determine the role of -GT in glutathione homeostasis and develop genetic screens for investigating glutathione homeostasis.

The earlier work had revealed the presence of an alternate pathway for glutathione degradation that was independent of the -GT enzyme.

In the present period a genetic strategy was taken to isolate mutants in the alternative pathway for glutathione degradation. Using this approach the group was able to show the participation of 3 previously uncharacterized genes in *S. cerevisiae*, *DUG1* (*YFR044c*), *DUG2* (*YBR281c*) and *DUG3* (*YNL191w*) (Defective in Utilization of Glutathione). A fourth gene was also picked up in this screen, *DUG4* that was identical to the glutathione transporter gene, *HGT1* (that has been previously described from the lab), and was responsible for the transport of glutathione into *S. cerevisiae* prior to its degradation by Dug1p/Dug2p/Dug3p. In this study the group was able to show that although dipeptides and tripeptides with a normal peptide bond such as cys-gly or glu-cys-gly required the presence of only a functional *DUG1* gene that encoded a protein belonging to the M20A metallohydrolase family, the presence of an unusual peptide bond such as in the dipeptide, -glu-cys, or in GSH, required the participation of the *DUG2* and *DUG3* gene products as well. The *DUG2* gene encodes a protein with a peptidase domain and a large WD40 repeat region, while the *DUG3* gene encoded a protein with a glutamine amidotransferase domain. The Dug1p, Dug2p and Dug3p proteins were found to form a degradosomal complex, through Dug1p-Dug2p and Dug2p-Dug3p interactions.

In addition to these studies the group has characterized a novel cysteine transporter in *Saccharomyces cerevisiae*. Cysteine transport in the yeast *Saccharomyces cerevisiae*

is mediated by at least eight different permeases, none of which are specific for cysteine. The team described a novel, high affinity, (K_m = 55 ì M), cysteine-specific transporter encoded by the ORF *YLL055w* that was initially identified by a combined strategy of data mining, bioinformatics and genetic analysis. Null mutants of *YLL055w*, but not of the other genes encoding for transporters that mediate cysteine uptake such as *GAP1*, *GNP1*, *MUP1* or *AGP1,* in a *met15* background, resulted in a growth defect when cysteine, at low concentrations, was provided as the sole sulphur source. Transport experiments further revealed that Yll055wp was the major contributor to cysteine transport under these conditions. The contributions of the other transporters became relevant only at higher concentrations of cysteine, or when *YLL055w* was either deleted or repressed. *YLL055w* expression was repressed by organic sulphur sources, and was mediated by the Met4p-dependent sulphur regulatory network. The results reveal that *YLL055w* encodes the principal cysteine transporter in *S. cerevisiae*, which the group has named as *YCT1* (Yeast Cysteine Transporter). Interestingly Yct1p belongs to the Dal5p family of transporters rather than the amino acid permease family to which all the known amino acid transporters belong.

Dr. Anand Bachhawat (standing, fifth from left) and group

P.R. Patnaik
pratap@imtech.res.in

MATHEMATICAL MODELLING UNIT

Project Leader: Dr. P.R. Patnaik

The Mathematical Modelling Unit (MMU) studies the applications of mathematical methods to biological problems. Its research is on bioreactor modelling and optimization under simulated industrial conditions.

During this two-year period, investigations were carried out in two projects:

(a) Exploration of noise-filtering strategies for performance enhancement of an oscillatory fermentation.

(b) Comparative evaluation of modelling approaches to bioreactor optimization under simulated industrial conditions: a case study of PHB production by *Alcaligenes eutrophus*.

Both projects were completed. The first project explored the effectiveness of conventional and neural filters for removal of noise present in the feed stream(s) of a continuous flow bioreactor sustaining an oscillating fermentation. The microbial system studied was *Saccharomyces cerevisiae*, which produces monotonic or oscillating or chaotic outputs. The nature of the outputs, mainly biomass, ethanol, dissolved oxygen (DO) and unconverted glucose, depends on the dilution rate and the DO concentration. Based on reported observations with large bioreactors, Gaussian noise containing a spectrum of variances was considered to be present in the glucose feed stream. An extended Kalman filter (EKF) and a low pass Butterworth filter (LPBF) were considered representative algorithmic filters, and their noise-reduction abilities were compared with different configurations of neural filters through simulation experiments. All neural filters were superior to both the LPBF and the EKF. However, the choice of the best neural filter depends on the performance index employed. These investigations also generated two interesting corollary sets of results. One was that a hybrid neural filter, that combined an algorithmic filter and a neural filter, was better than either of its components. Secondly, the presence of optimally filtered residual noise generated more of the product than the complete removal of noise. This was explained through resonance between the filtered noise and the biological system *per se*.

The Research Council of IMTECH in its 32nd meeting held on December 19-20, 2006 reviewed the work done under this project and declared the project as successfully completed.

The second project applied similar concepts to a fed-batch fermentation for the production of poly- -hydroxybutyrate (PHB) by *Ralstonia eutropha* (formerly called *Alcaligenes eutrophus*). Besides being a fed-batch operation, this fermentation also differed from the previous one in (a) the kinetics and (b) the use of two key substrates, one for nitrogen and the other for carbon. Glucose was the carbon substrate and ammonium chloride (or sulfate) provided nitrogen. Both substrates were utilized by the cells to grow and multiply, but PHB was synthesized only when there was a shortage of nitrogen. Furthermore, neither excess carbon nor complete deprivation of nitrogen was physiologically desirable, thus making the time-variant relative flow rates of the two substrates a controlling factor. Three key issues were investigated: (a) the merits of different kinetic modelling methods, (b) optimization of the degree of dispersion of the broth, and (c) different filtering strategies for noise in the two feed streams. For kinetic modelling, mechanistic and cybernetic models were employed from the literature, and neural models were proposed in this project. Since the dispersion in large bioreactors is rarely complete, the effect of controlling the dispersion becomes important. The results showed that dispersion corresponding to a Peclet number (Pe) of about 20 maximized the performance. For both Pe = 20 and for complete dispersion (Pe = 0), a neural description for the kinetics was more accurate than both mechanistic and cybernetic models. However, since neural networks have limitations also, the use of hybrid models as in the case of the *S. cerevisiae* work just described, was also studied. As expected, hybrid models were the best. Since different hybrid designs are possible, a pivotal question to be answered during the design of a hybrid neural system is: which variables are to be assigned to the mechanistic, cybernetic and neural aspects? The answer depends on the objectives of the hybrid system and it may differ from one culture to another. These aspects have been discussed in the publications arising out of both projects.

The work carried out under these projects is described in detail in the publications listed below:

1. Patnaik P.R., Neural network designs for poly- -hydroxybutyrate production optimization under simulated industrial conditions. **Biotechnol. Lett., 27**, 409-415, 2005.

2. Patnaik P.R., Application of the Lyapunov exponent to detect noise-induced chaos in oscillating microbial cultures. **Chaos Solitons Fractals, 26**, 759-765, 2005.

3. Patnaik P.R., Feasibility and uniqueness of stationary states of nonideal recombinant fermentations. **React. Kinet. Catal. Lett.**, **87**, 11-18, 2006.

4. Patnaik P.R., Synthesizing of cellular intelligence and artificial intelligence for bioprocesses. **Biotechnol. Adv.**, **26**, 129-133, 2006.

5. Patnaik P.R., Hybrid filtering to rescue stable oscillations from noise-induced chaos in continuous cultures of budding yeast. **FEMS Yeast Res., 6**, 129-138, 2006.

6. Patnaik P.R., External, extrinsic and intrinsic noise in cellular systems:

analogies and implications for protein synthesis. **Biotechnol. Mol. Biol. Rev., 1**, 123-129, 2006.

7. Patnaik P.R., Enhancement of PHB biosynthesis by *Ralstonia eutropha* in fed-batch cultures by neural filtering and control. **Food Bioprod. Process, 84 (C2)**, 150-156, 2006.

8. Patnaik P.R., Neural network configurations for filtering of feed stream noise from oscillating continuous microbial fermentations. **Bioautomation 4**, 45-56, 2006.

9. Patnaik P.R., Fed-batch optimization of PHB synthesis through mechanistic, cybernetic and neural approaches. **Bioautomation**, **5**, 23-38, 2006.

10. Patnaik P.R., Dispersion optimization to enhance PHB production in fed-batch cultures of *Ralstonia eutropha.* **Biores. Technol., 97**, 1994-2001, 2006.

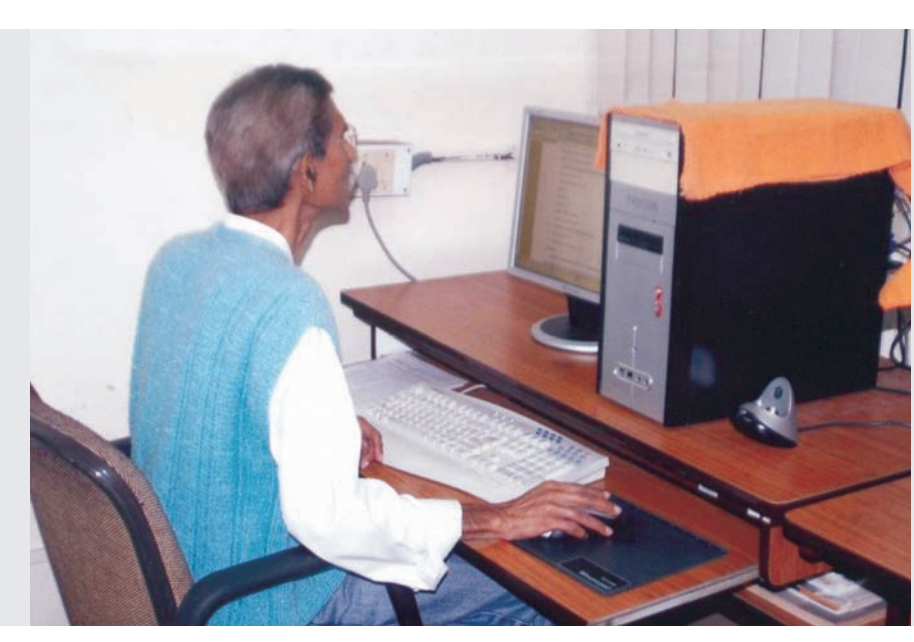
11. Patnaik P.R., A reappraisal of the plasmid stability problem in chemostat cultures. **Ind. J. Biotechnol., 6**, 267-270, 2007.

12. Patnaik P.R., Analysis of the effect of flow interruptions on fed-batch fermentation for PHB production by *Ralstonia eutropha* in finitely dispersed bioreactors. **Chem. Eng. Commun., 194**, 603-617, 2007.

13. Patnaik P.R., Sensitivity propagations in response to start-up perturbations during batch fermentations with complementary cultures. **Chem. Eng. Res. Des. , 85**, 1079-1085, 2007.

14. Patnaik P.R., Hybrid filtering of feed stream noise from oscillating yeast cultures by combined Kalman and neural network configurations. **Bioproc. Biosyst. Eng., 30**, 181-188, 2007.

Dr. Pratap R Patnaik



Sekhar Majumdar
majumdar@imtech.res.in

STUDY ON TNF- MEDIATED DEATH SIGNALING BY VIRULENT AND AVIRULENT MYCOBACTERIA

Project Leader: Dr. Sekhar Majumdar
Other Participant: Mr. Rohan Dhiman

The main objectives of this project are to understand the death signaling mediated by TNF-á and the role of NF-êB dependent anti-apoptotic protein molecules on apoptosis manifested by pathogenic and non-pathogenic mycobacteria. Studies will include examination of the apoptotic potential of virulent (*Mycobacterium tuberculosis* H37Rv) and avirulent (*Mycobacterium tuberculosis* H37Ra) mycobacteria on infected macrophages, role of NF-êB in apoptosis and mechanism of apoptosis in infected macrophages.

An emerging principle in intracellular parasitism is that successful pathogens such as *M. tuberculosis* have acquired the ability to persist in the host without always inducing disease and mortality. Despite the implementation of TB control programs, case rates continue to soar where the prevalence of HIV infection is high. The situation is further complicated by a worldwide increase in drug resistant and MDR-TB, and the recent reports of XDR-TB. Thus, the resurgence of TB truly constitutes a global health crisis. So, better understanding of host-pathogen interaction is the need of the hour.

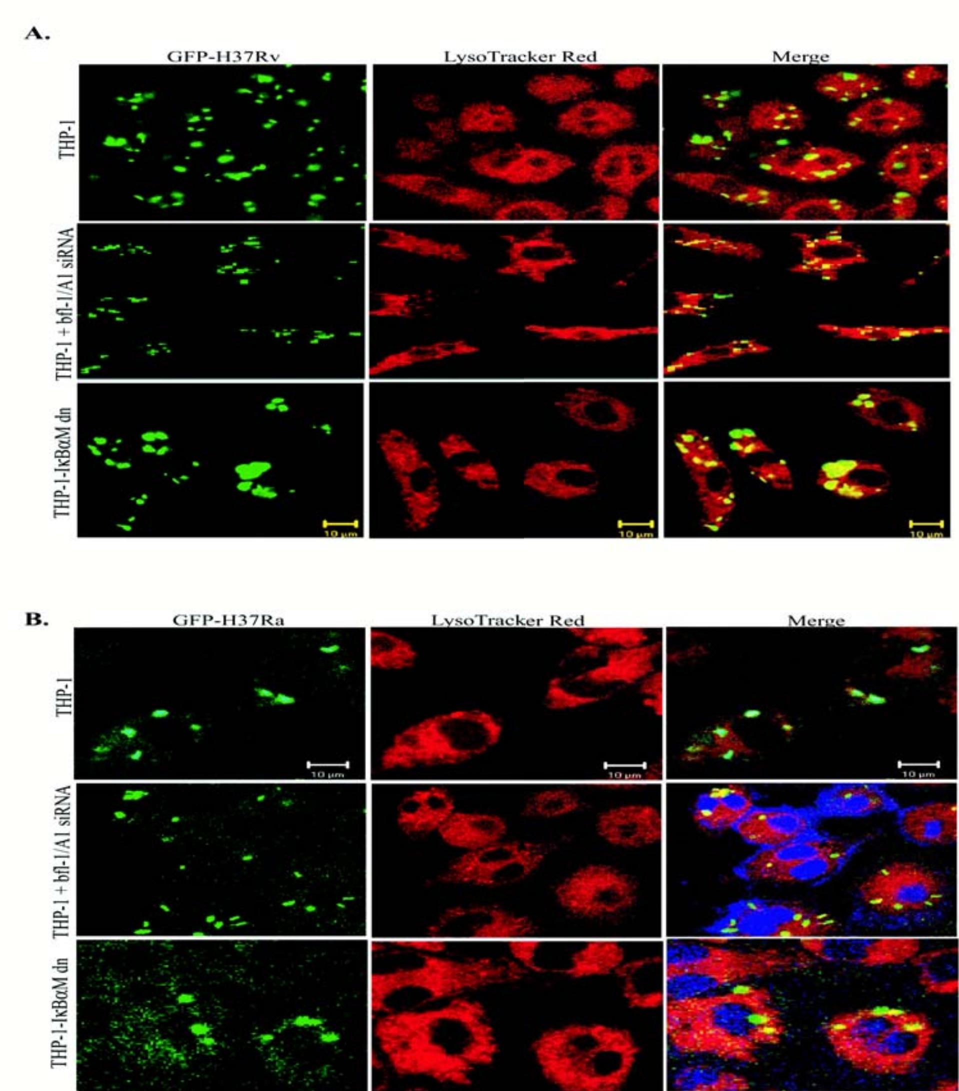
Preliminary experiments revealed that *M. tuberculosis* H37Rv and *M. tuberculosis* H37Ra differ in their apoptotic potential. *M. tuberculosis* H37Ra infected cells undergo more apoptosis than *M. tuberculosis* H37Rv in THP-1 cells. In order to understand the role of NF-êB in *M. tuberculosis* H37Rv mediated apoptosis, stable THP-1 cell-line (THP-1-IêBáM dn cells) over expressing IêBá mutated form was generated so as to inactivate NF-êB. Inactivation of NF-êB in THP-1- IêBáM dn cells was checked by studying NF-êB-DNA binding activity using EMSA and by seeing degradation of IêBá in cytoplasm. Annexin V staining clearly showed that the proportion of apoptotic macrophages in THP-1-IêBáM dn cells harboring *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv were more in comparison to *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv infected THP-1 cells respectively. This result was further confirmed by TUNEL, DAPI staining and DNA fragmentation where comparable fractions of *M. tuberculosis* H37Ra and *M. tuberculosis* H37Rv infected THP-1- IêBáM dn cells showed chromatin condensation and nuclear fragmentation suggestive of apoptosis than infected THP-1 cells. Further to deduce the mechanism of apoptosis and taking into account the earlier published report suggesting central role of mitochondria in TNF-á mediated

apoptosis of macrophages, mitochondrial membrane potential of infected THP-1-IκBαM dn and THP-1 cells was examined by flow cytometry as well as by confocal microscopy where significant change in mitochondrial membrane potential of infected THP-1- IκBαM dn cells was observed than infected THP-1 cells followed by significant release of cyt. c from mitochondria. Effect of cyt. c release was also studied on downstream caspases like caspase-3 where *M. tuberculosis* H37Rv and *M. tuberculosis* H37Ra infected THP-1- IκBαM dn cells showed more caspase-3 activity than infected THP-1 cells.

RNAse protection assay further gave insight into the mechanism of apoptosis where differential expression of bfl-1/A1 mRNA, an anti-apoptotic member was found with *M. tuberculosis* H37Rv or *M. tuberculosis* H37Ra infection in THP-1 cells at 48 h of post-infection. Since, bfl-1/A1 mRNA expression is regulated by NF- κB, activation of NF-κB was also studied at 48 h of post-infection where *M. tuberculosis* H37Rv infection showed more NF-κB-DNA binding activity than *M. tuberculosis* H37Ra. This was further confirmed by NF-κB-dependent reporter assay and p65 translocation by confocal microscopy.

It has been reported that TNF-α plays very important role in apoptosis of mycobacteria infected macrophages. Before studying the release of TNF-α in mycobacteria infected THP-1 and THP-1- IκBαM dn cells TNFR1 expression was first measured in PMA differentiated THP-1 and THP-1- IκBαM dn cells where THP-1- IκBαM dn cells showed higher TNFR1 expression than THP-1 cells. At 48 h post-infection, increased production of TNF-α was observed in THP-1- IκBαM dn cells than in THP-1 cells infected with *M. tuberculosis* H37Rv or *M. tuberculosis* H37Ra by ELISA. Role of TNF-α was investigated using neutralizing anti-TNF-α antibody where marked decrease in apoptosis after 72 h of post-infection were observed in both types of cells harboring *M. tuberculosis* H37Ra with anti-TNF-α antibody. In contrast, addition of anti-TNF-α antibody failed to show any effect on *M. tuberculosis* H37Rv infected THP-1 as well as THP-1- IκBαM dn cells.

Dr. Sekhar Majumdar (standing, fourth from right) and group



Bfl-1/A1 inhibition enhances P/L fusion in GFP-*M. tuberculosis* H37Rv or GFP-*M. tuberculosis* H37Ra infected cells after 48 h of infection. THP-1 cells were first transfected with bfl-1/A1 siRNA and then infected with GFP-*M. tuberculosis* H37Rv (A) or GFP-*M. tuberculosis* H37Ra (B) for 4 h. At 48 h post-infection, infected and control cells were stained for 2 h with LysoTracker and analyzed for P/L fusion under confocal microscope. THP-1-IkBaM dn cells were directly infected with GFP-*M. tuberculosis* H37Rv (A) or GFP-*M. tuberculosis* H37Ra (B) for 4 h and at 48 h post-infection, cells were analyzed for P/L fusion

Increased apoptosis in infected THP-1- IκBαM dn cells also showed its effect on the intracellular survival of *M. tuberculosis* H37Ra by 3.1 fold in THP-1- IκBαM dn cells in comparison to normal THP-1 cells and of *M. tuberculosis* H37Rv by 3.5 fold in THP-1- IκBαM dn cells in comparison to normal THP-1 cells. In conclusion, the study for the first time demonstrated that inhibition of NF-κB in *M. tuberculosis* infection lead to increase in apoptosis of infected macrophages by down-regulating bfl-1/A1 mRNA expression which affects intracellular bacterial viability.

Efforts are being made to target the expression of bfl-1/A1 so as to modify the outcome of infection.

Purnananda Guptasarma
pg@imtech.res.in

Engineering of proteins to understand determinants of protein folding, aggregation and stability, and to manipulate enzymatic or therapeutic function

Project Leader: Dr. Purnananda Guptasarma
Other Participants: Ms. Swati Sharma
Mr. Shubbir Ahmed
Mr. Sanjeev Kumar Chandrayan
Ms. Divya Kapoor
Mr. Neeraj Dhaunta
Ms. Uzma Fatima
Mr. Bipan Verma
Ms. Sharanjit Kaur
Principal Collaborators : Dr. Subramanian Karthikeyan
Dr. Balvinder Singh
Dr. Dibyendu Sarkar

Protein/enzyme surface re-engineering : The group has developed and experimentally tested a method, based on protein structural analyses, for the rational mixing of the interior features of a beta sheet-based protein with the surface features of a different structurally-homologous beta sheet-based protein, regardless of the level of sequence identity. Using this method, developed in collaboration with S. Karthikeyan and B. Singh, the group has successfully transplanted the cellulose-binding and catalytically-active surface of a mesophile endoglucanase (*Trichoderma reesei* Cel12A) onto a thermophile counterpart glucanase (*Rhodothermus marinus* Cel12A) to create a novel enzyme that combines the activity characteristics (the optimum temperature of function) of the mesophile enzyme with the structural stability characteristics (the temperature of structural melting) of the thermophile protein. The transplanted surface is made up of many tens of non-contiguous residues that come together to form the cellulose-binding groove, during folding, from all over the novel enzyme's polypeptide sequence, as established through determination of the X-ray crystal structure in collaboration with S.Karthikeyan. Besides the novelty of the engineering approach, and the obvious implications for the design and production of new industrial enzymes, the work also demonstrates a hitherto-unsuspected autonomy of structure and function of enzyme active surfaces, and shows that high global structural stability does not necessarily translate into high temperatures of function, in enzymes. Patents have been applied for. Besides this endoglucanase, we have also performed active surface transplantation with a *Pyrococcus furiosus* aminopeptidase, and are part-way through performing a whole surface transplant of the entire surface of a lens protein, beta crystallin B2, onto a structurally-homologous lens protein, gamma crystallin.

Therapeutic protein surface re-engineering : The group used structural analyses to arrive at potential modes of self-assembly of interleukin-2 into large soluble and insoluble aggregates, and are part-way through testing specific engineered variants for reduced proneness to aggregation. Similarly, the group used a combination of structural analysis and experimentation (principally mass spectrometry) to arrive at potential mutations to increase the proteolytic stability (shelf-life) of interferon gamma, and are part-way through testing these. In addition, in collaboration with D. Sarkar, the group designed variants of erythropoietin that are being tested for increased proteolytic stability in the absence of glycosylation.

Heterologous enzyme expression : The group cloned about ten different enzymes of importance from *Pyrococcus furiosus* and successfully expressed several of these in *Escherichia coli* and refolded the same to functional form, for in-house studies and transfer to interested industries. In particular, characterization of stability to water-miscible organic solvents, and function in the presence of solvents, has been carried out for a lysophospholipase of potential importance as a long-chain lipid de-esterification agent. Simultaneously, the group also modified an existing expression vector to create a novel vector for increased production of hyperthermophile proteins through fusion with a specific hyperthermophile protein that appears to both reduce proteolysis and improve the folding of the fusion partner; this vector also provides for affinity-based purification and post-purification proteolytic separation of fusion partners. A patent is being filed.

Stability of natural and engineered hyperthermophile proteins : The group achieved three important milestones in regard to understanding the kinetic stability of *P. furiosus* triosephosphate isomerase (TIM). **(A)** The group destroyed electrostatic interactions on the surface of *Pfu* TIM, through a combination of denaturant and heat, and without disturbing the secondary or tertiary structure, to demonstrably destroy kinetic stability and bring about cold denaturation upon cooling from 90-100 C. **(B)** The group similarly destroyed electrostatic interactions on the surface of *Pfu* TIM, through alterations in pH, again without disturbing the secondary or tertiary structure, to destroy the molecule's kinetic stability. **(C)** In partial support of the group's hypothesis regarding the mechanism of kinetic stabilization, the autonomous folding of numerous secondary and supersecondary structural elements (genetically excised from *Pfu* TIM and expressed in *E.coli*) was demonstrated.

Folding of sequence-scrambled proteins : The group discovered that folded beta sheet-containing structures with high polyproline type II (PPII) structural content are obtained by **(A)** backbone-reversal engineering of *E.coli* GroES, **(B)** conservative residue-substitution engineering of *Zea mays* chymotrypsin inhibitor 2 (CI2), and **(C)** genetic excision and tandem duplication of a large beta hairpin structure located on the surface of alpha hemolysin from *Staphylococcus aureus*. In all cases, the structure initially obtained was transformable into forms with further increased beta sheet content, through heating which converted PPII structures into beta sheets, or increased alpha helical content, through addition of trifluoroethanol. Retro-GroES folded to assemble into a multimer of 3 or 5 subunits. The other two engineered constructs remained monomeric, and displayed the structural features known to be characteristic of them in their original structural/sequence contexts.

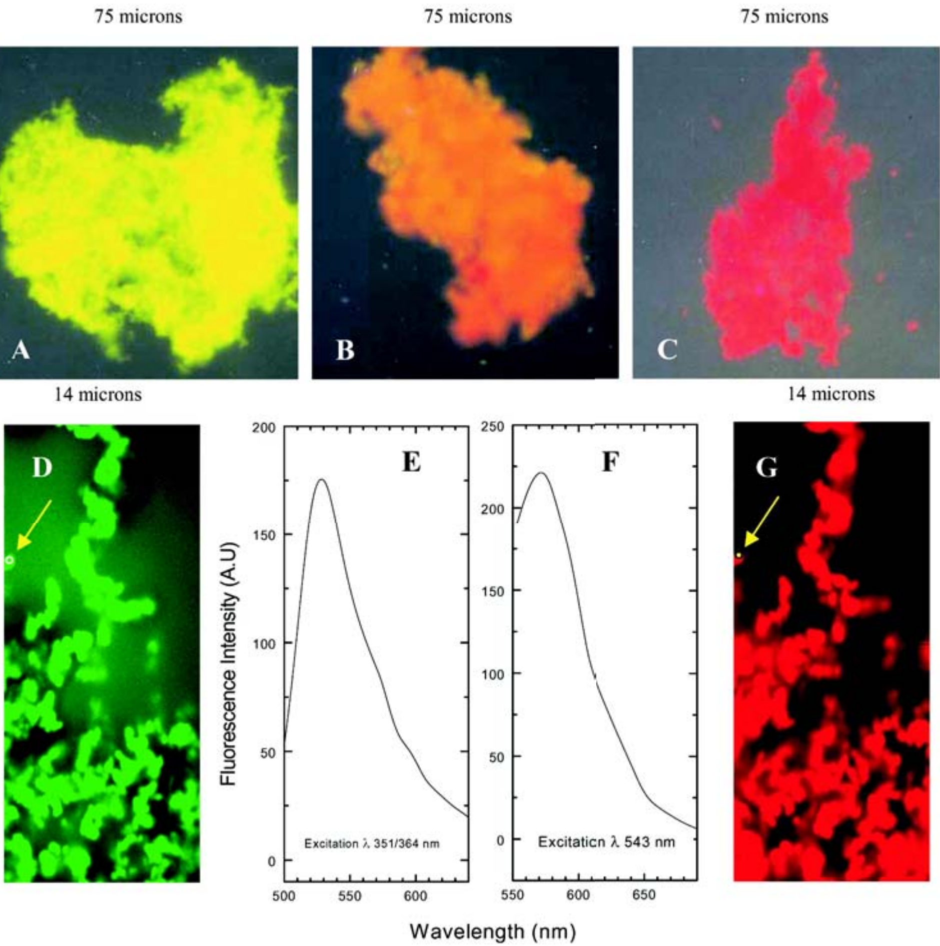


Figure. Heteroaggregation of the backbone-reversed forms of two all-beta proteins, retro-CspA and retro-HSP12.6. The three top panels show routine fluorescence microscopic images of FITC-labelled retro-CspA (panel A), TRITC-labeled retro-HSP12.6 (panel C) and a co-aggregate of the two proteins (panel B). Panels D and G show fluorescence from FITC, and TRITC, respectively, in the same confocal sections in a field of co-aggregates; the corresponding fluorescence emission spectra associated with regions of interest (marked by arrows) are shown in panels E and F.

Mechanisms of protein aggregation : These studies are continued on from the last biennium report. Briefly, during these two years, the group : (**A**) produced and studied a super-prionogenic variant of the human prion, initially identified through phage-display library creation and screening of prion variants mutagenized between residues 101-112, which spontaneously folds into a beta sheet-based structure, and also forms amyloid fibrils, establishing the importance of naturally occurring mutations in this region of the sequence which are associated with genetic prion disease such as new variant CJD; (**B**) established, using differential labelling and a

novel confocal spectrofluorimetric microscopy technique, that structurally unstable beta sheets can lose structure and associate in heteromolecular fashion to form aggregates; (**C**) established, using proteolytic probing of susceptible peptide bonds, that the structure of carbonic anhydrase chains within thermal aggregates conserves many native-like features; (**D**) created genetic fusions of amyloid-forming polypeptides with proteins that are extremely structurally stable (specifically, retro-CspA with green fluorescent protein, in one case, and Alzheimer's amyloid beta peptide with a thermophile-derived endoglucanase, in the other) to investigate whether in such fusion constructs the stable domain keeps its fusion partner from undergoing aggregation, or whether the aggregation-prone partner pulls the stable domain into the aggregate in structured, or unstructured form. While the results with the second fusion showed unequivocally that the stable domain resists aggregation, in the first fusion, GFP deposited with retro-CspA into an amyloid-like aggregate with reduced fluroescence quantum yield; **(E)** We have developed panels of phage display-derived monoclonal scFv antibodies against beta-2-microglobulin folded into two different conformations.

Dr. Purnananda Guptasarma (sitting, left) and group

R.K. Jain
rkj@imtech.res.in

CHEMOTAXIS AND DEGRADATION OF NITROPHENOLS AND NITROBENZOATES BY BACTERIA

Project Leader: Dr. Rakesh K. Jain
Other Participants: Mr. Gunjan Pandey
Ms. Debarati Paul
Mr. Janmejay Pandey
Ms. Archana Chauhan
Mr. Dhan Prakash

The objectives of the project were:

1. Characterization of nitrophenols and nitrobenzoates degrading chemotactic microorganisms. These compounds include o, -m and p-nitrophenols and o, -m- and p-nitrobenzoates.
2. Understanding of the pathways involved at the biochemical and genetic levels in the biodegradation of above compounds.
3. Understanding of the chemotactic behaviour of the microorganisms to the above listed nitroaromatic compounds.
4. Attempts to transfer the chemotactic property to more efficient nitroaromatic degrading non-chemotactic organisms studies to test the efficiency of such microorganisms for the degradation of these compounds.

Arthrobacter protophormiae and *Ralstonic* sp. are the two organisms, isolated by enrichment technique used in the present study, which are capable of utilizing p-nitrophenol (PNP), 4- nitrocatechal (NC) and o-nitrobenzoate (ONB) as sole source of carbon and energy. PNP and 4-NC are degraded by the oxidative route whereas ONB degradation followed a reductive route. The pathways for the degradation of above compounds have been determined on the basis of enzyme assays and characterization of the intermediates. These techniques involved TLC, GC, GC-MS, 1H NMR. In *Arthrobacter protophormiae*, a plasmid of approximately 65 kilobase pair was found to be responsible for encoding genes for the degradation of above compounds by ONB (0.5mM) could be degraded via the formation of anthranilic acid by *A. protophormiae*. Recently, it has been shown that this organism is capable of adapting itself to 90 mM ONB followed by its complete degradation. Following growth of this organism on higher concentration of ONB, presence of 3-hydroxyanthranilic acid (HAA) was clear and this was not found to be present as an intermediate in the degradation pathway earlier. The presence of HAA was identified by TLC and HPLC analysis following comparison with the authentic compound. Resting cell studies of ONB-induced cells showed that HAA was indeed an intermediate of the degradation pathway. This pathway is also a novel one and has been included in the Biocatalysis/Biodegradation database, University of Minnesota, USA.

Attempts were made to clone the gene(s) or gene cluster(s) involved in the degradation of some of the nitroaromatic compounds such as PNP and NC from *A. protophormiae*, *Burkholderia cepacia* and *Ralstonia* sp. These enzymes are benzenetriol dioxygenese and maleylacetate reductase. The above enzymes were found to be involved in the degradation of several aromatic pollutants including PNP and NC. This was achieved through PCR amplification using degenerate primers. The group was able to amplify a 300 bp fragment of maleylacetate reductase from *B. cepacia* which was further cloned into pBluescript II KS(+) vector and the above fragment was sequenced. Database search showed substantial homology with maleylacetate reductase gene. Similarly, in case of benzenetriol dioxygenase gene(s), using degenerate primers, the team were able to amplify a fragment of 540 bp in case of *B. cepacia* and *Ralstonia* sp. and a fragment of 300 bp in case of *A. protophormiae*. These fragments were then cloned into pBluescript II KS(+) vector and sequenced. The database search showed homology with benzenetriol dioxygenase enzyme. The Southern hybridixation of digested genomic DNA using 540 bp fragment as a probe confirmed the cloning of the gene(s) for benzenetriol dioxygenase enzyme. The group then prepared a genomic library of *Ralstonia* sp. from the DNA fragment that showed homology with benzenetriol dioxygenase, in order to clone the complete gene cluster, as the team knew that degradation genes are usually present in a cluster in many organisms. Clones were screened by dot blot analysis using the 540 bp DNA fragment(s) as probe and colony PCR. One of the clones that showed a high degree of homology in dot blot analysis was selected, its DNA was isolated and digested with EcoRI enzyme. This clone showed the presence of an insert of approximately 7 kb. Initial sequencing results of this fragment clearly showed the presence of maleylacetate reductase and benzenetriol dioxygenase. The sequence has now been completely annotated and submitted to the Genbank, NCBI (Accession No. AY574278). The group is further working on the characterization of the above clone. The genomic library obtained as above from *Ralstonia* sp. was screened for some other gene(s) for upper pathway enzyme(s) of PNP and NC by nitrite release. The clones were also screened for any possible chemotactic gene(s) involved in the chemotaxis of *Ralstonia* sp. toward different NACs.

The team had earlier shown that *Ralstonia* sp., isolated by the chemotaxis enrichment technique, is capable of utilizing PNP, NC, ONB and p-nitrobenzoate (PNB) as sole source of carbon and energy. The organism was found to be chemotactic toward these compounds as determined by the drop assay, swarm plate assay and capillary assay. However, it failed to show chemotaxis towards those compounds that are not degraded by the bacteria. Some of the intermediates of the degradative pathways have also been identified. Results obtained have indicated a correlation between chemotaxis and biodegradation of nitroaromatic compounds. The group has also determined that the above microbe is capable of utilizing 3-methyl -4-nitrophenol as sole source of carbon and energy, this compound is one of the major breakdown products of fenitrothion {(o, o- dimethyl o-(3-methyl-4-nitrophenyl) thiophosphate}which is a recalcitrant organophosphate insecticide used in agriculture. It has now been shown that *Ralstonia* sp. is also chemotactic toward

those nitroaromatic compounds that are partially degraded by the organism by using the above-mentioned assays. These compounds are m-nitrophenol, 2,4-dinitrophenol, 2,5-dinitrophenol, 2,6-dinitrophenol, o-nitrobenzene, m-nitrobenzene and 3,5-dinitrobenzoic acid. Some of the intermediates of the degradative pathways have also been identified using TLC, GC and GC-MS studies. The results obtained, therefore, have indicated a correlation between chemotaxis and biodegradation of nitroaromatic compounds. The chemotactic behaviour of this organism has also been determined in soil. A new soil-agarose plate method has been successfully developed in the laboratory. Encouraging results were also found in soil-agarose plate assay for other nitroaromatic compounds. A quantitative assay for soil chemotaxis has also been successfully performed.

The work carried out under this project, as published, is as below:

Pandey, G. and Jain, R.K. Bacterial chemotaxis toward environmental pollutants: Role in bioremediation. **Appl. Environ. Microb., 68**, 5789-5795, 2002.

Pandey, G.; Chauhan, A.; Samanta, S.K. and Jain, R.K. Chemotaxis of a *Ralstonia* sp. SJ98 toward co-metabolizable nitroaromatic compounds. **Biochem. Biophys. Res. Commun., 299,** 404-409, 2002.

Pandey, G.; Paul, D. and Jain, R.K. Branching of nitrobenzoate degradation pathway in *Arthrobacter protophormiae* RKJ100: Identification of new intermediates. **FEMS Microbiology Lett., 229**, 231-236, 2003.

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The Research Council of IMTECH in its 28th meeting reviewed the work done under this project and declared the project as successfully completed.

Dr. Rakesh K Jain (standing, sixth from right) and group

Jagmohan Singh
jag@imtech.res.in

UNDERSTANDING THE MOLECULAR MECHANISM OF LINKAGE BETWEEN THE RNAi MACHINERY AND HETEROCHROMATIN ASSEMBLY IN FISSION YEAST

Project Leader: Dr. Jagmohan Singh
Other Participants: Dr. Sharanjit Saini
Dr. Sumit Arora
Dr. Nandni Nakwal
Mr. Kamlesh K. Bisht
Mr. Ashok Kumar
Ms. Swati Haldar
Ms. Nupur Malhotra

The main objective of the proposal is to address the missing connection in the puzzle, i.e., how is Clr4 recruited to the heterochromatin sites by the RNAi pathway and how other heterochromatin proteins are assembled. In addressing these questions, the group envisages the following objectives:

i) Are Clr4 and Swi6 part of complex with siRNA in vivo?

Earlier published work has shown that the chromodomain region of the heterochromatin protein HP1, of which Swi6 is an ortholog, functions as an RNA-binding motif. It was proposed that Swi6 and/or Clr4, both of which contain the chromdomain, may bind to the RNA molecules being transcribed from the repeat sequences in the mating type and centromere regions. In agreement, unpublished data show that Swi6, and especially Clr4, exhibit binding towards siRNA, especially in the single-stranded form or as a hybrid with the siRNA-DNA hybrid, leading to the hypothesis that siRNA generated from the repeat sequences may bind to Swi6 and/or Clr4 and get chaperoned to the sites of homology, where they may initiate the histone modification (H3-Lys9 methylation), thus initiating the heterochromatin-specific mark. In addition the group found that the localization of Clr4 to the heterochromatin regions is sensitive to treatment with RNAseH, supporting the involvement of DNA-RNA hybrid in tethering Clr4 to the repeat sequences that transcribe RNA.

The group would like to further check whether Swi6 and Clr4 exist as a complex with RNA *in vivo* and to identify other protein components of such a hypothetical complex.

a) The identity of the RNA sequences bound by Swi6 and/or Clr4 will be checked by microarray technology, where the RNA isolated from

such complexes will be labeled with fluorescent dyes and used as a probe with the tiling arrays of *S. pombe*.

b) The identity of other protein components of such complexes will be determined by using strains harboring TAP-tagged copies of swi6 and or clr4 genes, isolation of the complexes using affinity chromatography and analysis of their protein components by mass spectrometry. Subsequently, the knockout of the genes corresponding to the identified protein components of the complex will be performed to investigate their role in silencing.

ii) Is there a direct physical interaction between RNAi and heterochromatin components?

The genetic data have indicated that of the three components of RNAi machinery, Rdp acts in the same pathway as Swi6 and Clr1-Clr4. Furthermore, the derepressed phenotype obtained at the mating type and centromere locus is suppressed by overexpression of the histone deacetylase Clr3. Thus, there may be direct interaction between some of the components of RNAi pathway and heterochromatin machinery, especially between Clr3 and Rdp. Therefore, the group will address the following questions:

a) Is Clr3 recruited to heterochromatin and whether this recruitment is dependent on Rdp, Dcr and Ago?

b) Is there a direct interaction between Clr3 and RNAi components, especially Rdp?

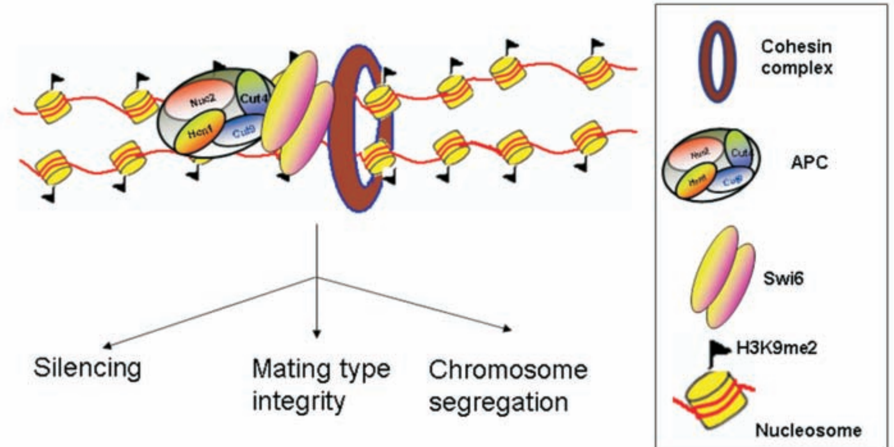
During the period under report the work carried out is as under:

i) Role of Anaphase Promoting Complex (APC) in Silencing:

Earlier work in the laboratory had shown the involvement of APC in gene silencing in fission yeast, which is mediated by its role in recruitment of the chromodomain protein Swi6 and histone methyltransferase Clr4 to the mat and cen loci. Further work has shown that APC subunits Cut4 and Cut9 directly interact with both Swi6 and Clr4 in vitro and in vivo and the mutant Cut4 protein fails to interact with Swi6 and Clr4. Thus, APC recruits Clr4 and Swi6 by direct physical interaction. Since Swi6 has been shown to be required for recruitment of cohesion complex, the group checked for recruitment of Rad21 and its degradation during metaphase-to-anaphase transition. Interestingly, both processes are abrogated in cut4 and cut9 mutants.

Further work has shown that, surprisingly, both Cut4 and Cut9 are enriched at the mat and cen loci and interestingly, this localization is dependent on Swi6. These results suggest that APC on one hand and Swi6 along with Clr4 on the other are involved in a positive feedback loop to bring about heterochromatin assembly.

The team have further addressed the order of events and found that while Swi6 can suppress the silencing defect of cut9 mutant, cut4 cannot suppress the silencing defect of swi6 mutant. These results suggest that Swi6 may act



Speculative model based on our studies shows a contiguous localization of APC and Cohesin at heterochromatin regions through their interaction with Swi6, which may further facilitate heterochromatin assembly, silencing, mating-type integrity, centromeric cohesion and segregation.

downstream of APC. Based on other genetic results, a model was proposed wherein APC may be recruited at the time of DNA replication; APC then recruits Swi6 and/or Clr4 and thereafter, a positive feedback loop of interaction ensures assembly of heterochromatin and its further stabilization by recruitment of Cohesin.

ii) Role of DNA polymerases α and δ in silencing:

Following up on the earlier work showing that Polá and ä are involved in silencing, interestingly, the group now finds that both Polá and Polä interact with Swi6 and Clr4 as well and the *pol*á and *pol*ä mutants are defective in recruitment of Swi6, H3-K9 methylation as well as cohesin recruitment at mat and cen loci. These results show a tight coupling between DNA replication and heterochromatin machineries and suggest the possibility that chromatin structure at the heterochromatin loci may be copied in a semi-conservative mode, like DNA. A proposal to test this possibility experimentally has been supported by the New Idea Fund scheme of CSIR.

iii) Role of Chromatin Assembly Factor 1 and Pap1 in Gene Silencing:

In another line of work the team have investigated the role of CAF1 and Pap1, which perform roles in DNA replication coupled assembly of chromatin and in response to oxidative stress, respectively, in gene silencing. The three subunits of CAF1, namely Cac1, Cac2 and Cac3 have been shown to be non-essential for viability but essential for protection from UV damage in *S. cerevisiae*. However, in *S. pombe*, the team found that *cac3* is essential and allelic to previously identified gene *mis16* in *S. pombe*. As expected, the strains having *cac1Δ*, *cac2 Δ* and *mis16 /cac3* mutations are defective in silencing at mating type and the outer repeats of centromere in *S. pombe*. In

addition, *cac1 Δ* and *cac2 Δ* strains show a modest defect in rDNA silencing but no effect on telomere silencing. Radiation sensitivity experiments showed that *cac1 Δ* strain shows a modest sensitivity to UV rays but not to ã-radiation. Most interestingly, *cac1 Δ* and *cac2 Δ* strains show significant sensitivity to histone deacetylase inhibitor TSA and *mis16* mutant is sensitive to the DNA replication inhibitor hydroxyurea. In presence of HU, *mis16* mutant exhibits increased septation, and chromosome segregation, referred to as 'cut' (chromosomes untimely torn) phenotype, which appears coincidentally with decline in viability, suggesting that mis16 may be defective in chromatin assembly checkpoint. Such a checkpoint although speculated to occur has not been demonstrated in any system so far.

Pap1 is a global transcription factor which is induced by mild oxidative stress. Since the Pap1 also functions in regulating the expression of a chromatin regulator Uhp1 that affects silencing, the team explored the possibility whether it might be involved in regulating silencing as well. In this regard, it has been shown that another factor Atf1, which is induced upon strong oxidative stress, is also required for silencing of mating type loci independently of RNAi and its function is mediated through Atfi1 binding sites neighbouring the *mat3* locus and through recruitment of the histone methyltransferase Clr4. The results show that Pap1 is required for silencing of *mat3* and outer repeat locus *otr1::R* of centromere I. In addition, it is also required for silencing of reporter genes inserted at *cnt1* and rDNA loci. In support of its role in silencing, deletion of the individual binding sites in the *mat2-mat3* interval also partially abrogates silencing. Interestingly, *pap1 Δ* strain is also sensitive to TSA suggesting that Pap1 may be required for global chromatin assembly and histone acetylation.

Dr. Jagmohan Singh (sitting, centre) and group

Alok Mondal
alok@imtech.res.in

UNDERSTANDING MOLECULAR GENETICS OF HALOTOLERANCE IN YEAST *Debaryomyces hansenii*

Project Leader: Dr. Alok Mondal
Other Participants: Dr. Monika Aggarwal
Mr. Netrapal Meena
Ms. Dipanwita Biswas
Ms. Anupriya Minhas
Mr. Deepak Bhatt
Mr. Randeep Sharma

Debaryomyces hansenii is one of the most osmotolerant and halotolerant species of yeast. It can grow in the medium containing as high as 4M NaCl, whereas the growth of *S. cerevisiae* is limited in medium containing more than 1.7M NaCl. It is the yeast most frequently found in traditional cheese and sausages, with a recognized contribution to special flavors in these products thus, has an enormous bio-technological potential for food industry. In the recent past, *D. hansenii* has become an important model for understanding halotolerance and osmotolerance. The group is working towards understanding the extremophillic nature of this yeast at genetic and biochemical level. During the period under report the work carried out is as under:

A. Cloning and characterization of DSLN1, DNIK1 and DSHO1

In yeast, the osmosensing signal transduction pathway (HOG pathway) plays cardinal role in regulating its growth and survival under high osmolar conditions. The core component of this pathway is the MAP kinase, Hog1p. HOG pathway is ubiquitous in yeast and fungi. Although this pathway has been studied quite well, how cells sense the change in the osmolarity of the environment remained an enigma. In *S. cerevisiae* a trans-membrane protein Sho1p and a membrane bound two-component histidine kinase Sln1p appeared to be involve osmosensing. In case of *N. crasa* and *C. albicans* Nik1p, a hybrid histidine kinase has been implicated to function as osmosensor. To identify putative osmosensor/s in *D. hansenii*, the group conducted a blast search (TBLASTN) of *D. hansenii* genome sequence utilizing *S. cerevisiae* Sho1p & Sln1p and *C. albicans* Nik1p sequences. The search revealed presence of Sho1 homologue in chromosome C (E value $5e^{-34}$); Sln1 homologue in chromosome E (E value $9e^{-148}$); Nik1p homologue in chromosome G of *D. hansenii*. The corresponding genomic region was PCR amplified *D. hansenii* strain CBS767 and cloned in yeast vector pRS425. The amplified fragments were sequenced to rule

out any mutation. Phenotypic analysis showed that *DSHO1* could complement *sho1* mutation in *S. cerevisiae*. Similarly, the cloned *DSLN1* could complement *sln1* mutation in *S. cerevisiae*. Interestingly, the Sln1 homologue in *D. hansenii* has been predicted to be a pseudogene due to the presence of a stop codon. However, this stop codon was absent in the clone as observed from DNA sequencing. Therefore, this gene is bona fide *SLN1* homologue. The cloned *DNIK1* gene was also found to complement *sln1* mutation in *S. cerevisiae*.

B. Creation of salt-insensitive 3'(2'), 5'-bisphosphate nucleotidase by modeling and mutagenesis approach

Previously the group has identified a 3'(2'), 5'-bisphosphate nucleotidase (Dhal2p) from a highly halotolerant yeast *Debaryomyces hansenii.* Molecular characterization revealed a number of unique features in Dhal2p, indicating this is an extraordinary member of the family. The team therefore carried out the structure-function analysis of Dhal2p. The group has used molecular modeling approaach to identify key amino acid residues and region that are important for its function and salt tolerance. By doing *in vitro* mutagenesis as well as *in vivo* studies, the team have established, for the first time, the crucial role of the 'flap region' and the 'loop region', in the salt tolerance of Dhal2p. Moreover, this structural knowledge has provided the team an opportunity to create novel and salt insensitive mutant of Dhal2p.

C. Development of genetic tool for *D. hansenii*

For a development of the *D. hansenii* transformation system, four histidine auxotrophs were isolated. Cells of the wild-type strain CBS767 were UV-irradiated at approximately 10% survival and plated to achieve 200-300 viable colonies per plate, on YPD. After testing ~20000 colonies, 4 his- mutants were isolated. Among these, DB9 appeared to be very stable and carry a defective DhHIS4 gene. The group has constructed a plasmid pDH100 by cloning ARS element of *Candida famata* and DhHIS4 gene. This plasmid was transformed into DB9 by electroporation and transformants were selected on YNB plate. Plasmid rescue experiments from the transformants showed that pDH100 was maintained episomally in *D. hansenii*. Subsequently, by screening a genomic library of D. hansenii strain CBS767, the group has isolated eight genomic fragments that could function as ARS element. High frequency transformation of *D. hansenii* could be achieved with some of these fragments.

Dr. Alok Mondal (sitting, centre) and group

Pradip K. Chakraborti
pradip@imtech.res.in

CHARACTERIZATION OF MYCOBACTERIAL SERINE / THREONINE KINASES

Project Leader: Dr. Pradip K. Chakraborti
Other participants: Ms. Meghna Thakur
Mr. Rahul Saxena
Mr. Haider Hussain Dar
Ms. Payal Mittal
Ms. Pavitra Kanudia
Mr. Jankey Prasad

The objectives of this project were:

i) Cloning, overexpression and purification of mycobacterial serine/threonine protein kinases utilizing a heterologous (*E. coli*) expression system.
ii) Biochemical characterization of mycobacterial serine/threonine protein kinases.
iii) Identification of natural substrates of mycobacterial serine/threonine protein kinases.
iv) Evaluating contributions of mycobacterial serine/threonine protein kinases.

The genome sequence of *Mycobacterium tuberculosis*, the causative agent of the dreadful disease, tuberculosis, has unveiled the presence of a family of eleven eukaryotic-type Ser/Thr kinases. The existence of such a large number of regulatory proteins in this intracellular facultative pathogen could be attributed to its unique characteristics of being able to survive within the hostile environment inside the host together with its long quiescent dormant state. The work in this project was focussed on one such homologue of mycobacterial Ser/Thr kinases, PknA. Previously this goup has reported molecular, and biochemical characterization of this kinase (Chaba et al., **Eur. J. Biochem.**, 269: 1078 - 1085, 2002.) and its interaction with the only serine/threonine phosphatases, PPP. This work has further been extended towards in depth understanding of autophosphorylation activity of this kinase, identification of its different subdomains and their functional significance. Finally, attempt has also been made to identify the natural substrate of this kinase.

Structural Analysis of PknA: Ser/Thr or Tyr kinases are annotated based on the presence of a catalytic domain harbouring eleven signature motifs, termed as Hank's subdomains. Analysis of the nucleotide derived amino acid sequence of PknA indicated the presence of these conserved subdomains towards its amino terminus end. PknA showed the ATP binding motif GXGXXG (alanine is present instead of first glycine and X is a variable amino acid) in subdomain I and an invariant lysine

residue in subdomain II, characteristic of Ser/Thr or Tyr kinases. The essentiality of this lysine for exhibiting the kinase activity of PknA was highlighted by the inability of K42N mutant (lysine at amino acid position 42 of PknA was mutated to asparagine) to autophosphorylate. Furthermore, typical DXKPXN and GTXXYXAPE sequences of Hank's subdomain VI and VIII respectively argued the inclusion of PknA as a member of eukaryotic-type Ser/Thr protein kinases. An Ala/Pro-rich (alanine/proline-rich) region was identified downstream to the catalytic domain of PknA, followed by a hydrophobic stretch of 23 amino acid residues. The charge distribution around this sequence of PknA revealed it to be a transmembrane kinase acting as a signalling molecule between the cell exterior and interior. The amino acid sequence following the transmembrane domain shared negligible homology with other eukaryotic-type Ser/Thr kinases and thus is unique to PknA. This stretch of amino acids presumably represents the regulatory domain of PknA.

To analyze the molecular mechanism of PknA autophosphorylation, various deletion mutants were constructed. The mutants were expressed and purified as MBP fusion proteins. The autophosphorylating status of the deletion mutants was analyzed by an in vitro kinase assay and further confirmed by immuno blotting with anti phosphothreonine antibody. On analyzing the kinase activity we found that the 1-338 deletion mutant of PknA harboring the catalytic domain and juxtamembrane (Ala/Pro) region was capable of autophosphorylation. However, further shortening of this domain by deleting the putative juxtamembrane region contiguous to the catalytic domain completely abolished the kinase activity. This demonstrated that the catalytic domain along with the juxtamembrane region was essential for PknA autophosphorylation. This was further supported by the expression of catalytic domain deletion mutants, which showed no phosphorylation. Hence, the team concluded that the juxtamembrane region was essential for autophosphorylation of the kinase domain and this 1-338 deletion region was the minimum autophosphorylating core of PknA. The core was capable of phosphorylating itself in a concentration dependent manner like the full length PknA, although 5µg of the protein exhibited phosphorylation comparable to 800 ng of full-length protein. Further, the effect of bivalent cations was also concurrent with full length PknA, as autophosphorylation was detectable only in the presence of Mn^{2+} and to some extent with Mg^{2+}. Similarly, ammonium molybdate and sodium vanadate, the potent kinase inhibitors, affected the phosphorylation of the core region as the wild type PknA. All these results led us to conclude that the autophosphorylation behavior of the core region mimics the wild type protein.

Several lines of evidence indicate that PknA undergoes autophosphorylation, however, the mechanism underlying phosphorylation is poorly understood. To determine whether the autophosphorylation was mediated through cis or trans acting mechanism, the team conducted an *in vitro* kinase assay wherein different concentrations of the kinase inactive mutant of PknA core carrying altered Mg^{2+}-ATP orienting lysine (K42) were incubated with wild type protein. Interestingly, the K42N mutant, which is unable to phosphorylate itself, underwent phosphorylation on incubation with wild type protein indicating that PknA autophosphorylation was intermolecular or trans.

After establishing the autophosphorylation behavior of the core region, it was

tempting to envisage whether the core is able to transfer phosphate to exogenous substrates known to be phosphorylated by wild type PknA. To investigate this, purified core protein was incubated with -^{32}P ATP and casein. In addition to an autophosphorylating band of MBP-PknA (1-338) at 75kDa, substrate phosphorylation was also observed. All the aforementioned evidences argued that the juxtamembrane region in PknA encompassing residues 269-338 is indispensable not only for autophosphorylation but also for substrate phosphorylation ability. These results are discordant with the recent reports strongly suggesting that juxtamembrane phosphorylation is not required for the catalytic activity of other mycobacterial Ser/Thr kinases, like PknB and PknF.

Protein kinases exhibit a multitude of mechanisms for their regulation. The best understood aspect of regulation recognized in recent years is phosphorylation on a residue(s) located in a particular segment in the center of the kinase domain, which is termed as the activation segment or T-loop. The activation loop in several kinases has been found to be highly disordered in the crystal structure and is capable of undergoing large conformational changes when the kinase switches between active and inactive states that may determine the catalytic state of the enzyme. The recently solved crystal structure of PknB has highlighted the importance of activation loop in supporting the universal activation mechanism of the kinase. The loop thus identified, comprises of two threonines actively participating in the activation of the protein. The team now wished to examine whether PknA undergoes activation through the same mechanism. Notably, on aligning the sequence of PknA with that of PknB, two threonines corresponding to the phosphothreonines in PknB, were identified. Comparable threonines have also been found to exist in other mycobacterial Ser/Thr kinases, PknD, PknE and PknF. To confirm and further analyze the role of the two mapped threonines in the activation loop on the kinase activity of PknA, these residues were mutated to alanine singly. The single mutants T172A and T174A were constructed by site directed mutagenesis and purified as MBP fusion proteins. The results argued that T172 is the predominantly phosphorylating residue in the activation loop of PknA. Nonetheless, unlike PknB, T174A did not show a remarkable effect on phosphorylation. The data corroborated well with the PknH, where only one phosphothreonine T170 corresponding to T172 of PknA and T171 of PknB could be identified in the activation loop. This threonine in the activation loop has been recognized as the target for PknH autophosphorylation. The significant decrease in kinase activity of PknA on replacing the phosphothreonine to alanine suggested its direct regulatory role and hence favors the universal activation mechanism of the kinase.

Evaluating the contribution of PknA in the process of cell division: Alteration in cell shape is the initial event in bacterial cell division that involves ordered assembly of proteins. These proteins are fairly conserved among different prokaryotes. This was evident from the fact that a ~56 kDa soluble protein of *E. coli* interacted with mycobacterial PknA. To investigate the involvement of PknA in this process, the protein was expressed constitutively in the *E. coli* strain DH5 using a *Mycobacterium* - *E. coli* shuttle vector, p19Kpro. As evaluated by scanning electron microscopy, expression of PknA resulted in dramatic alteration in the phenotype of *E. coli* cells. The elongation of cells was about 20 to 30 fold compared to the normal

rods of *E. coli*. This observation was not an experimental artifact or the effect of plasmid load since *E. coli* transformed with the vector (p19Kpro) as well as the antisense construct (p19Kpro-aPknA) did not show such phenotypic alteration. The elongation of cells did not seem to result in any toxicity from 'out of context' expression of the mycobacterial gene since growth curves between experimental and controls were very similar. Furthermore, the kinase dead mutant, K42N (cloned in vector p19Kpro), did not result in the elongation of *E. coli* cells, thereby indicating that the kinase activity of mycobacterial PknA is involved in regulating these morphological changes.

Since the core region of PknA is capable of auto as well as substrate phosphorylation in a manner similar to wild type protein, it was intriguing to know whether the region is capable of generating the morphological changes exhibited by the wild type protein. In order to see the phenotypic effect of the deleted construct (core), it was cloned under a constitutive expression vector p19kpro, an *E. coli-Mycobacterium* shuttle vector. While the wild type protein was able to remarkably elongate the cells, unexpectedly, the core fragment when cloned in the constitutive expression vector did not show any cell elongation. This led the group to speculate that the C-terminal region is likely to play a sensor role, which thereby relays signals downstream, since no morphological changes could be identified in the absence of regulatory cues from extra cellular domain. Therefore, to verify the role of the regulatory domain, the group next wanted to determine if these subdomains could interact in trans in *E. coli* cells. The core region was cotransformed with regulatory and transmembrane domains. Interestingly, coexpression of core and C-terminal region (harboring transmembrane and regulatory domain) restored the wild type phenotype. The cells exhibited elongated morphology, further supporting that the domains associate in trans to reconstitute a functional protein *in vivo*. However, the self-association was affected by deletion of a single region or domain, as the core region transformed with regulatory or transmembrane domain alone was unable to restore full-length phenotype.

Identification of natural substrates of PknA: To elucidate the possibility of interaction of PknA with unknown protein(s), soluble fractions of cell lysates from *E. coli* strain DH5á was incubated with MBP-PknA fusion protein that was immobilized on amylose resin. *In vitro* kinase assays with aliquots of resin after thorough washing indicated the phosphorylation of a ~56kDa protein in addition to ~97kDa autophosphorylating MBP-PknA.

Further, to identify these interactive proteins/natural substrate(s) of PknA, the group concentrated on the elongated cell morphology of *E. coli* that resulted due to constitutive expression of this kinase. The cells when examined by DAPI staining under a confocal microscope showed beaded structure indicating that the elongation event was a result of inadequate septation. FtsZ, a homolog of eukaryotic tubulin is a crucial molecule in bacterial cell division. It initiates Z-ring formation and congregates several other proteins to form a septosomal complex. The group, therefore, focused on FtsZ from *E. coli* and *M. tuberculosis* and their interaction with a signaling protein, PknA. The group found FtsZ either from *E. coli* (eFtsZ) or *M. tuberculosis* (mFtsZ) was phosphorylated on co-expression with PknA. Consistent with these observations, solid phase binding and *in vitro* kinase assays revealed the

ability of PknA to interact with mFtsZ protein and also to phosphorylate it. The group, therefore, ascertained mFtsZ as a substrate of PknA. Furthermore, the phosphorylated mFtsZ exhibited impairment in its GTP hydrolysis and polymerization abilities. Thus the results highlighted the ability of PknA to phosphorylate as well as to regulate functionality of FtsZ, the protein central to cell division throughout the bacterial lineage (Thakur and Chakraborti, **J. Biol. Chem.,** 281:40107-40113, 2006).

Elucidation of the contribution of PknA in M. tuberculosis : Western blot analysis with cell lysates from *in vitro* culture of *M. tuberculosis* using antisera generated against MBP-PknA revealed the downregulation of PknA in stationary phase, which represents a state of limited metabolism with little or no cell turnover. In order to evaluate the contribution of mycobacterial PknA, a disruption construct containing kanamycin cassette inserted into the *pknA* has been generated in a suicidal vector pPR27. The transformation of the construct in *M. tuberculosis* strain H37Ra did not result in any viable colony. At this juncture it is worth mentioning that *pknA* has already been speculated to be an essential mycobacterial gene through in silico analysis.

Regulation of activities of PknA : All lines of evidence, presented so far convincingly established the participation of mycobacterial PknA in the process of cell division/differentiation. However, such morphological events need to be tightly regulated in order to maintain a state of homeostasis. Inspection of the bacterial genomes has revealed the genetic linkage between Ser/Thr kinases and phosphatases, suggesting that phosphatases are reversing protein phosphorylation reactions catalyzed by linked kinases. The previous *in vitro* studies with PPP revealed its ability to remove phosphate from autophosphorylated PknA as well as the substrates phosphorylated by this kinase. In order to study the *in vivo* interaction of PknA and PPP, p19Kpro-PknA and pMAL-PPP were cotransformed in *E. coli* DH5á and the effect of coexpression on phenotype of the cells was examined under the microscope. On co-expressing PknA and PPP, the majority of cells were normal rods and only a small population showed the elongation phenotype. These studies indicated that PPP had the ability to reverse the phenotypic alterations shown by PknA and thereby established that genetically linked mycobacterial Ser/Thr kinase and phosphatase (PknA-PPP) form a functional unit *in vitro*.

Conclusion : The results revealed that *pknA* from *M. tuberculosis* encode an active Ser/Thr kinase. An interesting finding of the present study is the remarkable elongation of *E. coli* cells observed upon the expression of PknA. Although, the involvement of this kinase in the process of cell division/differentiation in mycobacteria needs to be envisaged, nonetheless the evidence presented here strongly suggested the participation of *pknA* in regulating these morphological changes. Since bacterial Ser/Thr kinases and phosphatases have been reported to have opposing physiological roles, it remains to be seen how *ppp* behaves in this situation. However, the ability of PPP to interact with PknA is definitely pertinent information towards elucidating the role of phosphorylation/dephosphorylation cascade in the process of mycobacterial cell division/differentiation. In conclusion, the interaction of PknA and FtsZ needs to be characterized in greater detail. This may

help in developing rapid assays for screening of inhibitors of this important serine/threonine kinase.

The work carried out under this project, as published, is as below:

Chaba, Rachna; Raje, M. and Chakraborti, P.K.
Evidence that a eukaryotic-type serine / threonine protein kinase from *Mycobacterium tuberculosis* regulates morphological changes associated with cell division. **Eur. J. Biochem., 269**, 1078-1085, 2002.

Tiwari, Sangeeta; Radha Kishan, K.V.; Chakrabarti, T. and Chakraborti, P.K. Amino acid residues involved in autophosphorylation and phosphotransfer activities are distinct in nucleoside diphosphate kinase from *Mycobacterium tuberculosis.* **J. Biol. Chem**., **279**, 43595-43603, 2004.

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GTPase activity of mycobacterial FtsZ is impaired due to its transphosphorylation by the eukaryotic type Ser/Thr kinase, PknA. **J. Biol. Chem., 281,** 40107-40113, 2006.

The Research Council of IMTECH in its 31st meeting held on July 3-4, 2006 reviewed the work done under this project and declared the project as successfully completed.

Dr. Pradip K Chakraborti (standing, fifth from left) and group

Kanak L. Dikshit
kanak@imtech.res.in

MOLECULAR AND BIOCHEMICAL STUDIES ON NOVEL HEMOGLOBINS OF *Mycobacterium tuberculosis*: STUDIES ON CELLULAR FUNCTIONS OF MYCOBACTERIAL HEMOGLOBINS AND THEIR EVALUATION AS A PROBABLE DRUG TARGET

Project Leader: Dr. Kanak L. Dikshit
Other Participants: Dr. G. Rajamohan
Ms. Amrita Lama
Ms. Sudesh Pawaria
Ms. Swati Arya
Mr. Muthukrishanan

The prime objective of this research project is to understand structure-function and molecular mechanism of hemoglobins functions in *Mycobacterium tuberculosis* to explore their relevance in cellular metabolism and pathogenicity and evaluate the feasibility of using these novel hemoglobins as potential drug targets.

Mycobacterium tuberculosis (MTB) is one of the most successful human pathogens, claiming more lives annually than any other infectious diseases. One of the major contributing factors to the success of MTB is its ability to survive under hypoxic and nitrosative stress during its intracellular regime in spite of being an obligate. *M. tuberculosis* synthesizes two novel hemoglobins, HbN and HbO. Detailed spectral, biochemical and subunit association properties of these mycobacterial hemoglobins have been studied. The distinct structural features of HbN and HbO, their different temporal expression pattern and distinct ligand binding characteristics suggested that these mycobacterial hemoglobins may have very different physiological roles. The group demonstrated that HbN carries a potent oxygen dependent nitric oxide deoxygenase 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). The group observed that the properties of HbO with respect to oxygen and NO binding are significantly different. The group demonstrated that HbO associates with the respiratory membranes and stimulate its respiratory activity that is dependent on cytochrome *o* (Pathania *et. al.*, **J. Biol. Chem.,** 2002). These experimental evidences suggest important contributions of these hemoglobins in proliferation and persistence of *M. tuberculosis* within the hypoxic and nitric oxide enriched environment of intracellular habitat. The research during last couples of years have been directed to understand how these oxygen binding proteins contributes to the cellular metabolism of pathogenic mycobacteria and whether functional properties of HbN and HbO would provide any benefit to its host during intracellular regime..

Studies on role of mycobacterial hemoglobins on growth and cell survival during macrophage infection: Implications of *M. tuberculosis* HbN and HbO on intracellular survival

Since the previous studies indicated that HbN provides distinct growth advantage to its recombinant hosts, *E. coli* and *M. smegmatis*, after nitric oxide (NO) exposure (Pathania *et. al*., **Molecular Microbiology**, 2002) and initial intracellular niche of *M. tuberculosis* is macrophages which is NO enriched, the group attempted to check whether the presence of hemoglobins provide any effect on cell survival during macrophage infection. HbN and HbO encoding genes of *M. tuberculosis* were expressed in *hmp* mutant of S. *typhimurium*, which is unable to metabolize NO and grow within the macrophages. To check the implications of HbN and HbO on infectivity and survival within the intracellular environment, Hb carrying *S. typhimurium* was allowed to infect mouse peritoneal macrophages and intracellular growth and survival of cells were determined after lysis macrophages every after 30 min. Viable cell counts of Hb carrying S. typhimurium gradually increased and was 3-4 fold higher as compared to control and HbO carrying cells suggesting HbN is providing substantial protection within the macrophage environment. Since the level of NO in the activated macrophages increases significantly, the group tested the effect of IFN-gamma on intracellular survival of HbN and HbO carrying cells. In activated macrophages HbN carrying cells still exhibited 2-3 fold higher numbers of cells as compared to control. Overall results, thus, clearly indicated that HbN plays crucial role in protecting its host from microbicidal activity of macrophages particularly in the context of NO toxicity.

Studies on *M. tuberculosis* hemoglobins gene expression and promoter activity through transcriptional fusion

The initial studies on temporal expression of HbN and HbO indicated that expression of HbN is induced significantly during stationary phase, whereas, HbO remains expressed throughout the growth cycle of *M. bovis* and *M. tuberculosis*. To get an insight into the molecular mechanism of their genetic regulation the group studied the promoter activity of HbN and HbO through transcription fusion with GFP. 250 bp upstream of glbN gene and 500 bp upstream of glbO gene were PCR amplified from the genomic DNA of *M. tuberculosis* and cloned on promoter probe shuttle vector, pSC301. The promoter-GFP fusion constructs were transformed into *M. smegmatis* and *M. tuberculosis* and promoter activity was monitored under different physiological growth conditions through fluorimeter. Results indicated that promoter activity of HbN is enhanced several fold at stationary phase, whereas, level of HbO promoter activity remains same under normal growth condition. Under microaerophilic condition, HbO promoter activity was sustained at a level higher than that of HbN. Experiments are underway to study the promoter activity under different stress and nutritional conditions.

Studies on Role of Pre-A helical region of HbN in protein function: Deletion and site directed mutagenesis of Pre-A region

HbN of *M. tuberculosis* carry an unique N-terminal motif which has been designated as Pre-A region which distinguishes it from other homologous HbN type trHbs.

Genome analysis of various mycobacterial species revealed that only few HbN might carry an additional Pre-A helical region. It was found that HbN from fast growing mycobacterium, *M. smegmatis*, lacks this structural motif and display very low NO metabolizing activity. To explore the relevance of Pre-A helical region in protein function, 12 residues N-terminal motif of HbN of *M. tuberculosis* was deleted and truncated protein was expressed and characterized. Deletion of Pre-A region did not alter heme binding and oxygen binding properties of HbN of *M. tuberculosis* but it drastically reduced the NO metabolizing activity and ability to resist acidified nitrite and nitrosative stress of its host suggesting that it may be important for the function of the protein. The group further swapped this motif on the HbN of *M. smegmatis* to check whether addition of this motif to similar heterologous protein would affect its function. Surprisingly, integration of pre-A region of HbN of *M. tuberculosis* on HbN of *M. smegmatis* significantly enhanced its ability to resist acidified nitrite and nitrosative stress suggesting that Pre-A region of HbN of *M. tuberculosis* may be playing vital role in modulating its NO scavenging ability. Role of Pre-A region of HbN was further studied by site-specific mutagenesis to find out if any particular residue is involved in modulating the function of HbN. 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. The data, thus, provided strong evidence for the novel role of Pre-A region in modulating the NO scavenging function of HbN of *M. tuberculosis.* Attempts are underway to explore if this region is in anyway involved in regulating the protein-protein interaction of hemoglobin to catalyze NO dioxygenase function.

Cloning and expression of hemoglobin encoding genes from *Mycobacterium smegmatis*

Since the above results indicated that both HbN and HbO of *M. tuberculosis* may be having important functions in facilitating growth under oxygen limited and NO rich environment present in macrophages where the tubercle bacillus primarily resides, the group initiated work on looking into the characteristics of hemoglobins of non-pathogenic mycobacterium, *Mycobacterium smegmatis* to analyze functional differences between Hbs of these two groups of mycobacteria. Hemoglobin encoding genes of *M. smegmatis* have been cloned and expressed in *E. coli.* Primary spectral and ligand binding properties of *M. smegmatis* hemoglobin has been determined and compared with that of *M. tuberculosis* which indicated that NO scavenging properties of HbN of *M. smegmatis* is significantly lower.

Technology Transfer: Process for the production of recombinant Staphylokinase

Under the ongoing research programme on thrombolytic proteins, the group has been working on structure-function and molecular mechanism of function of an anti-

clotting agent, Staphylokinase, which promises immense potential as a clot buster for thrombolytic therapy due to its fibrin selectivity. A recombinant strain for overproduction of recombinant staphylokinase has been developed and a facile purification protocol for high level production of recombinant protein has been developed at laboratory scale. This system has been successfully upgraded at batch and fed- batch fermentation level in collaboration with BRPDC division of IMTECH and the process, thus, developed for high level production of recombinant has been successfully transferred to a pharmaceutical company " M/s Stride Arcolabs Ltd., Bangalore".

Dr. Kanak L. Dikshit (sitting in the centre) and group

R. Kishore

kishore@imtech.res.in

DESIGN OF SELF-ASSEMBLING -TURN MOTIFS: STABILIZATION BY HYDROPHOBIC INTERACTIONS

Project Leader: Dr. R. Kishore
Other Participants: Mr. Samir K. Nath
Mr. Nigam Kumar

The objectives of this project are to undertake the chemical synthesis and characterization of a few designed peptide sequences that can adopt self-assembling well-defined folded - turn like motifs using a design strategy where it is aimed to examine whether the high proportion of hydrophobicity imparts additional stabilizing tendency.

To design, synthesize and characterize the peptides that are capable of *mimicking* a well-defined â-hairpin *like* topology, the group proposed to synthesize and characterize a few model peptides with general sequence: Naa-Pro-Xaa-Caa (where Naa and Caa are hydrophobic moieties at the N- and C-terminals, respectively, and Xaa = non-chiral proteinogenic or non- proteinogenic residues). In these model sequences, the Pro residue has been incorporated in view of its strong propensity to induce folded topology in the main-chain (*i.e.*, for L-Pro the ö value is restricted close to -65±10º). Under specific environments, such peptides are expected to undergo self-association and suggested to have biological implications. To establish whether central dipeptide segment adopts a folded topology, the corresponding model peptides Boc-Pro-Xaa-$NHCH_3$ (devoid of Naa- and Caa moieties) have also been developed.

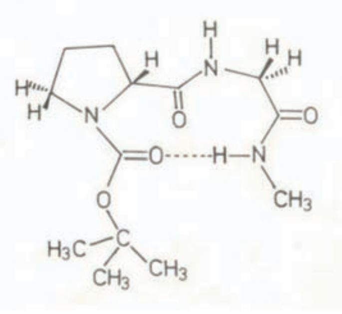

Figure : turn structure across Pro-Gly segment

A few peptides have already been synthesized, purified and characterized. Their covalent structures have been established. In combination with Fourier-Transform Infra-red (FT-IR) and circular dichroism (CD) spectroscopic analyses, the group is currently performing high-resolution one- and two-dimensional ^{1}H NMR experiments to establish solution conformation. Whether flanking hydrophobic entities have any influence on the folded topology will be the subject of detailed investigation. Apparently, the available results tend to suggest that in some cases the Pro-Xaa segment tends to accommodate a folded -turn like topology where the Pro and Xaa residues occupy the *i*+1 (left-corner) and *i*+2 (right-corner) positions, respectively.

In order to determine the complete three-dimensional structure of the model peptides, the group has been able to obtain single crystals, suitable for X-ray diffraction analysis, of a few model peptides and currently the team is in the process of analyzing the crystal molecular structures.

Employing high-resolution ^{13}C solution NMR and circular dichroism (CD) spectroscopic techniques, the distinctive influence of two intimately related hexafluoro solvents: hexafluoro-2-propanol (HFIP) and hexafluoroacetone trihydrate (HFA), on the structural characteristics of *Bombyx mori* silk fibroin and its two synthetic peptides: $(\text{Ala-Gly-Ser-Gly-Ala-Gly})_5$ and $(\text{Ala-Gly})_{15}$ have been examined. The detailed structural analysis revealed that of the two prevalent helical structures: -helix and 3_{10}-helix, the evidence emerged for the fibroin protein and its derived peptides in favor of 3_{10}-helcal structure in HFIP solvent however, its significant disruption has been observed in HFA as deducted from the characteristic CD data.

The proposal on model peptides *mimicking* â-hairpin *like* topology has received financial support from the Department of Science & Technology, Govt. of India.

Dr. Raghuvansh Kishore (sitting, centre) and group

G.P.S. Raghava
raghava@imtech.res.in

BIOINFORMATICS APPROACH FOR IDENTIFICATION OF VACCINE CANDIDATES BASED ON T-CELL EPITOPE PREDICTION

Project Leader: Dr. G.P.S. Raghava
Other Participants: Mr. Harpreet Singh
Mr. Manoj Bhasin
Mr. Sudipto Saha

Traditional vaccines based on killed or attenuated bacteria and viruses are responsible for preventing millions of deaths each year from infectious diseases. These vaccines have dramatically decreased the incidence of diphtheria, measles, mumps and tetanus. This traditional approach has potentially harmful or toxic effect if due to some reason the pathogen reverts, thus causing disease in the patients. In order to overcome some of the limitations of traditional vaccines, antigens of disease causing pathogens were introduced as vaccine candidates instead of the full pathogen. Though the antigens have much lower toxic effects than the pathogen still they have a few unwanted toxic regions. In order to over come these problems, subunit vaccine design has emerged as a promising strategy for the rapid development of safe vaccines. Since 1995, subunit vaccine design has become an integral part of vaccine design strategy. In a subunit vaccine the immunogenic region of the antigen is used instead of a complete antigen as the vaccine candidate. It is based on the assumption that immunologically active regions can spark the immune response to eliminate the pathogens or kill the self-altered cells. A peptide or segment of antigen can be used as a vaccine candidate if it can simulate the B-cells or T-cells. Thus identification of immunologically active regions/epitopes recognized by B or T cells play a crucial role in subunit vaccine design. In the last 5 years, a large number of methods have been developed at the Bioinformatics Centre, Institute of Microbial Technology, which allow a user to predict B or T cell epitopes with high accuracy. These methods are available to the public in the form of web servers and are heavily used by the scientific community.

Details of the work done:

ProPred (Prediction of HLA class II binders): It is a graphical web tool for predicting MHC class II binding regions in antigenic protein sequences. The server implement matrix based prediction algorithm, employing amino-acid/position coefficient table deduced from literature. The predicted binders can be visualized either as peaks in graphical interface or as colored residues in HTML interface. This server might be a useful tool in locating the promiscuous binding regions that can bind to several HLA-DR alleles.

ProPred1 (Prediction of Promiscuous MHC class I binders): It is an on-line tool for predicting the peptides bind to MHC class – I alleles. This is a matrix-based method, which allows prediction of binding sites for 47 MHC class-1 alleles in an antigen sequence. The server presents the binding sites on antigen in a user-friendly format, which can assist the user in identification of promiscuous binding regions that can bind to multiple alleles. ProPred1 allows to predict the standard proteasomal and immuno-proteasomal cleavage sites in an antigen sequence. The simultaneous prediction of MHC binders and cleavage sites in an antigen sequence can assist the user in identification of potential T-cell epitopes. Server is available at http://www.imtech.res.in/raghava/propred1/

MHCBench: A general-purpose web server called MHCBench has been developed in evaluating the performance of prediction algorithms for the MHC binding peptides. This server allows, threshold dependent and independent evaluation of algorithms. The evaluations can be performed on-line, on peptide data sets available at server as well as on user-defined sets. In order to demonstrate the capabilities of server, the team present an evaluation of twelve HLA-DRB1*0401 binding peptide parediction algorithms, in this study. These include five motif based, six matrix based and one Artificial Neural Network (ANN) based method. The evaluation is performed on different data sets including the sets of i) non-redundant peptides, ii) natural occurring peptides, and iii) non-homologues peptides. The analysis shows that the matrix-based algorithms are better than the motifs and at par with the ANN based methods. The best algorithm showed an ROC of 0.787 while the lowest performer had an ROC of 0.542. The performance of all the algorithms was found poor at amino acid level. The users can evaluate performance of their prediction algorithms for any MHC allele using MHCBench server. In brief, server provides a platform for evaluating prediction algorithms for MHC binding peptides.

CTLPred (Prediction of CTL epitopes using QM, SVM and ANN Techniques): A systematic attempt has been made to develop a direct method for predicting CTL epitopes from an antigenic sequence. The method is based on quantitative matrices, Support Vector Machine (SVM) and Artificial Neural Network (ANN). The quantitative matrix based method achieved an accuracy of 70%. Later on, the team has developed a method using feed-forward backpropagation ANN and obtained an accuracy of 72.2%. The team has also developed SVM based method and achieved an accuracy of 75.2%. Finally, SVM and ANN based prediction methods had been combined for utilizing their full potential. The highest accuracy obtained by consensus and combined prediction approaches are 77.6% and 75.8% respectively.

HLADR4Pred (SVM based Method for Predicting HLA-DRB1*0401 binding Peptides in an Antigen Sequence): This is a SVM based method developed for identifying HLA-DRB1*0401 binding peptides in an antigenic sequence. SVM was trained and tested on large and clean data set consisting of 567 binders and equal number of non-binders. The accuracy of the method was 86% when evaluated through 5-fold cross validation technique.

Peleavage (Improved Prediction of 20S Proteasome Cleavage sites with Support Vector Machine): In this study a systematic attempt has been made to improve the accuracy of prediction of 20S proteasomal cleavage sites using following new

machine learning classifiers; i) Support Vector machine (SVM), ii) Waikato Environment for Knowledge analysis (Weka), and iii) Parallel exemplar based learnings (PEBLS). All the classifiers have been trained and tested using five fold cross-validation on 645 peptides (including 458 non-cleavage sites and 187 cleavage sites) obtained from experimental degradation data of yeast enolase I and casein. It has been observed from results that SVM classifier outperformed the rest of classifiers used in this study as well as previously published proteasomal cleavage prediction algorithms. The SVM based classifier yielded an accuracy of 68% and MCC of 0.43 on independent dataset. A webserver Peleavage, has been developed for the prediction of proteasomal cleavage sites in antigenic sequences using above SVM classifier (http://www.imtech.res.in/raghava/pcleavage/).

TAPPred: Analysis and Prediction of affinity of TAP binding peptides using Cascade SVM: In this study, 409 peptides that bind to human TAP transporter with varying affinity were analyzed to explore the selectivity and specificity of TAP transporter. The abundance of each amino acid from P1 to P9 positions in high, intermediate and low affinity TAP binders were examined. It was observed that the binding affinity of peptides to human TAP transporter is mainly affected by three residues at –NH2 terminal and one residue at –COOH terminal. The –COOH terminal of TAP binders does not favour hydrophilic and buried residues whereas the –NH2 terminal favours charged, aliphatic and hydrophilic amino acids. The rules for predicting TAP binding regions in an antigenic sequence were derived from the above analysis. The quantitative matrix was generated on the basis of contribution of each position and residue in binding affinity. The correlation of r=0.65 was obtained between experimentally determined and predicted binding affinity using quantitative matrix. Further a Support vector machine (SVM) based method has been developed to model the TAP binding affinity of peptides. The correlation (r=0.80) was obtained between the predicted and experimental measured values using sequence based SVM. The reliability of prediction was further improved by cascade SVM that utilizes features of amino acids along with sequence. An extremely good correlation (r=0.88) was obtained between measured and predicted values, when cascade SVM based method was evaluated through jack-knife testing. A web service TAPPred (http://www.imtech.res.in/raghava/tappred/orhttp://bioinformatics.uams.edu/mirror/ tappred/) has been developed based on this approach.

MHCBN 3.1: A comprehensive database of T cell epitopes and MHC, TAP binding peptides: The current version 3.1 of MHCBN has nearly 24000 MHC binding and non-binding peptides, ~1100 peptides interacting with TAP, ~3500 antigenic sequences and nearly 1000 MHC sequences and more than 1500 published references. MHCBN also contains structures of nearly 850 antigenic proteins and 120 MHC molecules. It is a curated database where entries are compiled from published literature and public databases. Each entry of the database provides full information like (sequence, its MHC or TAP binding specificity, source protein) about peptide whose binding affinity (IC50) and T cell activity is experimentally determined. MHCBN has number of web-based tools for the analysis and retrieval of information. These include tools for mapping of antigenic regions on the query sequence, creation of allele specific dataset, BLAST search against MHC or antigen database and online submission tool etc. All database entries are hyper linked to

major databases like SWISS-PROT, PDB, IMGT/HLA-DB, PubMed and OMIM to provide the information beyond the scope of MHCBN. MHCBN database can be accessed via internet from http://www.imtech.res.in/raghava/mhcbn/. The updated database has been present in the collection of premier databases of the world at European Bioinformatics Institute. The database can be accessed from the EBI at http://srs.ebi.ac.uk/srs6bin/wgetz?-page+LibInfo+-id+1X2XW1JU5_L+-lib+-lib+MHCBN. The database is largest of its field.

BCIPEP (A Database of B cell Eiptopes): BCIPEP is a database of experimentally determined B-cell epitopes of varying immunogenicity. This is a curated database where detailed information about the peptides is collected and compiled from published work. The current version of Bcipep contains 2479 entries, including 654 immunodominant, 1617 immunogenic and 208 null immunogenic epitopes. The Bcipep contain on-line web tools for extraction and analysis of data, which includes keyword search, peptide search, peptide mapping and BLAST search. The database also facilitates identification of such B cell epitopes, which can also act as T cell epitopes. It provides links to various databases such as GenBank, PDB and SWISS-PROT. The database is available athttp://www.imtech.res.in/raghava/bcipep , http://bioinformatics.uams.edu/mirror/ bcipep/ (Mirror Site) and http://srs.ebi.ac.uk/srs6bin/egi-bin/wgetz?-page+LibInfo+-id+1X2XW1JU5_L+-lib+BCIPEP

During this project the team developed number of web servers and databases which are used heavily world wide for subunit vaccine design. The team became top group in the field of immunoinformatics in last 4 years. The team developed two databases which are largest in their field and used by community as reference databases. Both databases are recognized by world-wide and distributed by European Bioinformatics Institute (EBI), only databases from India distributed by EBI. A commercial package VAXIPRED has been developed with ELQUEST private company which integrate 10 software and two databases.

The work carried out under this project, as published, is as below:

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xiii. Bhasin, M. and Raghava, GPS (2002) TAPPred: A software package for predicting TAP binding affinity of peptides via internet (Submitted).

The Research Council of IMTECH in its 30th meeting held on July 26-27, 2005 reviewed the work done under this project and declared the project as successfully completed.

Dr. Gajendra PS Raghava (standing, third from right) and group

C. Raman Suri
raman@imtech.res.in

DEVELOPMENT OF DIPSTICK BASED IMMUNOSENSOR SYSTEM FOR SPECIFIC AND SENSITIVE DETECTION OF METHYL PARATHION IN WATER SAMPLES

Participants: Dr. C. Raman Suri
Other Participants: Mr. Rajesh Kumar

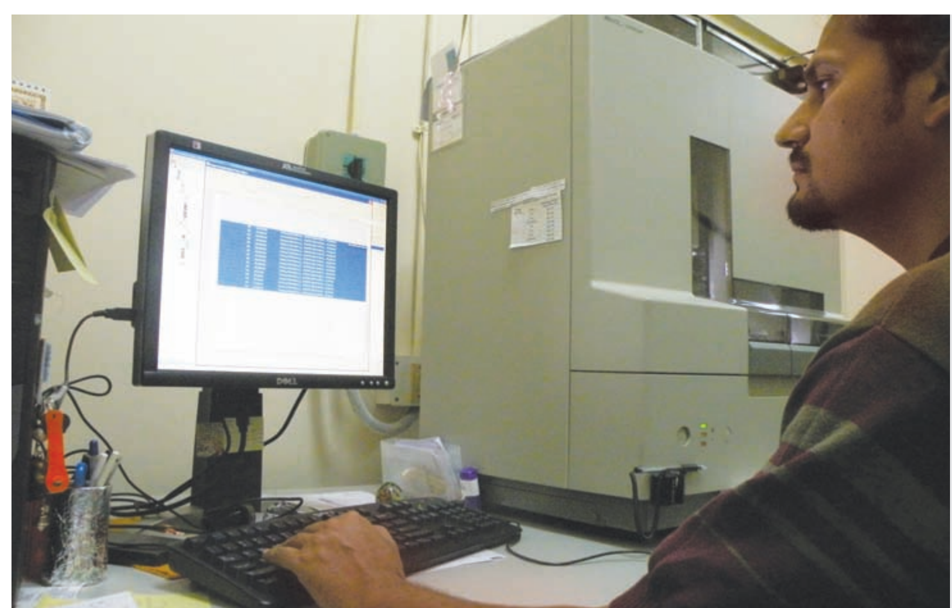
The objective of this project is to develop a rapid screening methodology for the monitoring of pesticides in samples by concentrating on following aspects:

1. Synthesis of hapten for making hapten-protein conjugates
2. Generation of specific antibodies (IgG and IgY) against MP and their purification
3. Generation of tracers (hapten-gold and antibodies-gold conjugates)
4. Development of dipstick based immunoassay
5. Validation of developed immunochromatographic assay

Antibodies against methyl parathion using well characterized immunogen (hapten-protein conjugates) were generated and used as receptor molecules for immunoassay development. A lateral flow based dipstick kit has been developed using a nitrocellulose membrane on which anti-pesticide antibody is immobilized at the test line on the detection zone. The sample is introduced through the sample pad affixed at

Dr. Raman Suri (standing, fourth from right) and group

one end of the nitrocellulose membrane while on the other end; an absorbance pad is attached to increase the flow of molecules onto the membrane. The sample along with the conjugate move onto the nitrocellulose membrane where these two different molecules react competitively to the available binding sites of the anti-MP antibodies coated on the membrane. A hand held reflectrometer has been developed indigenously for the semi-quantification of sample concentrations at 540 nm. The intensity of color developed (reversibly) give the presence of analyte in the sample. The intensity of signal is correlated with the concentration of sample with standard curve.



D.K. Sahoo
debsahoo@imtech.res.in

DEVELOPMENT AND SYNTHESIS OF BIOPROCESSES FOR PRODUCTION OF RECOMBINANT BIOMOLECULES

Project Leader: Dr. D.K. Sahoo
Other Participants: Dr. K.L. Dikshit
Mr. Arshad Jawed

With increase in the number of people suffering from thrombolytic disorders, the market for better and efficient thombolytic agents is growing rapidly. The studies aimed at developing a process for production and purification of a promising thrombolytic molecule, staphylokinase (SAK). Staphylokinase is a plasminogen activator from *Staphylococcus aureus*. Compared to second-generation thrombolytic agents, third generation ones such as staphylokinase result in greater angiographic potency rate in patients with acute mycocardial infraction. SAK is highly fibrin-specific, with a pharmacological profile that avoids systematic plasminemia. Staphylokinase gene, cloned and expressed in *Escherichia coli* BL21 under T7 promotor, was used for development and synthesis of a bioprocess for production of recombinant staphylokinase. The fermentation medium composition plays a major role in the growth of the cells and formation of product(s). Since in case of recombinant SAK the product is accumulated inside the cells, its yield is directly proportional to both cell concentration and specific expression of SAK. Hence, a fermentation medium, capable of supporting higher cell densities and sustaining higher specific yield of staphylokinase was highly desirable. Since Luria Bertini medium is not known to yield higher cell concentrations (O.D.~3.0), there was a need to formulate and optimize a synthetic/ semi-synthetic medium to achieve higher cell density. Initially LB medium along with a synthetic medium was evaluated for SAK production. The addition of complex nitrogen sources like yeast extract and tryptone to the designed synthetic medium increased SAK expression. Initial studies on media optimization using classical optimization method showed glucose to be the best carbon source for cell growth. As the classical methods suffer from some major drawbacks, further experiments were designed by statistical methods and glucose as carbon source was used for statistical optimization studies.

It was observed that the components present in the culture medium interact in a very complex fashion, and the interactions were affected by their individual concentrations and other factors. Though major interacting components were carbon and nitrogen sources, other components such as phosphate were found to have variable share in interactions. As classical methods do not consider these interactions, the optimization results from classical methods are based on the effect of individual medium component rather than the whole system. Statistical methods are one of the best tools to study the effect of the various components present in the medium simultaneously and Response surface methodology was used to maximize

biomass production and staphylokinase yield, while D-Optimal design was used to find out the best combination of the participating nitrogen sources, namely yeast extract, peptone and tryptone. The trials were done with each component at five different levels of concentration. The parameters included glucose as the main source of carbon in every experiment and separate experiments were done for studying the effect of glucose with individual nitrogen sources (yeast extract, tryptone and peptone).

After statistically analyzing and optimizing the production conditions glucose concentration of 0.7% was found to be optimum and the nitrogen sources were optimized with yeast extract and tryptone ratio of about 1:4. In the optimized medium, the cell growth was OD_{600nm} = 4.1 as compared to OD_{600nm} = 3.0 in LB medium and the production of staphylokinase increased to 150 mg/L from 90 mg/L (in LB medium).

SAK production was further optimized in fermenter at 5 L scale and then, the process was scaled up to 20 L. As process conditions in the fermenters are better controlled as compared to shake flasks, the process becomes robust and reproducible. An optimized medium concentration was used and various parameters like the effect of inoculum concentration and inoculum age were standardized. Effects of inducer concentration, fermentation time and pH control (e.g. by addition of NaOH and liquid ammonia) were also studied in batch fermentation process. Maximum cell concentration of OD_{600}=12.0 and staphylokinase yield of 450 mgL^{-1} were obtained in batch process under experimental conditions. In order to further increase the cell density and product yield, the process was operated in fed-batch mode, starting with batch mode for 4 hours and then shifting to fed-batch mode with feeding of a mixture of glucose, complex nitrogen sources and other nutrients for about 4 h. After 8 h of fermentation, the culture was induced with IPTG to synthesize staphylokinase. Optimization and scale up of the process from shake flask to fed-batch process increased the cell density by 23 folds reaching OD_{600nm}=70 and the staphylokinase production was enhanced by more than 28 folds to 2566 mgL^{-1}. The cell concentration is high in case of fed-batch fermentation and so is the demand for nutrients that need to be met for production of desired proteins. One of the important nutrients is oxygen and hence, dissolved oxygen concentration is critical in the production of recombinant proteins. In order to study the effect of *Vitreoscilla* hemoglobin, a binder of oxygen, on SAK expression at low dissolved oxygen concentration, it was co-expressed along with staphylokinase. Fermentation was carried out at different concentrations of dissolved oxygen (DO), 15 – 35 %, with the control strain and the clone co-expressing *Vitreoscilla* hemoglobin. In case of clone with *Vitreoscilla* hemoglobin, the growth was increased by 12 – 16 % and the production of staphylokinase increased by 15 – 25% as compared to the control strain.

As the cloned staphylokinase molecule is expressed intra-cellularly and accumulates in the periplasmic space of *Escherichia coli*, the need was for lysis of cells to recover the product in intact and active form. Bead milling is a process scale cell disintegration (lysis) method and the capacity to handle high cell density and large volume of cell suspensions and addition of no other chemical substances for lysis are some of its advantages. Hence large-scale cell lysis was optimized using bead mill and the important operational parameters, such as feed rate (of the cell slurry), cell loading,

bead loading and bead milling time were optimized by statistical design, response surface methodology. The cell lysate was centrifuged and the clear supernatant was separated and used for purification studies. An array of ion-exchange chromatography matrices such as Sepharose Q, Sepharose XL, DEAE-sepharose, Granular silica, Cellufine A and SP-Sepharose was examined for the preferential binding of staphylokinase and to purify it to maximum homogeneity preferably in single step. Granular silica and SP-Sepharose were found to be the potent candidates for the purification of staphylokinase. Though, granular silica bound to staphylokinase with higher affinity, it required higher salt concentration to detach the molecule from the chromatography matrix, the elution was slow and the results were not consistent under experimental condition. Hence, SP-sepharose was studied in detail for capture of staphylokinase from the total cell lysate (as the initial step). The unbound protein was washed and elution of bound protein was carried out with 0-500 mM NaCl where staphylokinase was eluted at 300-400 mM NaCl concentration. The elute containing staphylokinase was collected and added with NaCl to a final concentration of 2 M and this solution was loaded onto hydrophobic interaction column packed with phenyl sepharose as the chromatography matrix. The staphylokinase was eluted with decreasing salt concentration from 500-200 mM NaCl. The fractions were run onto a SDS-PAGE gel for confirmation and quantification of staphylokinase. Staphylokinase was also analysed for its activity and to quantify the active protein. In the first stage of chromatography the yield was 75 – 80 %, while yield of 60 – 65 % was obtained in the second chromatographic step. Using a pH based elution in ion-exchange chromatography with Streamline SP sepharose in expanded bed chromatography (EBC) where cell lysates directly fed into the column after cell disruption (without clarifications), about 75% yield of purified SAK (to homogeneity) was obtained in a single step. The purity and homogeneity of staphylokinase was checked in SDS-PAGE (single band of SAK with no presence of contaminating protein bands in silver stained gel) and further confirmed by Matrix Assisted Laser Ionization and Desorption (MALDI-TOF).

The Research Council of IMTECH in its 33rd meeting reviewed the work done under this project and declared the project as successfully completed.

***Licensing of Technology*:** This work contributed to the development of a process for the production of recombinant staphylokinase and the technology demonstrated at 5 L and 20 L scale was licensed to M/s Strides Arcolab Limited, Bangalore.

Dr. Debendra K Sahoo (standing, fourth from right) and group

Grish C. Varshney
grish@imtech.res.in

ANTIGENIC AND MOLECULAR TARGETS IN MALARIA-INFECTED ERYTHROCYTES

Project Leader: Dr. Grish C. Varshney
Other Participants: Mr. Souvik Bhattacharjee
Mr. Ajit Tiwari
Ms. Sukhwinder Kaur
Ms. Ashu Shah

The objectives of the project were to characterize the antigenic and molecular components present on malaria-infected erythrocytes, using monoclonal antibodies.

The malaria parasite alters the host erythrocyte during its intracellular growth. These alterations include overall changes in the erythrocyte cytosol along with structural changes in the host erythrocyte membrane. A plethora of parasite-derived proteins directed at the erythrocyte surface possibly actuate some of these observed modifications. Consequently, there is an increasing interest in identifying the parasite antigens as well as host-cell altered components expressed on the erythrocyte surface as potential candidates for therapeutic interventions. The earlier studies in the lab demonstrated the efficacy of homologous immunization with pathogen-infected cell membrane towards the generation of infected cell-surface reactive antibodies (Chaudhury, 1996; Goel *et al*., 1998, Majumdar *et al*., 2000). Among panel of monoclonal antibodies (mAbs) generated against *Plasmodium berghei* infected erythrocytes, two mAbs; mAbF10 and mAbD2, reacted with the infected cell surface. Monoclonal antibody, mAbF10, efficiently targeted chloroquine-encapsulated liposomes to the infected erythrocytes *in vivo* (Owais *et al*., 1995; Chaudhury, 1996). Besides, few mAbs were also generated against *P. falciparum* parasite in earlier studies. This panel of mAbs was expanded by generating more clones secreting mAbs. These antibodies were used as tools to identify the neo-antigenic determinants on the infected cell surface. Since the sequence conserved and/or antigenically conserved portions of the target molecules may provide better options in identifying the functionally or structurally important target molecule(s) in malaria parasite and in infected erythrocytes, reactivity of the mAbs was also simultaneously tested with both the species of Plasmodium parasite as well as with respective infected erythrocytes by ELISA, immunofluorescence assay and western blotting. Further, RBC invasion inhibitory/ intracellular growth inhibitory potential of these mAbs was also tested by ^{3}H-hypoxanthine incorporation assay. mAbs were selected for characterization based on their growth inhibitory potential as well as binding pattern with the parasite and infected erythrocytes.

i) Identification and characterization of mAbs D2 and F10 reactive components

In order to identify components recognized by F10 and D2, immunoprecipitation experiments were performed, both with metabolically labelled and unlabelled IRBC. Immunoprecipitaion with D2 revealed two antigens of molecular weights approximately 146- and 105-kDa, both found to be metabolically labelled, signifying a parasite-derived source. mAbF10, on the other hand, failed to immunoprecipitate any antigen following conventional protocol using uncoupled antibody. However, mAbF10-conjugated beads precipitated labelled MSP-1 (having molecular masses 230kDa, 195kDa, 156kDa and 95kDa). Further studies involving cellular fractionations of metabolically labeled IRBC into infected-erythrocyte membrane-rich fraction (MRF) and parasite rich fraction (PRF), and subsequent immunoprecipitation involving mAbD2 revealed interesting results. Immunoprecipitation from PRF revealed the presence of both 146- and 105-kDa proteins the earlier described antigens, while in MRF only 105-kDa protein could be observed. Since the intensity of 146-kDa band was much more in PRF as compared to the 105-kDa band, a significant parasite contamination in the MRF was ruled out. In case of mAbF10, all recognized components were present both in parasite as well as membrane rich fractions. Infected cell membranes, affinity purified using rabbit anti-mouse NRBC antibody-sepharose column, showed the presence of RhopH3 and all the recognized components of MSP-1, when probed with mAbD2 and F10 respectively. Further, confocal microscopy using MSP-1 and RhopH3 specific antibodies strongly indicated that these molecules are also associated with the infected cell membranes (Figs. 1 and 2).

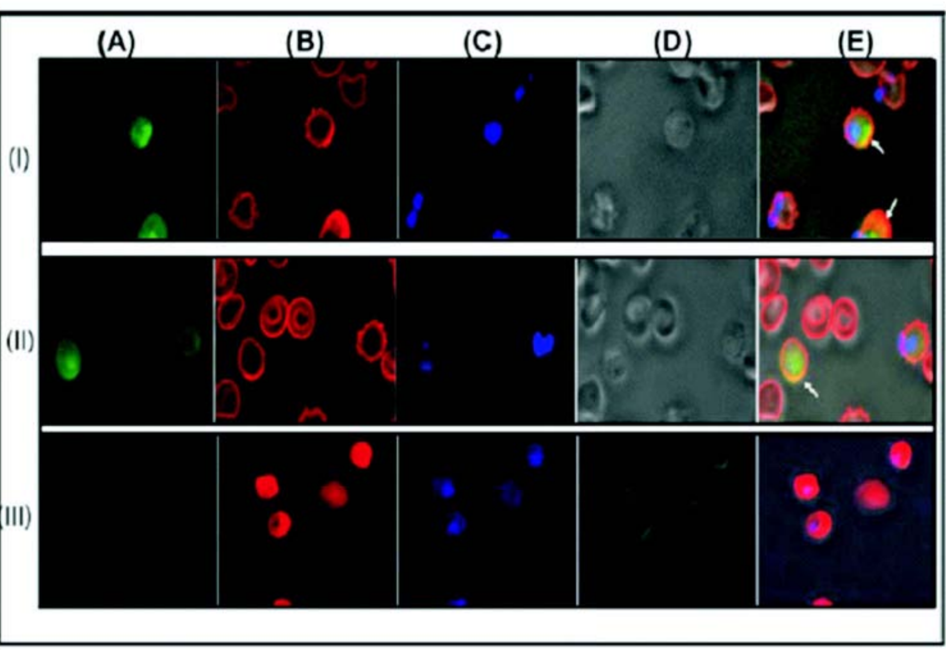


Fig.1 : Fluorescence micrograph showing localization of RhopH3 in intact *P. berghei* infected RBC : Infected cells were incubated simultaneously with rabbit anti-NRBC membrane antibodies and anti-RhopH3 antibodies, mAbB6 (row I) or mAbG6 (row II) or control-mAb (row III). The primary antibodies binding was localized using FITC labeled rabbit anti-mouse lg (panel A), TRITC labeled goat anti-rabbit lg (panel B). The cells were counterstained with DAPI for nuclear staining (panel C). Panels D and E represent phase contrast image and co-localization (indicated by arrows) of RhopH3 binding mAb and membrane binding rabbit antibodies respectively.

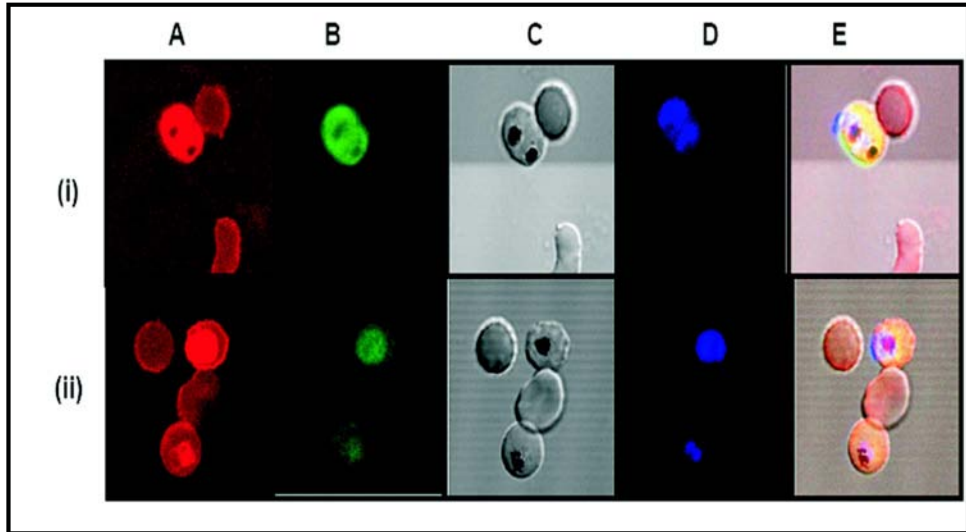


Fig.2 : Fluorescence micrograph showing localization of MSP-1 in *P. falciparum* infected erythrocyte: Localization of MSP-1 in IRBC was analysed by incubating the infected cells simultaneously with anti-MSP-1 antibodies mAbF10 (row I) or mAbC3 (row II) and rabbit anti-NRBC membrane antibodies. The primary antibodies binding was localized using TRITC lebeled goat anti-rabbit lg (panel A) and FITC labeled rabbit anti-mouse lg (panel B). Panel C represents phase contrast image of field corresponding to field B. Panel D shows paasite counterstained by nuclear stain DAPI. Merged image is depicted in panel E.

mAbs D2 and F10 reactive molecules were purified using their respective mAb-affinity columns. Isolation of antigen(s) from IRBC extracts using D2-Sepharose column yielded antigens similar to that of immunoprecipitation experiments, *i.e*., molecular weights 146- and 105-kDa. Additionally, F10-Sepharose column also eluted an antigen of molecular weight greater than 205-kDa, which could not be obtained in conventional immunoprecipitation experiments. N-terminal sequencing of the components purified using mAbF10 column revealed that all the molecules *i.e*., 230-kDa, 195-kDa and 156-kDa shared the same N-terminus. The obtained N-terminal sequences were then searched for homologous counterparts using the BLAST server at www//ncbi.nlm.nih.gov. The retrieved hits revealed homology to variants of merozoite surface protein/antigen (MSP/MSA-1) of rodent malaria parasites. Identity, of the order of 90-100% to precursor of major merozoite surface antigen (PMMSA)/MSP-1 of *P. yoelii* and *P. berghei* parasite and 77% to major merozoite surface antigen precursor/ merozoite surface protein-1 of *P. chabaudi,* were observed. For D2 reactive components, the homology search of N-terminus obtained for 105-kDa molecule revealed 93% identity with high molecular weight rhoptry protein 3 (RhopH3) of *P. yoelii* while repeated efforts failed to yield a conclusive N-terminal sequence of 146-kDa, possibly due to its blockade. Nevertheless, mAb25 reactive against RhopH2 member of HMW complex, procured from Dr. Kaneko (University School of Medicine, Shigenobu-cho, Ehime, Japan), reacted well with 146kDa protein, confirming that it is RhopH2 protein, a member of HMW complex. Thus, mAbs D2 and F10 were reactive with RhopH3 and MSP1 respectively.

Stage-specificity of MSP-1 and RhopH3:
For studying the expression profiles of MSP-1 and RhopH3 in infected erythrocytes at different stages during intracellular growth of the parasite, IRBC harboring the early and mature stages of parasite were fractionated and metabolically labeled with [^{35}S] methionine and immunoprecipitated using respective mAbs. With mAbF10-Sepharose beads, as observed earlier, all forms of MSP-1 i.e. MSP-1_{230}, MSP-1_{195} and MSP-1_{156} were immunoprecipitated from schizont stage of the parasite harboring IRBC. On the other hand, from trophozoite stage mAbF10 could only detect MSP-$1_{230.}$ Immunoprecipitations, using schizonts, by mAbD2-Sepharose yielded the RhopH3 component of 105-kDa and the associated 146-kDa molecule. While, only the RhopH3 component of 105-kDa was found to be present in the trophozoite stage.

Brefeldin A-sensitivity
Studies in *P.falciparum* have strongly supported the existence of a functional Golgi and secretory protein transport pathways. So, experiments were designed in order to see the fate of mAbF10- and mAbD2- reactive MSP-1 and RhopH3 components in presence of brefeldin A, a fungal metabolite that causes disassembly of the Golgi complex and blocks protein export and post-endoplasmic reticulum (ER) processing events in mammalian cells. IRBC were first incubated either in the presence or absence of brefeldin A, followed by [^{35}S] labeling and immunoprecipitation using mAb-Sepharose beads. No change in immunoprecipitation profile as well as intensity of labeled proteins could be seen both for MSP-1 and RhopH3 components. Thus, the occurrence of observed components recognized by mAbF10 and mAbD2 were brefeldin A-insensitive.

ii) Studies on MSP-1:

Association of membrane bound MSP-1 with spectrin
In the literature, with limited experimental evidence, the existence of an association between MSP-1 and spectrin during late trophozoite or early schizont developmental stages has been suggested. Since the results have demonstrated the association of MSP-1 with *Plasmodium berghei*- infected erythrocytes membranes, so it was thought pertinent to check its association with spectrin, if any. For this, affinity purified molecule was immunoblotted using mAbF10, anti-IRBC membrane antisera and anti-human spectrin antibody. It was observed that anti- spectrin antibody did not show any reactivity indicating that membrane bound MSP-1 is not associated with spectrin.

Release of MSP-1 from infected erythrocytes
Many soluble antigens are released from erythrocyte infected with *Plasmodium* at the time of schizont rupture and also during merozoite reinvasion, thus showing their presence within the infected serum. It has been reported that smaller fragments of proteolytically processed form of MSP-1 are secreted in culture medium or in the serum of infected mice. In order to check for the presence of observed higher molecular mass forms of MSP-1 in the plasma of *P.berghei* infected mice, western blotting was performed with the plasma of mice having 10-15% infection and using MSP-1 specific mAbF10. It was observed that mAbF10 recognized proteins in the range of 230, 195 and 156-kDa corresponding to MSP-1_{230}, MSP-1_{195}, and MSP-1_{156}.

Control antibody and rhoptry specific mAbD2 did not react with any proteins in the infected plasma thus confirming that not only the smaller fragments but also the observed higher forms of MSP-1 are also secreted/released within the plasma of the infected mice**.**

Released MSP-1 does not associate with NRBC

In order to confirm that mAbF10 recognized only the parasite encoded protein on to the surface of infected cell and not due to non-specific association of the released MSP-1 with normal red blood cells (NRBC), NRBC binding assay was performed. For this, NRBC were incubated with infected plasma (from 20-30% parasitemia) at 37^0C for one hour and then the cells were lysed and centrifuged. Pellet enriched with NRBC membranes was taken for western blotting with mAbF10. The antibody did not recognize any of the protein thus ruling out the nonspecific association of released MSP-1 with the surface of NRBC**.**

Active uptake of mAbF10: A possible involvement of MSP-1

The earlier studies showed a time-dependent increase in binding of ^{125}I-mAbF10 to IRBC at 37^0C, while no such difference in binding of mAbD2 to IRBC was observed. In order to establish the specificity of F10 uptake, IRBC were incubated with respective iodinated mAbs, in the presence of ten-fold excess non-specific cold antibody (mAb44G3), at both 37^0C and 4^0C. Results clearly indicated a specific uptake of mAbF10 without any change in transport kinetics even in the presence of non-specific mAb. On the contrary, mAbD2 showed an overall decrease of 25-30% cell-associated counts in the presence of cold mAb44G3. References in literatures site a non-specific transport of macromolecules to the IRBC, in addition to 'receptor-mediated' uptake. A reduction in cell-associated counts involving ^{125}I-mAbD2 clearly emphasizes the mentioned fact, contributing towards non-specificity of D2 transport.

As an extension to radiolabeling studies, experiments involving immunoelectron microscopy was undertaken further. Briefly, both the test mAbs (F10 and D2) and control mAb (44G3) were covalently conjugated with 1 nm gold particles. Monomaleimido nanogold, procured from M/s Nanoprobes Inc. were conjugated to reduced mAbs, in a reaction condition where a single gold particle gets covalently linked to the reduced disulfide bond at the hinge region between the heavy chains of IgG. The overall conjugation leaves the antigen-binding site unaltered. These gold-conjugated mAbs served as a better tool than conventional gold solution since the latter is attributed to exhibit non-specific gold leaching properties. These gold conjugates were incubated with IRBC at 37^0C for 30 min, following which the samples were fixed and processed for immunoelectron microscopy. Silver enhancement of ultra-thin grid sections revealed an increased intracellular localization of mAbF10 as compared to D2. Though the compartment-specific localization could not be established, yet a greater number of gold particles could be seen in sections incubated with F10 gold conjugates. The control mAb44G3 revealed negligible gold particles in IRBC sections, thus confirming the uptake of mAbF10 by intracellular parasite.

Experiments were further planned to check the uptake of mAbs under ATP-depletion conditions. Before this, viability status of IRBC was checked by [^{3}H] hypoxanthine

incorporation assay. Cells incubated with ^{125}I-mAbs were taken at different time points for measuring cell-associated radioactivity. No difference in the kinetics of ^{125}I-mAbF10 uptake could be observed in IRBC incubated under normal or ATP-depleted conditions. Similarly, IRBC showed no difference in ^{125}I-mAbD2 binding at both the above-mentioned conditions, thus indicating that binding and uptake of mAbs was independent of the metabolic status of IRBC. Secondly, the possible involvement of caveolae in mediating ^{125}I-mAbF10 uptake was studied in the presence or absence of caveolae-disrupting agents such as nystatin or phorbol-12-myristate 13-acetate (PMA). No change in cell-associated radioactivity or binding kinetics of ^{125}I-mAbF10 was seen in the presence or absence of either nystatin or PMA. Thus, it is likely that some other alternate pathway of antibody uptake exist in IRBC.

MSP-1 epitope mapping

Merozoite surface protein-1 (MSP-1) is one of the most abundant protein reported on the merozoite surface and its role have been implicated in the parasite entry into erythrocytes. In the earlier studies, anti *Plasmodium berghei* MSP-1 MAbF10 was also actively taken up by infected erythrocytes in temperature dependent manner. Further, the comparison of deduced *P.berghei* sequence of MSP-1 with that of *P. falciparum* indicated around 37% identity. So in order to characterize infected cell membrane associated MSP-1 molecule the group first selected MSP-1 reactive MAbs from a panel of MAbs, generated against *P. berghei* and *P. falciparum.* Results showed MAbs: PbF10, PbG_8C_3 and PbC_5C_3 (anti -*P.berghei* mAbs) and one MAb PfE_4C_7 (anti-*P. falciparum* mAb*)* reacted with rodent MSP-1 which was affinity purified using mAb F10 column. It was interesting to observe that two antibodies mAbs PbG_8C_3 and PfE_4C_7 showed *in vitro P. falciparum* growth inhibition as analysed by ^{3}H-hypoxanthine incorporation and LDH assays. While, MAb F10 showed the temperature dependent uptake in *P.berghei* parasite as well as infected erythrocytes. However, this antibody didn't cross react with *P. falciparum.* Thus, it was pertinent to dissect the regions (epitopes) in MSP-1, functionally and/or immunologically conserved in these two plasmodium species. Epitope mapping was first carried out by screening 12–mer phage display peptide library using biopanning tecnique. Phages bound to antibody coated plates were eluted and then further amplified for subsequent pannings. DNA sequences of these phages were determined. Homology search of mAbF10 specific peptide against *Plasmodium* genome using BLAST resulted in ~70% sequence homology to MSP-1. Further ClustalX alignment of this peptide with MSP-1 revealed the presence of eleven identical residues, indicating that this mAbF10 reactive epitope resides at N-terminal region of the MSP-1. This observation was further corborated by the earlier observation that all forms of MSP-1: 230kDa, 195kDa and 156kDa recognized by MAbF10 had same N-terminal sequences. Thus, the epitope responsible for specific uptake of mAbF10 was present towards N-terminus of MSP-1 while mAbs G_8C_3 and E_4C_7 reacted with the epitope conserved among *P.falciparum* and *P.bergei* parasite.

(iii) Studies on RhopH3.

Gene sequence analysis and homology search

Homology search of the N-terminal sequences of MSP-1 and RhopH3 identified

similar molecules in different rodent malaria parasites. However, no report of the RhopH3 protein from *Plasmodium berghei* NK65 strain was found in the literature. Also, the search for MSP-1 homologues revealed a sequence from *P. berghei* ANKA strain, so it was thought pertinent to clone the sequence of these proteins. Some part of this work was done with the help of Dr. S.K. Batra, UNMC Omaha. Primers were designed based on N- and C- terminal sequence of MSP-1 and RhopH3 genes of Pla*smodium yoelii* .The gene encoding RhopH3 was reverse transcribed from the poly A^+ mRNA of *P. berghei* NK65. However, despite repeated attempts, the same for MSP-1 could not be amplified, probably due to its large size. While for RhopH3 gene cloning, the reverse transcribed product was cloned in the expression vector pCR2.1. The entire RhopH3 encoding gene of 2.6 kb was sequenced and the putative open reading frame (ORF) with deduced amino acid translation was obtained using Gene runner software.

The N-terminal sequence (KDYFNGVLNQKLDDL) of the RhopH3 protein was compared to the translated gene sequence of *P. berghei* RhopH3 (*Pb*RhopH3), closest similarity was observed at the 25-39th amino acid position (KDYFNGVLNQKLSDL). Both the sequences were found to be identical except at the 13th amino acid position, where a transition from serine to glutamate was observed in the N-terminally sequenced protein. Use of Signal P program to predict the signal sequence of the theoretical ORF, identified a putative signal peptide sequence at the amino acid residues 1-24 of the translated sequence, thus explaining that the N-terminal region of the 105-kDa RhopH3 protein was a product obtained after the cleavage of the signal peptide.

In order to further confirm the structural similarities between RhopH3 of *P.berghei* and other rodent and human malaria parasite the entire RhopH3 encoding gene of 2.6 kb was sequenced and the putative open reading frame (ORF) with deduced amino acid translation was taken for subsequent analysis. Multiple alignments of PbRhopH3 with the known *P. yoelii* RhopH3 (*Py*RhopH3), using Clustal W indicated a complete identity in the signal sequence, except a substitution of isoleucine with valine at the 19th position of *Pb*RhopH3. A similarity of 94.5% and an identity of 87.8% were observed between *Pb*RhopH3 and *Py*RhopH3. Manual adjustments for the best fit divided the *Pb*RhopH3 protein into two types of blocks. The conserved blocks composed of long stretches of sequence were clustered at the N-terminal, central region and C-terminal, while the short stretches of variable blocks interspersed these conserved sequences and mainly concentrated in the central region. All the 12 cysteine residues, located in the conserved blocks were conserved between *Pb*RhopH3 and *Py*RhopH3. Clustal W alignment of *Pb*RhopH3 with *P. falciparum* RhopH3 (*Pf*RhopH3), yielded three types of blocks: conserved, semi-conserved and variable blocks. Although minor variations were evident, the overall patches of identity essentially remained similar between *Pb*RhopH3 and *Py*RhopH3. Thus, in the comparison of RhopH3 sequences between *P. berghei*, *P. yoelii* and *P. falciparum*, the overall identity pattern remained similar, thereby indicating conservation across the three species of *Plasmodium*.

Cloning and expression of *Rhoph3* gene

This part of study was carried out in collaboration with Dr. K.L. Dikshit's group. For structural & functional characterization, attempts made to clone & express RhopH3

gene initially resulted in expression of truncated form of *RhopH3* (corresponding to 48-1106 nucleotide from N-terminal). However, the protein was found in inclusion bodies. Hence, attempts were made to solublize the recombinant protein. The accumulation of the recombinant protein as aggregates could be a result of incorrect folding of the expressed protein. Based on the observation that lower growth temperatures often lead to decreased inclusion body formation, culture was grown at 25^0C and 30^0C. But at both temperatures more than 95% of the protein was found in inclusion bodies. Further, extraction buffer containing 8M urea resulted in only partial solubilization but also denatured the protein. Besides, various conditions using different buffers having salt, detergents and sugar also failed to effectively solublize the protein. As the recombinant protein contain 5 cysteine so oxido-shuffling method was applied but in this case also protein was unable to fold. Finally, success was achieved by using a mild ionic detergent sarkosyl which effectively solubilized the protein. Both the mAbs D2 and E_2B_6, reactive with RhopH3, did not show any reactivity with this recombinant RhopH3(16-294) protein, thus it is likely that the epitopes recognized by these two antibodies are not present in this region of RhopH3.

RhopH3 associated molecules and epitope mapping

Molecules localized in rhoptries, particularly erythrocyte binding proteins, have been studied as vaccine targets as these are important for erythrocyte invasion. Conserved regions in *P.falciparum* RhopH3 and that of *P.yoelii* RhopH3 have been identified. Literature shows that HMW rhoptry proteins of malaria parasite exist in multiprotein complex which are not affected by chaotropic agents. In line with these observations, the studies revealed the association 105 kDa with 146 kDa protein. However, among these, only 105 kDa (RhopH3 of HMW) was recognized by mAbD2 in western blotting. In order to further check the presence of other members of multiprotein HMW complex, affinity purification of HMW was carried out by passing IRBC extract through mAbD2 column. Immunoblotting studies with this eluate using polyclonal antiserum revealed other components, although present at a low concentration. Accordingly, it was thought pertinent to select the HMW reactive mAbs from a panel generated against *P. berghei* and *P. falciparum.*

Immunoblotting of mAb-D2 column purified fraction (containing RhopH3 and associated molecules) was performed using above panels of mAbs. PfG_5D_7 and PfG_6F_2 from anti-*P.falciparum* antibody panel and PbE_9G_6, PbD_3F_7 and $mAbE_2B_6$ from anti-*P.berghei* infected red blood cell membrane panel, showed good reactivity with 105 kDa , 95 and 45 kDa members of HMW complex. While other antibodies of the panels showed no reactivity with the complex. mAbE2B6 also showed *P.falciparum* growth inhibition indicating that it is reactive against functionally conserved domain of RhopH3 protein. Interestingly, the recent observations have also indicated the possible association of a protease with the HMW complex

Epitope mapping was first carried out for D2 reactive region of RhopH3 by MALDI and peptide phage display methods. For MALDI, immune complex was digested with TPCK-trypsin, V8 protease, AspN and Endo Lys C. The region bound to antibody after protease treatment was analyzed by mass spectrometry. In parallel, antibody binding peptides were also identified by using 12-mer peptide phage

library. Homology search of MAbD2 specific peptide (YLGPLEDTNLGY) against *Plasmodium* genome using BLAST showed ~60% sequence homology to RhopH3. Further, ClustalX alignment of this peptide with RhopH3 protein revealed the presence of eight identical residues, indicating that possible epitopic region of mAbD2 resides towards C-terminal. Moreover, the epitope identified by MALDI coincided with this peptide sequence, thus confirming that this region constitute the epitope of mAbD2.

Insight thus gained towards the conserved/functional domains of RhopH3 and MSP-1 might be useful in further testing these epitopes/molecules as targets against malaria.

The Research Council of IMTECH in its 32nd meeting held on Dec. 19-20, 2006 reviewed the work done under this project and declared the project as successfully completed.

Dr. Grish C Varshney (standing, sixth from left) and group

Girish Sahni
sahni@imtech.res.in

BASIC AND APPLIED STUDIES ON CLOT-DISSOLVER AGENTS USING PROTEIN ENGINEERING APPROACHES

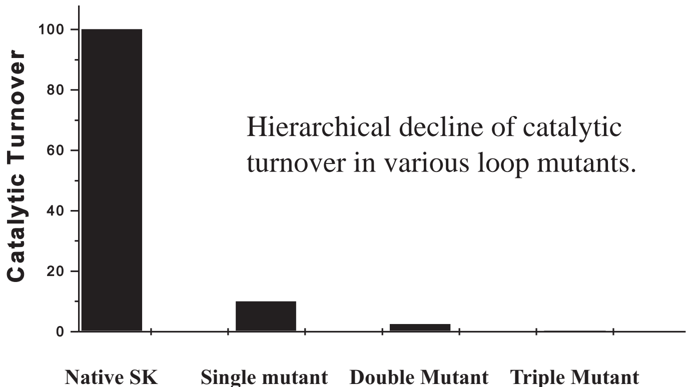
Project Leader: Dr. Girish Sahni
Other Participants: Ms. Rachna Aneja
Ms. Suman Yadav
Mr. Shekhar Kumar
Mr. Kishore Kumar Joshi
Mr. Neeraj Maheshwari
Mr. Prakash Kr. Sinha
Ms. Paramjit Kaur

The overall aim of the project is to obtain mechanistic insights into the molecular action of plasminogen activator enzymes of human as well as bacterial origin so as to redesign newer, more efficacious clot-busters.

Catalytic synergy between multiple loci located in the three domains of streptokinase during substrate plasminogen processivity.

Plasminogen activation mediated by the streptokinase-plasmin (SK.PN) activator complex and its macromolecular substrate, plasminogen involves dynamic protein-protein interactions during docking, scissile peptide bond cleavage and product release/dissociation steps of catalysis. Although biochemical studies from our lab such as Yadav, *et al.,* (Biochim Biophys Acta. 2008), Dhar *et al.,* (J. Biol. Chem. 2002) and Chaudhary *et al.,* (Protein Sci. 1999) have indicated in the past that the several possible epitopes, especially surface-exposed loops, participate in the first, enzyme-substrate docking step, the identity of a 'higher order' synergistic process, if any, in streptokinase (SK) action has not been demonstrated so far. The possible 'global' function of prominent surface-exposed loops resident in different domains of SK in substrate plasminogen processivity was explored through simultaneous multi-point mutations in these epitopes, namely the alpha domain's 88-97 loop, the beta domain's 170 and 250-loops and the region in gamma domain known as coiled coil region. Various combinations of mutations in all these four loci caused retarded catalytic activity up to varying extents e.g. a number of different mutations in the 88-97 loop exhibited nearly 4-fold decreased co-factor activity as compared to the native activator complex, while site-specific alanine substitution mutations of the 170-loop exhibited decreased catalytic activity of an order of magnitude likewise, various charge substitution mutations in the close vicinity of the 250-loop caused a decrease in catalysis by a factor of five, as also mutation of key residues in the coiled coil

region of streptokinase caused a significant fall in catalytic rates of substrate plasminogen activation by the preformed SK mutant plasmin complexes . Double 'hybrid mutants' of these different loci showed a clear phenomenon of co-operativity in plasminogen (PG) activation, for example, hybrids in which mutations in the 88-97 and 170 loops were introduced simultaneously, showed a much more reduction in catalytic activity, of the order of nearly 35-fold. Similarly, a double-locus mutant in which charge mutations of the 170 and 250 loops were recombined in a single hybrid SK mutant exhibited a concerted decline (40-fold) in its cofactor activity. 'Triple hybrid' mutants of these distinct loci in which mutations of the 88-97, 170, 250 loops and coiled coil region were combined in a single construct, showed a still greater retardation, nearly of three orders of magnitude in substrate processing, compared to the native complex. From these studies, the group infer that the three epitopes in SK likely work in “symphony” as catalytic motors during the conversion of substrate plasminogen to plasmin.



Bacterial over-expression and *in vitro* refolding of cysteine-rich proteins of the human thrombolytic system

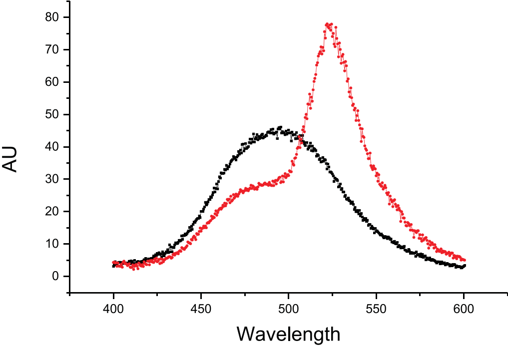
The group has successfully developed and optimized *in vitro* refolding capability for complex, large proteins of the human thrombolytic system that contain multiple disulfide bonds in their native state. Inability to refold *in vivo* during bacterial over-expression leads many recombinant proteins into the inclusion bodies. Screening for multiple conditions optimal for oxidative refolding was made simple by adopting a MATRIX based refolding approach and devising a 96-well micro plate based activity assay. This approach provided a high-throughput method to examine multiple conditions of refolding in several multi-well plates simultaneously. Refolding can be set under 96 different conditions for one plate and the activity generation can be quantified in a single absorption scan for hydrolyzed substrate. A systematic investigation exploring different combinations of salt, glutathione, co-solvents such as glycerol, and refolding promoters such as L-arginine etc. resulted in successful refolding of various plasminogen derivatives that are rich in cysteine and difficult to refold with conventional approaches. The methods so optimized are promising for other cysteine rich proteins, in particular those that contain serine-protease like catalytic domains and/or the extensively disulfide- bonded or simply the kringle domains that are present in many other proteins besides plasminogen with therapeutic importance.

High-level secretory expression of human plasminogen derivatives in *Pichia pastoris*

In order to understand the differential contributions of kringles present in plasminogen molecule for catalytic turnover by the SK-PN complex, the expression of different molecular forms of plasminogen is highly desirable. For the expression of relatively large, disulphide-rich proteins, the yeast expression system,. *Pichia pastoris* was optimized for secretory expression. Different signal sequences and culture conditions have been optimized to increase the production of plasminogen derivatives. Using the various plasminogen derivatives as substrate, the team observed that upon addition of each successive kringle at the N-terminus of the catalytic domain there is an increment in catalytic rate which clearly suggests its role in the formation of multiple contacts in the process of catalysis by SK-PN complex. These studies reveal the unique molecular mechanism behind action of SK-PN, which utilizes protein-protein contacts spatially far away from the catalytic domain/the target peptide bond. Future studies on the mechanistic aspects of the enzyme-substrate interactions are being carried out using bacterially and yeast expressed proteins and their mutational forms (see below).

Physical mapping of streptokinase and plasminogen interaction sites and their concerted action in catalysis probed by steady-state and real-time FRET studies.

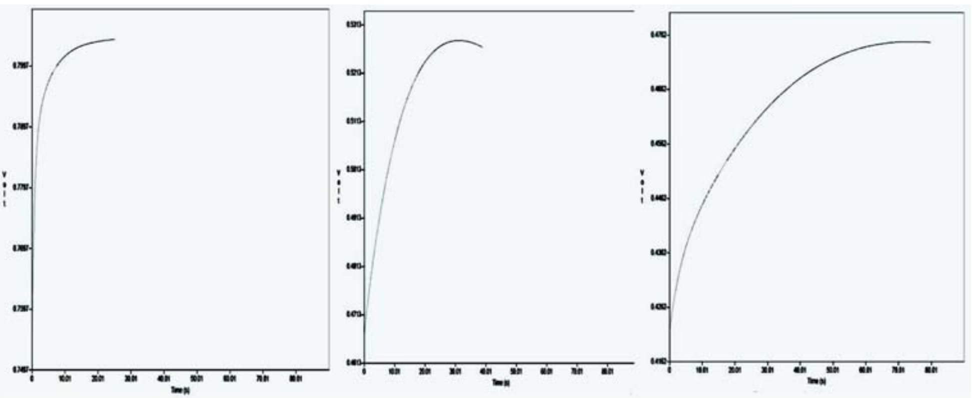
Despite the determination of X-ray crystal structures of SK in complexation with microplasmin (catalytic domain devoid of all kringles), the group lacked critical information about the conformational transitions that may either be transient or steady in nature but are, nonetheless, indispensable for expression of the active site in the plasminogen. Also, the crystal structure of the SK.microplasmin binary enzymatic complex is devoid of the docked substrate. This leaves a big gap in the understanding of how the substrate interacts with the binary enzymatic complex in a highly substrate and site-specific manner. To address these mechanistic issues, the team have now developed fluorescence based assays. To explore the interaction of docked substrate in the SK.PN complex the group took the approach of steady-state Fluorescent Resonance Energy Transfer (FRET) that utilized extrinsic fluorophore attachment to strategically placed cysteine residues at different loci of both streptokinase and substrate plasminogen. The extent of energy transfer between the donor and acceptor sites in this system enabled the team to map some of the proximal and distal loci of the docked substrate from one reference point of SK.



Typical FRET spectra showing quenching of donor fluorophore present in SK beta domain due to proximal placing of acceptor fluorophore containing Kringle domain of docked substrate

The distance mapping validated the previous solution studies based on loop deletion and mutagenesis (Dhar *et al.,* 2002; J. Biol. Chem.) where the group first proposed the role of substrate kringles in catalytic amplification. The distance geometries calculated by FRET are being utilized to develop a ternary interaction model of SK.PN and docked substrate that could answer many underlying queries of enzyme-substrate interactions in the SK system.

The group have also developed a Stopped-Flow FRET based rapid kinetic technique in order to examine and explore the dynamic changes in both SK and PG and their allostery in controlling the different steps of catalysis, such as substrate capture, catalytic transformation and, finally, product release.



Substrate dependent rates of product formation probed in real-time with Stopped-Flow FRET studies. Traces show rapid kinetics of product formation for three different substrate derivatives *viz.* midi-plasminogen, mini-plasminogen and micro-plasminogen of Plasminogen.

By using time-dependent FRET studies the group examined the rapid kinetics of catalysis for different truncated plasminogen derivatives and showed their altered competencies as substrates. This pre-steady state examination enabled to calculate the real-time rate constant of product formation for different plasminogen derivatives under a single catalytic turn-over situation. This method has also enabled to examine the catalytic processivity of different mutants of streptokinase that show co-opertivity in engendering full blown plasminogen activation capability. These results reveal an undisputed synergism between different loci spread over the SK structure that interact with the substrate and co-operate during the plasminogen activation phenomenon.

PEGylation of Streptokinase to increase *in vitro* and *in vivo* stability

Use of various thrombolytic protein drugs is hampered by the generally short *in vivo* half-life (10-15 min) as well as their immunogenicity. This situation becomes particularly acute in case of the heterologous agents, such as streptokinase, where the protein can elicit antibodies sometimes leading to allergic response makes repeated administrations of the drug essentially ineffective and even dangerous. To maximize the pharmacological benefits of streptokinase through reduction of immunogenicity

and increased *in vivo* stability, Poly-Ethylene Glycol (PEG) polymer addition was attempted. Covalent PEG additions to protein are known to increase their circulating half-life, reduce the susceptibility to degradation by proteolytic enzymes, increase the solubility of proteins, and lessen their propensity to generate neutralizing antibodies. To achieve these objectives, cysteines were strategically introduced at different regions in streptokinase. The mutational plan was designed on the basis of solvent accessibility of the site and its proteolytic lability. The Cysteine variants were further chemically modified by attaching sulfhydryl reactive PEG reagents. This strategy resulted in the development of a handful number of potentially improved SK derivatives. PEGylated streptokinases, so obtained can be useful in treating subjects with circulatory disorders such as venous or arterial thrombosis or, in particular, myocardial infarction with the advantages being that the PEGylated streptokinases have the potential for increased efficacy due to extended action thereby requiring lesser doses, and also allows the possibility of repeated administration with minimal immune reactivity (a patent application has been filed for this invention).

Dr. Girish Sahni (standing, in centre) and group

Balvinder Singh
bvs@imtech.res.in

ANALYSIS AND EVALUATION OF COMMONLY USED FORCE FIELDS FOR DERIVING RULES FOR DNA-PEPTIDE/PROTEIN DOCKING

Project Leader: Dr. Balvinder Singh

Other Participants: Mr. Manish Dutt
Ms. Ruchi Sachdeva

Protein-nucleic acids complexes have been the subject of study for past many years. Proteins that bind to specific DNA target sites play essential roles in all aspects of gene regulation. Large amounts of sequence information for genes and transcription factors etc. from genome analyses are presenting a great challenge in the field of bioinformatics. Despite tremendous amount of sequence and structural data on protein-DNA complexes, mechanisms of target recognition are not well understood. Sequence similarity searches are the most commonly used methods for extracting functional information from sequence data. Structural data contain valuable functional information as well. Many a time, conformation of DNA plays an important role in protein binding. Molecular dynamics based on a solid body of physical theory can simulate biomolecular interactions of the system such as protein-DNA complex using force field based methods. Molecular dynamics and mechanics methods employ a number of force fields such MM3, CVFF, AMBER, CHARMm and GROMOS. The functional form remains more or less the same in all force fields that incorporate a large number of empirical parameters. The strategy of developing force fields by fitting as many properties of simple systems as possible has resulted in the emergence of many slightly different parameterizations and functional forms. The force fields do not treat the electrostatic interactions effectively; limits (cut off, smoothing algorithm etc.) applied during force field calculations also differ; the solvent effects are not addressed satisfactorily. There has been little known on the comparative evaluation of these force fields on these macromolecular systems. Also, it is interesting to simulate and explore the interactions/docking between the proteins and DNA.

The protein DNA complexes have been subjected to analysis, firstly for their classification based on the comparison of structures of proteins associated in the complexes by the group. The structural homology of proteins has been verified by visual comparison to assign the classes and families. The number of already reported groups (by Thornton *et al.*) remained the same with more protein chains being observed belonging to the groups 'Enzymes' & 'Helix-turn-helix' as compared to other groups. A few new families of protein chains (such as Tn5 transposase family, lambda integrase, P4 origin binding domain like, endonuclease IV family, DNA

glycosylase etc.) under various groups have been observed. A new dataset of protein–DNA complexes have been generated by taking information from well known public databases such as PDB and NDB. The interactions occurring between protein and DNA in their respective complexes have been analyzed for the preference of type and kind of amino acid residues to base pair(s). A particular set of charged residues have been found to be involved in interactions predominantly as compared to others. The study of these interactions will help in deriving rules for the docking of proteins and DNA into the respective complex. Also, the strengths and qualities of commonly employed force fields such as CVFF, AMBER, CHARMm are being tested on a few representative complexes.

This study is essential and critical in the post genomic era where a number of nucleic acid, protein sequences and structures are available for analysis and simulations.

Dr. Balvinder Singh
(Sitting in the centre) and group

K. Ganesan
ganesan@imtech.res.in

GENETIC ANALYSIS AND MANIPULATION OF *Candida albicans*

Project Leader: Dr. K. Ganesan
Other Participants: Dr. Sanjoy Paul
Mr. M.A. Mannan
Ms. Sushma Sharma
Mr. Yashpal

Development of Genetic Tools for Functional Analysis of Candida albicans Genes

Candida albicans normally exists as a harmless commensal in most healthy humans, along the mucosal surfaces of the gastrointestinal and genitourinary tracts and, to a lesser extent, on the skin and buccal cavity. However, when the immune system of the host gets compromised, it can become an opportunistic pathogen causing painful, superficial infections, and systemic infections that are often fatal. *Candida* infections are also on the rise due to a steady increase in the immunocompromised population of patients. A molecular level understanding of the biology and pathogenicity of *Candida* will go a long way towards prevention and treatment of infections.

Though the genome of *C. albicans* is fully sequenced, the function of large proportion of genes is still unknown. Genes crucial for survival and/or pathogenesis of *C. albicans* are identified and studied individually, primarily by gene disruption/deletion. However, this is quite cumbersome since *C. albicans* is naturally diploid, and both alleles of a gene need to be mutated to obtain a phenotype. Moreover, efficiency of transformation as well as targeted integration is poor in *C. albicans*. Thus, mutating both alleles typically involves generation of deletion constructs (by cloning or by PCR), two sequential transformations, and confirmation of targeted integration after each transformation. This makes the generation of homozygous mutants a rate limiting step in studying *C. albicans* genes. Failure to generate homozygous mutants can lead to inconclusive results, and it may be hard to determine whether the gene is essential or not. Also, the phenotype cannot always be attributed to the disrupted gene due to the possibility of transformation induced chromosomal abnormalities/mutations elsewhere in the genome. A straightforward approach to accurately determine the biological role of a gene is its conditional expression, by bringing it under the control of a regulatable promoter. The presence/absence of phenotype when the promoter is induced as opposed to when it is shut down would clearly indicate the role of the regulated gene in contributing to the phenotype. The effect of unintended changes elsewhere in the genome, if any, contributing to the phenotype can also be easily ruled out. In *C. albicans*, a few

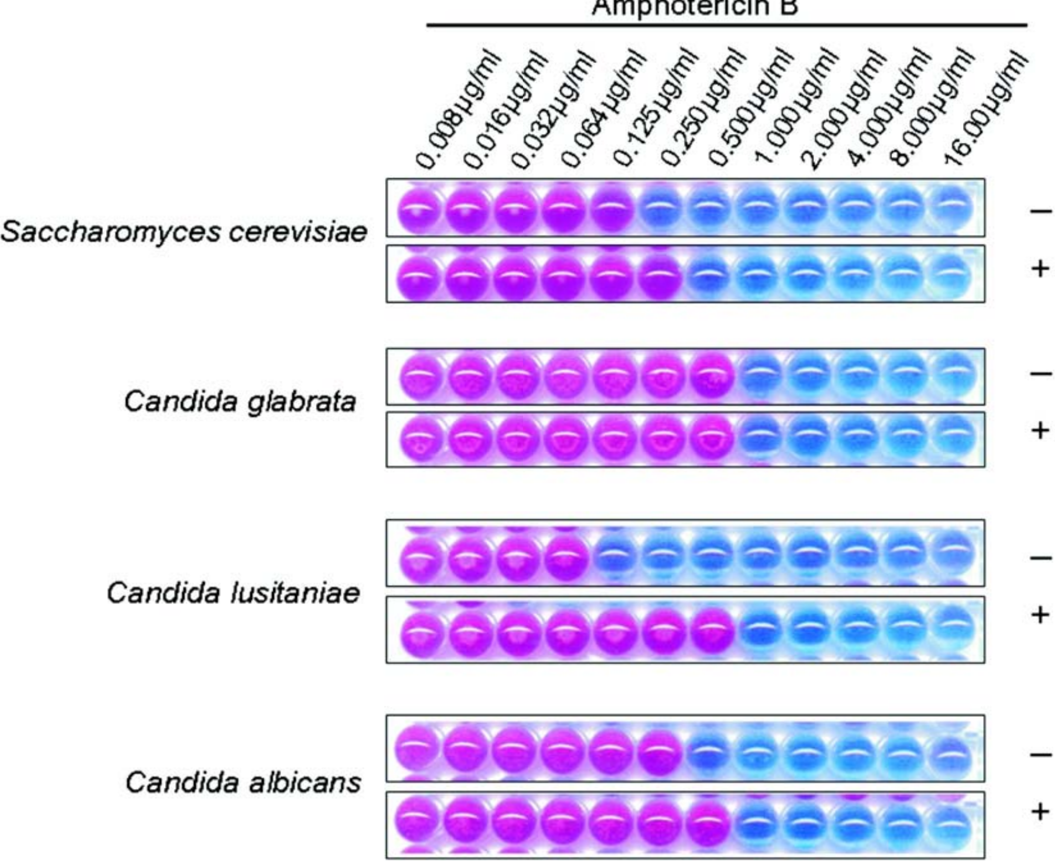
regulatable promoters have been used to study function of genes by conditional expression, where one of the alleles is deleted, and the other allele brought under the control of the regulatable promoter. However, the limitations of this approach are the need to generate two constructs (one for disruption, and another for regulated expression), the need for two transformations as well as two confirmations of targeted integration. These limitations, along with inefficient targeted integration, make the generation of strains with conditional expression of test genes very laborious and time consuming.

To circumvent the above limitations, we have developed a set of modular cassettes with which both alleles of a target gene can be conditionally expressed after only a single transformation. These cassettes employ auxotrophic markers *HIS1* and *ARG4*, which have the advantage of not having any effect on virulence, and methionine repressible (MET3) and maltose inducible (MAL2) promoters. These cassettes are designed such that after initial selection for arginine prototrophy, *in vivo* gene conversion, recombination and further selection for histidine prototrophy would bring both alleles of a target gene under the control of regulatable promoter. These cassettes could be successfully used to check the essentiality of certain genes for growth and survival of *C. albicans in vitro*. The cassette with MET3 promoter could also be used to shut down the expression of *C. albicans* genes during infection of mice, thereby facilitating the study of the role of these genes in pathogenicity.

<u>Molecular Mechanisms of Amphotericin B (AmB) Resistance in Candida</u>

As mentioned above, the incidence of invasive, and often fatal, *Candida* infections is becoming more common. There is also an increase in the number of clinical isolates resistant to amphotericin B, a frontline antifungal, frequently resulting in treatment failures. Hence, in another project we are trying to identify and characterize genes and regulatory pathway(s) that are involved in AmB resistance. A molecular level understanding of this resistance is likely to help in devising strategies to minimize development of AmB resistance during therapy.

Dr. K. Ganesan (standing, second from left) and group



Effect of farnesol on amphotericin B (AmB) resistance, tested in multiwell plates with increasing concentration of AmB, in the presence (+) or absence (–) of 50 µM farnesol. An indicator dye is included in the wells which turns pink upon growth of cells, but stays blue when there is no growth. Farnesol effect on AmB resistance varies depending on the species -- while it has no effect on *C. glabrata*, maximal effect is seen with *C. lusitaniae.*

During preliminary experiments done in our laboratory to study AmB resistance, it was observed that farnesol, a quorum sensing molecule, increased the resistance by about 4-8 fold. To explain this, first we tested the involvement of known mechanisms of AmB resistance to see if farnesol effect is mediated through these. Farnesol is synthesized from farnesyl pyrophosphate, an intermediate in the ergosterol synthesis pathway, and thus it is likely that exogenous addition of farnesol affects ergosterol levels. Since inhibition of ergosterol biosynthesis is known to increase resistance to AmB, we initially suspected that farnesol increases AmB resistance by modulating ergosterol levels. Mutants impaired in ergosterol biosynthesis were tested for AmB resistance in the presence and absence of farnesol. Farnesol increased the AmB resistance of all the mutants tested, indicating that farnesol effect is not mediated through this pathway. Since AmB causes oxidative damage to the cells, and since farnesol increases resistance to oxidative stress, another possibility is that farnesol influences AmB resistance by enhancing oxidative stress response. However, results from mutants impaired in oxidative stress response show that farnesol effect is not mediated through this pathway as well. Thus, efforts are on to identify other pathway(s) through which farnesol increases AmB resistance, to better understand the molecular basis of this phenomenon.

Pradip Sen
psen@imtech.res.in

DEFINING THE MOLECULAR MECHANISM OF HEPATOCYTE GROWTH FACTOR-MEDIATED IMMUNOREGULATION OF DENDRITIC CELL FUNCTION

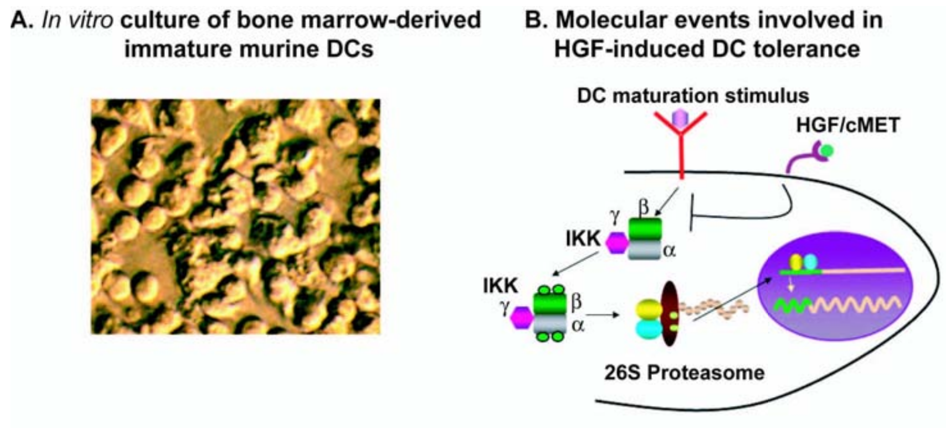
Project Leader: Dr. Pradip Sen
Other Participants: Ms. Eshu
Ms. Alpana Singh
Mr. Selvan

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.

Dr. Pradip Sen (standing, second from right) and group

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 HGF-induced inhibition has not been defined in DC. Therefore, the main focus of the group is to define the molecular basis of HGF-mediated inhibition of DC activation and effector function via c-MET receptor.

The studies demonstrate that HGF pretreatment of bone marrow derived DC (BMDC) results in inhibition of NF-κB DNA binding activity, IκḄdegradation and IKK activation induced by LPS stimulation. Furthermore, HGF induced inhibition of NF-κB activation results in reduced NF-κB binding to IL-12p35 and IL-12p40 promoter. Importantly, NF-κB activation regulates DC maturation and APC function. Therefore, regulation of NF-κB activation pathway by HGF in DC will give a novel molecular insight of HGF-induced DC tolerance. Currently studies are ongoing to elucidate the molecular mechanism how HGF/c-MET signaling exhibits inhibitory effect of NF-κB activation pathway in DC and its impact on DC APC function. 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.



Karthikeyan Subramanian
skarthik@imtech.res.in

ANTIBACTERIAL DRUG DISCOVERY: STRUCTURE BASED DRUG DESIGN AGAINST A POTENTIAL TARGET

Project Leader: Dr. Karthikeyan Subramanian
Other Participants : Mr. Mirage Singh
Mr. Pankaj Kumar
Mr. Zeyaul Islam

The objectives of this project comprise:

1. To clone the RFK/FAD synthase gene from *Bacillus subtilis/ M. tuberculosis/S. typhimurium/V. cholerae* into a suitable vector
2. Expression and optimization of RFK/FAD synthetase
3. Purification of RFK/FAD synthetase suitable for crystallization
4. Screening for potential crystallization condition
5. Optimization of crystallization condition to yield diffractable crystals
6. Data collection, structure determination, refinement of the model
7. Analysing the structure
8. Determination of crystal structures with substrates and products.
9. Identification of potential inhibitor using virtual drug screening and docking studies.
10. Kinetic and structural studies of the FAD synthetase with its potential inhibitor.
11. To develop the inhibitor with pharmacological properties (with suitable collaboration)

During the period under report the following objectives were achieved:

RFK/FADS of S. *typhimurium* :

The gene that encodes for Riboflavin kinase/ FAD synthetase from *S. typhimurium* has been PCR amplified from its genomic DNA and cloned into pET-28C vector. The sequence of the cloned gene was verified by automated sequencing in both directions. The protein was over expressed in *E.coli* and the conditions were optimized for over expression. The purification protocol has been standardized to yield high quantity of purified enzyme. The activity was checked using HPLC. The molecular mass was determined by MALDI experiments.

RFK/FADS of *M. tuberculosis* :

The gene encoding the RFK/FADS has been PCR amplified from its genomic DNA and cloned into pET-28C vector. The sequence of the cloned gene was verified by

automated sequencing in both directions. The protein was over expressed in E.coli and the conditions were optimized for over expression. Yet, the protocol has to standardized for better yield and purification.

RFK domain of *M. tuberculosis* :

In addition to the full length the team cloned and expressed only the riboflavin kinase domain of *M.tuberculosis*. The protein over expressed in *E.coli* and purified to its homogeneity. Screening of the crystallization conditions were started for this domain.

RFK/FADS of *V. cholerae* :

The full length gene of RFK/FADS was PCR amplified from its genomic DNA and cloned into pET-28C vector. Expression, purification and characterization of this enzyme is currently going on.

Full length and RFK domain of *B.subtilis*:

The full length and RFK domain of B.subtilis was PCR amplified from its genomic DNA and cloned intopET-28C vector. Expression and purifications has been standardized.

Dr. Karthikeyan Subramanian (standing, second from left) and group

Manoj Raje
manoj@imtech.res.in

CHARACTERIZATION OF A MEMBRANE EXPRESSED MULTIFUNCTIONAL GLYCOLYTIC PROTEIN AND ITS ROLE IN MACROPHAGE FUNCTION

Project Leader: Dr. Manoj Raje
Other Participants : Dr. Chaaya Iyengar
Mr. Rohan Dhiman
Mr. Santosh Kumar
Ms. Pooja Rawat
Mr. Anil Theophilus

GAPDH is known primarily as a cytosolic protein. The group has demonstrated for the very first time that GAPDH has a novel cell surface localization in mammalian cells. It was of interest to understand how an essentially cytosolic protein is localized on the cell membrane surface and also what are its functions there. In order to answer this question, the complete characterization of the membrane expressed GAPDH has been completed. It has now been established that this molecule functions as a novel receptor for the important iron transport protein Transferrin.

These findings describe the presence of an entirely new uptake mechanism for the iron transport protein transferrin into mammalian 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. It is now 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. Further studies are underway to elucidate the detailed mechanism and regulation of this novel transferrin based iron uptake pathway.

Significance of the Contribution: The 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. This recent discovery identifies a new type of ubiquitously expressed Transferrin receptor that bears no homology to the two previously known receptors. The group has also established that this receptor is regulated by the levels of extracellular iron.

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 -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. Current research is also focused on this aspect with relevance to immunity and infection. This study opens up a new avenue of research in attempting to understand the survival mechanisms of intracellular pathogens.

Dr. Manoj Raje (standing, first from right) and group

National Facilities

National Facilities

BIOINFORMATICS CENTRE ON PROTEIN MODELING & ENGINEERING

Project Leader : Dr. G.P.S. Raghava
Other participant: Dr. Balvinder Singh
Dr. Manoj Kumar
Mr. Harvinder

Bioinformatics centre at IMTECH has shown consistent growth in the different areas of bioinformatics whether in carrying out peer reviewed research or providing services to the world wide. This Centre has developed three immunological databases and databases for general uses. Following immunological databases are used as reference databases i) MHCBN consists of MHC-binders, T-cell epitopes, TAP binders etc.; ii) BCIPEP a database of B-cell of epitopes and iii) HaptenDB having haptens. BIC has developed a number of web servers based on peer reviewed research carried out at the bioinformatics centre (http:/imtech.res.in/raghava/www.html). BIC staff has published more than 60 research papers in reputed international journals, more than 10 chapters in books, around *35* Copyright softwares and more than 25 abstracts/poster/papers presented in national and international conferences (http://imtech.res.in/raghava/pub.html). The servers are getting more than 15,000 hits per day.

BIC is equipped with state-of-the-art computing facility that includes i) Apple cluster integrated with 200 bioinformatics software; ii) Apple macintosh nodes; iii) SGI graphics workstations and SGI origin server; iv) Alpha workstations/server v) Sun servers for email and webserver; vi) large number of intel/itenum based machines; vii) two dedicated Internet connections, viii) domain name server, router, proxy server and ix) firewall. The Centre has powerful infrastructure in the area of protein modeling and Engineering. All computers are on local area network as well as on Internet.

Staff of the BIC

MICROBIAL TYPE CULTURE COLLECTION & GENE BANK (MTCC)

Dr. Tapan Chakrabarti
Dr. R.K. Jain
Dr. G.S. Prasad
Dr. D. Ananthapadmanaban
Mr. S. Mayil Raj
Dr. K. Suresh
Ms. S. Pandey
Mr. Sudhir Kumar
Mr. Sushil Kumar
Mr. Upendra Singh
Mr. Ashok Yadav
Mr. Ramesh Singh
Ms. Girja Ditya
Mr. Malkhan Singh
Mr. Paramjit
Mr. Dhan Prakash
Mr. Malkit Singh

Microbial Type Culture Collection and Gene Bank (MTCC) a national facility supported jointly by DBT and CSIR has been functioning since 1987. MTCC is an affiliate member of the World Federation of Culture Collection (WFCC) and is registered with the World Data Centre on Microorganisms (WDCM, Registration Number 773). The main objectives of MTCC are:

i) Procurement of microbial cultures and their long term preservation
ii) Supply of authentic cultures to scientific community
iii) Provide services in the area of identification of microorganisms and freeze-drying of cultures.
iv) Act as a depository of microorganism including deposit of cultures for patent procedure
v) Conduct research in microbial diversity, taxonomy and in related areas.
vi) Impart training/organize workshop in the area related to culture collection, microbial taxonomy and diversity.

MTCC is now recognized as an IDA. The MTCC catalogue and related information are available on its website (www.imtech.res._Hlt165717350i_Hlt165717350n/mtcc) and is now being extensively used by regular and potential customers around the country and abroad.

Staff of the MTCC

At present MTCC has more than 7000 characterized cultures of Actinomycetes, Bacteria, Fungi, Plasmids and Yeasts in its collection. In addition about 6000 potentially important cultures of bacteria and fungi are also being maintained. During this period (2005-2007) deposits under the Budapest Treaty was 119. For filing patents in India and for safe deposit MTCC received 8 and 3 microorganisms respectively.

In 2005-2006, a total of 5162 and in 2006-2007 a total of 5291 cultures were supplied to different colleges, universities, research institutions and to industries. During this period (2005-2007) more than 700 cultures were identified as a service. Besides these freeze-drying service was also provided to a large number of investigators.

Focus of research in MTCC has been mainly on microbial diversity and taxonomy. During this period two new genera and many new species from diverse ecological niches of India have been described

BIOCHEMICAL ENGINEERING RESEARCH AND PROCESS DEVELOPMENT CENTRE

Project Leader: Dr. D.K. Sahoo

Other Participants: Dr. Vijay Sonawane
Mr. Hemraj Nandanware
Mr. Anirban Roy Choudhury
Mr. E. Senthel Prasad
Dr. Lata Verma
Mr. R.K. Kanojia

Objectives:

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

R&D on the below mentioned projects was undertaken during the period under report:

1. Media and process optimization for production of staphylokinase.
2. NMITLI Project on "Biotechnology for Leather: towards cleaner processing Phase II".
3. CSIR network project on "Standardization of technologies for bioresources for and from leather".
4. Production of cerulomycin
5. Bio-extract making and screening for bio-actives: Exploration of microorganisms for secondary metabolites
6. Screening for proteases, lipases and cellulases

Consultancy, Technology Demonstration and Licensing

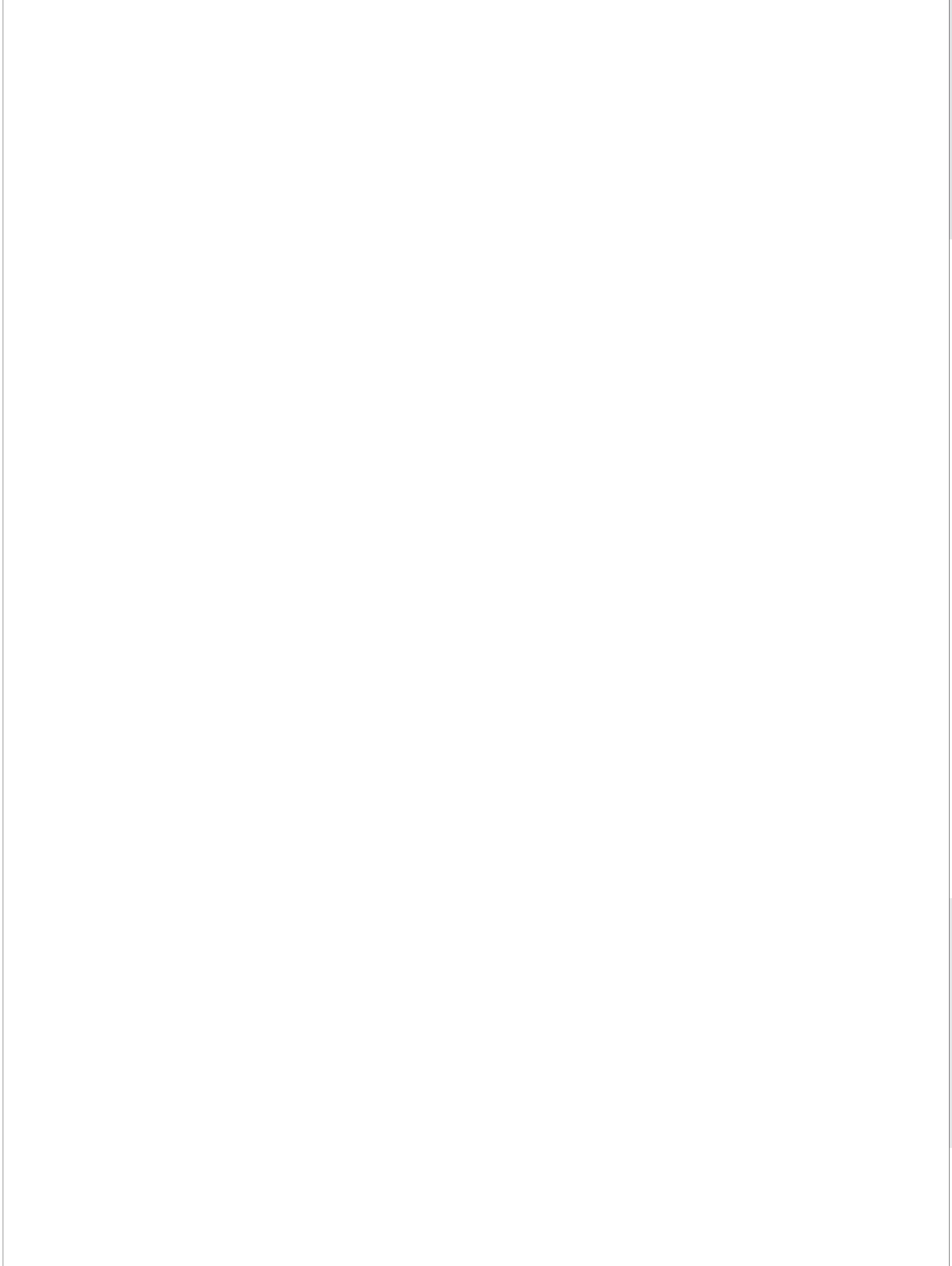
- Consultancy assignment of Bigtec, Bangalore was successfully completed.
- Technology on "A process for the production of recombinant staphylokinase" at 5 L and 20 L scale was demonstrated and licensed to M/S Strides Arcolabs Limited, Bangalore.
- Technology on "Production of -amylase" and "A process for production of alkaline protease" at 20 L and 100 L scale were demonstrated and licensed to Celestial Labs Limited, Hyderabad.

Human Resource Development

- BERPDC imparted training to students from a number of Universities/ Institutes in areas of Biochemical Engineering and Applied Microbiology.

Staff of the BERPDC

Extra-mural Human Resource Development



Extra-mural Human Resource Development

- Summer Training Programme for M.Sc. (Biotechnology) students from Universities of Calcutta, Panjab, Banaras Hindu, Kurukshetra, Goa, Pondicherry, Aligarh Muslim, Punjabi, Guru Nanak Dev, Devi Ahilya, Calicut, Jawaharlal Nehru, Nagarjuna, Delhi, etc. was conducted during the period under report. A total of 54 students underwent this training.

- Winter Training Programme for M.Sc. (Biotechnology) students from Universities of Himachal Pradesh, Shimla; Kumaun and Tejpur was also conducted during the period under report. A total of 9 students underwent this training.

- Long Term Training Programme (6 months) for M.Sc. & B. Tech. students from Universities of Calcutta, Panjab, Banaras Hindu, Kurukshetra, Goa, Pondicherry, Aligarh Muslim, Punjabi, Guru Nanak Dev, Devi Ahilya, Calicut, Jawaharlal Nehru, Nagarjuna, Delhi, etc. was also conducted during the period under report. A total of 100 students underwent this training.

- The Institute of Microbial Technology signed an agreement with the Panjab University, Chandigarh on Sept. 19, 2005. According to the agreement, IMTECH scientists will participate in the various teaching programs of Panjab University and the University will recognize and accredit IMTECH scientists, on their expressed requests for guiding research leading to Ph.D. degree and the two research Institutes will explore the possibility of joint efforts for seeking for funds and conduct of collaborative research, training and consultancy with industry / institutions in India / abroad. The MoU would be valid for a period of 5 years commencing from 1st Sept., 2005.

- The Institute celebrates **"Hindi Pakhwara"** every year to promote the progressive usage of Hindi language in official work. During the period under review the same was celebrated from Sept. 1-14. Several competitions viz., Hindi Essay Writing, Hindi Vocabulary, Hindi Calligraphy, Hindi Typing and Hindi Quiz were held.

The Bioinformatics Centre successfully conducted, from October 18-21, 2005, a National Workshop on **"Machine Learning Techniques in Functional Proteomics"** in which 33 participants from several academic institutions and industries underwent an intensive training.

Participants of the workshop on Machine Learning Technique in Functional Proteomics

- IMTECH conducted the seventh **"CSIR Programme on Youth for Leadership in Science"** on November 10-11, 2005. A total of 27 students attended the two day meet. The programme was initiated by a welcome address by the Dr. P.R. Patnaik, Scientist 'G'. Thereafter Dr. Naresh Kumar gave a brief introduction about CSIR and the aims and objectives of the CPYLS programme. The Inaugural talk was delivered by Dr. Tapan Chakrabarti, Scientist 'G' on the topic "**Prokaryotic Diversity- The unseen majority**". A special lecture entitled "**Development of Scientific Culture in India"** was delivered by Dr. Nitya Nand, Ex-Director, CDRI, Lucknow on Nov. 10, 2005. The lecture generated considerable enthusiasm among the participants. A scientific exhibition on IMTECH's ongoing research programmes and existing capabilities, live demonstration of its computer modelling capabilities, and several laboratory experiments were also held and the students actively participated in all these events. Besides this, a poster making competition and a quiz contest entitled "**Creativity Olympiad**" were held in which all the students actively participated.

Participants of the CPYLS Programme

- IMTECH conducted the eighth **"CSIR Programme on Youth for Leadership in Science"** on October 9-10, 2006. A total of 33 students attended the two day meet. The programme was initiated by a welcome address by the Dr. P. Chakraborti Scientist 'F'. Thereafter Dr. Naresh Kumar gave a brief introduction about CSIR and the aims and objectives of the CPYLS programme. The Inaugural talk was delivered by Prof. B.N. Ganguli, Consultant, IMTECH on the topic "**Some Things Small but Wonderful**". A scientific exhibition on IMTECH's ongoing research programmes and existing capabilities, live demonstration of its computer modelling capabilities, and several laboratory experiments were also held and the students actively participated in all these events. The programme concluded with a lively discussion with the participants about their expectations and the career propects as they see it. They were also encouraged to give their views & inputs on the programme.

- IMTECH organised two workshops on **"Leadership Renewal and Reinforcement" and "Leadership and Change Management"** from February 13-14 and Feb. 15-17, 2006 respectively which were attended by scientists and senior personnel from administration, finance and stores and purchase. The workshop was facilitated Mr. Ian Dean of the Groman Consultancy Group of South Africa. These workshops were well received and highly appreciated.

- Provided training on "Maintenance and Identification of Cultures" to 3 persons from Nagarjuna Fertilizers & Chemicals Ltd., Hyderabad from March 20-31, 2006.

- Provided training on “Maintenance and Identification of Cultures” to a staff member from Saguna Poultry Farm Ltd., Pondicherry, from March 20-31, 2006.

- The Bioinformatics Centre successfully conducted from November 14-17, 2006 a National Workshop on “**Bioinformatics Resources on miRNA and siRNA**” in which 28 participants from several academic institutions and industries underwent an intensive training in the aforesaid field.

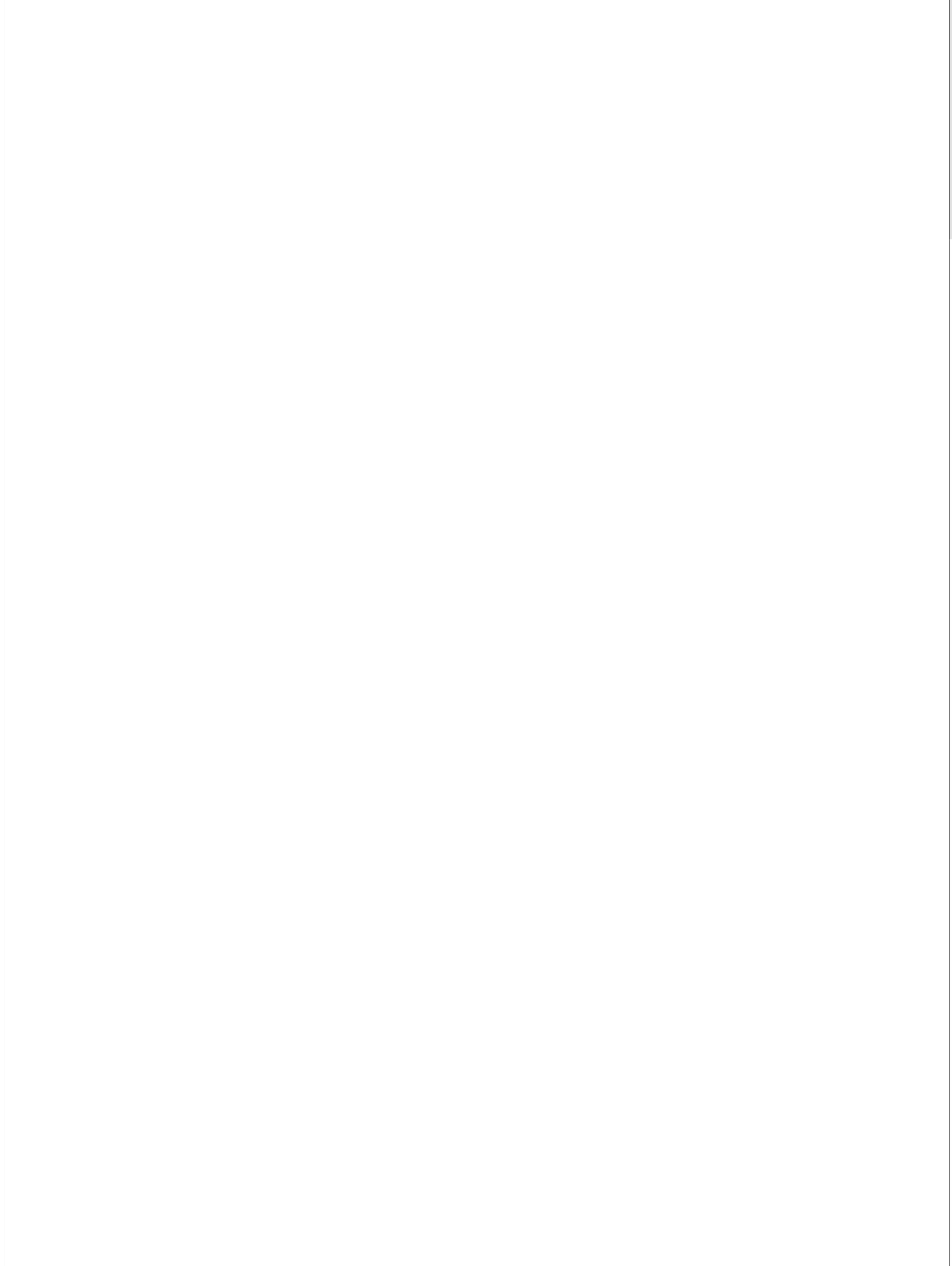
- The Institute organised, on behalf of HRDG, Ghaziabad a 2-day conclave of the Controllers of Finance & Accounts / Finance & Accounts officers of CSIR system from Dec. 1-2, 2006. A total of 54 participants participated in this meet.

- The Institute organised, on behalf of EU-India TIDP, a 2-day workshop on patent drafting, IPR licensing, Technology Transfer etc. for scientists and senior faculty of IMTECH, CSIO, NIPER and Panjab Univeristy from February 26-27, 2007. A total of 58 participants participated in this workshop.

Signing ceremony of an MoU with Panjab University, Chandigarh.

Research Publications & IP

Research Publications & IP



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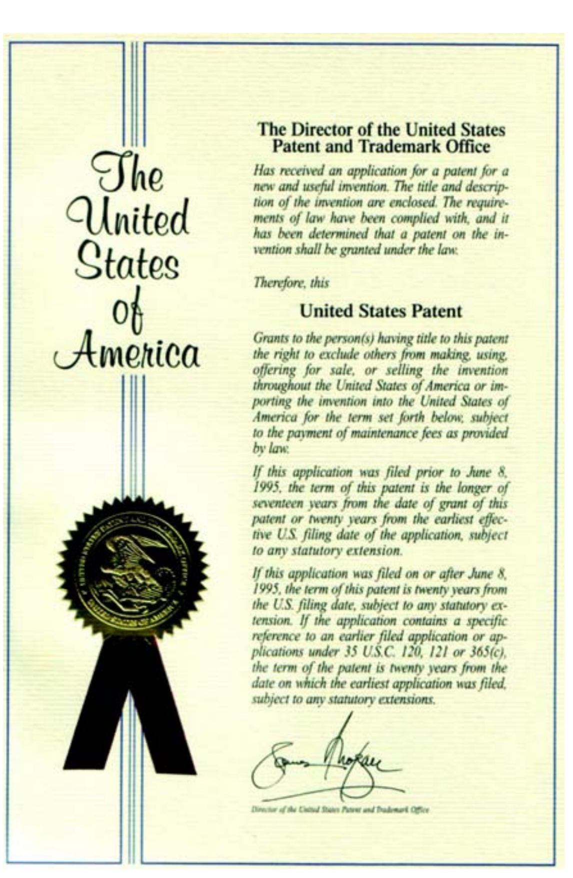
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The Director of the United States Patent and Trademark Office

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person(s) having title to this patent the right to exclude others from making, using, offering for sale, or selling the invention throughout the United States of America or importing the invention into the United States of America for the term set forth below, subject to the payment of maintenance fees as provided by law.

If this application was filed prior to June 8, 1995, the term of this patent is the longer of seventeen years from the date of grant of this patent or twenty years from the earliest effective U.S. filing date of the application, subject to any statutory extension.

If this application was filed on or after June 8, 1995, the term of this patent is twenty years from the U.S. filing date, subject to any statutory extension. If the application contains a specific reference to an earlier filed application or applications under 35 U.S.C. 120, 121 or 365(c), the term of the patent is twenty years from the date on which the earliest application was filed, subject to any statutory extensions.

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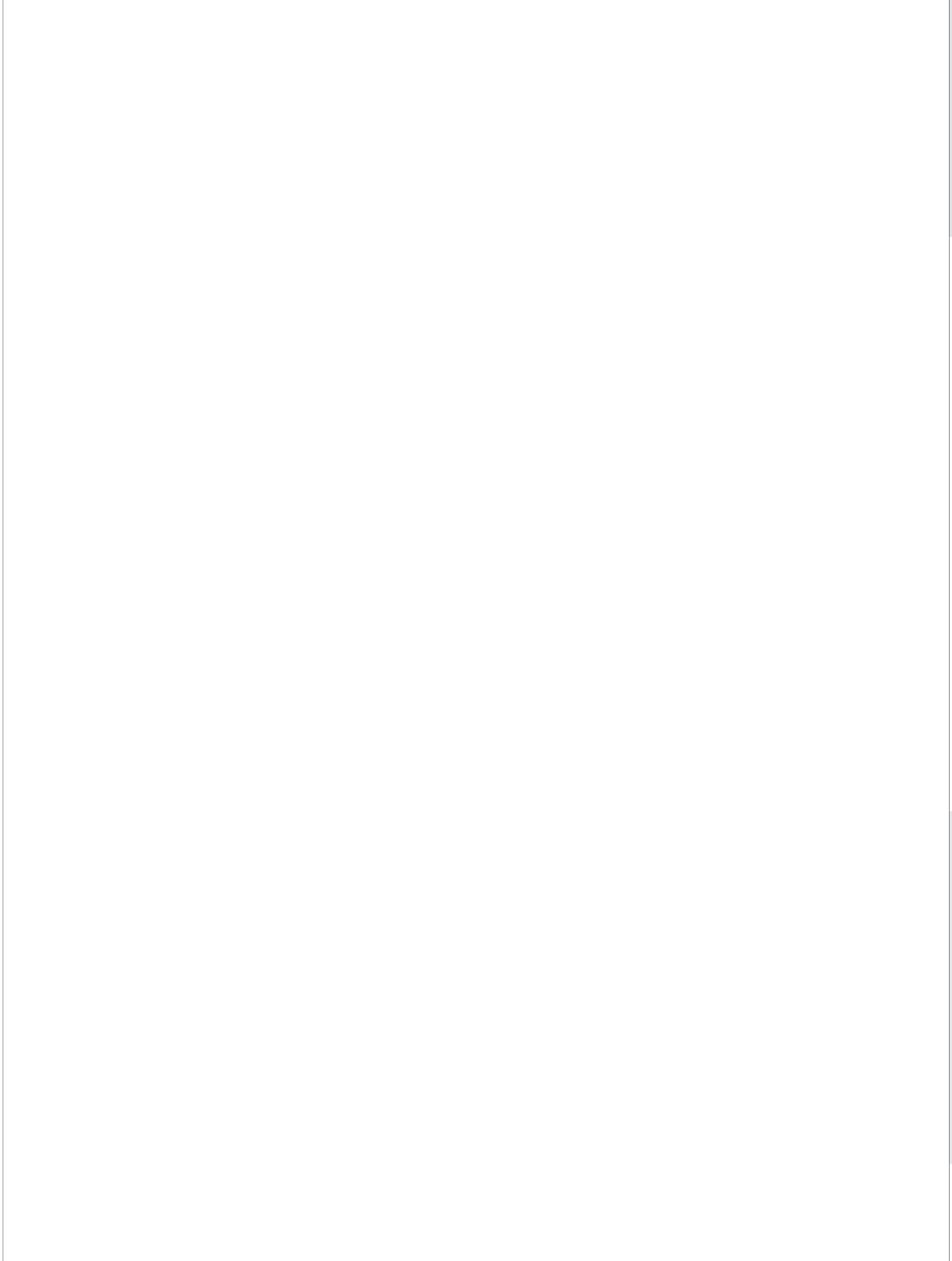
Awards

Awards



Awards/Honours

- Dr. J.N. Agrewala was conferred the prestigious “Dr. S.S. Bhatnagar Award” for the year 2005. Dr. Manmohan Singh, Prime Minister of India gave the award to Dr. Agrewala for his outstanding contributions in the field of Medical Sciences.

- Dr. R.K. Jain was conferred the prestigious "Pitamber Pant National Environment Fellowship Award" for the year 2004. The award was in recognition of his contribution in the field of Environmental Biotechnology and for undertaking research on "Chemo toxic and biodegradation of nitro aromatic compounds".

- Dr. G.P.S. Raghava was selected for the prestigious “National Bioscience Award for Career Development” for 2005-2006.

- Dr. P. Guptasarma was conferred the “AVRA Young Scientist Award” for the year 2005.

- Dr. J. N. Agrewala was selected for the prestigious “National Bioscience Award for Career Development” for 2006-2007.

- Dr. Girish Sahni: Elected Fellow of Association of Microbiologists of India.

Dr. Javed Agrewala receiving the S.S. Bhatnagar Award - 2005 from Hon'ble Prime Minister of India.

Dr. Javed Agrewala being honored during IMTECH Foundation Day Celebration

Dr. G.P.S. Raghava being honored during IMTECH Foundation Day Celebration

Dr. R.K. Jain being honored during IMTECH Foundation Day Celebration

Memorable Events

Technology Transfer Tie-ups

Clot-specific Streptokinase

A team of IMTECH scientists lead by Dr Girish Sahni, has designed a new-generation clot buster protein. Although the most used thrombolytic protein, Streptokinase, has generally proven to be as efficacious in large scale field trials on myocardial infarctions as its more expensive counterparts (Urokinase and tPA), the administration of the drug at clinically effective doses often results in rapid plasminogen activation in the blood stream, leading to the unwanted proteolytic degradation of clotting factors, causing bleeding and other serious side-effects in many patients. Hence, a clot-buster drug with minimal side-effects is highly desirable. This function i.e. the ability to dissolve the pathological blood clot without plasminogen activation through the circulatory system is highly coveted and a premium property in any clot-buster drug of choice. The new clot-buster drug developed at IMTECH is unique in its functions, where the engineered clot buster circulates in an inactive state without activating the blood plasminogen. However, once it encounters and binds to the blood clot, it is activated and thereby generates plasmin in and around the vicinity of the clot, which, in turn, cleaves the fibrin clots without the occurrence of generalized proteolysis, as often happens during SK therapy.

Signing Ceremony of technology licensing agreement between IMTECH, Chandigarh and Nostrum Pharmaceuticals Inc, USA in New Delhi on July 26,2006.
Seen in the picture are : Dr. Naresh Kumar, Head RDPD, CSIR (extreme left); Dr. Girish Sahni, Director, IMTECH (second from left); THE HON'BLE MINISTER for Science and Technology, Shri Kapil Sibal (third from left); Dr. R.A Mashelkar, Director General, CSIR (fourth from left); Mr. Kalra (fifth from left) and Dr. Nirmal Mulye, President, Nostrum Pharmaceuticals Inc, USA (sixth from left) during the signing of technology licensing agreement

A bio-process for the preparation of the new clot buster has been standardized at laboratory scale. The process begins with culturing, growing and harvesting the organisms, either bacteria or yeast, and purification of biologically active protein of about 90-95% purity.

The technology has been successfully transferred to a US firm (Nostrum Pharmaceuticals Inc., USA). Based on the tie-up made between Nostrum pharmaceuticals, USA, and CSIR (IMTECH), Chandigarh, the new, engineered Clot-buster will be developed and commercialized both in developing countries and in other markets worldwide.

Recombinant Staphylokinase

A recombinant strain and a high-efficiency facile two step protocol for purification of staphylokinase has been developed by IMTECH scientists for high level intracellular production of Staphylokinase in E. coli.

The complete technological package for the production for recombinant staphylokinase has been transferred to M/S Strides Arcolab Ltd., Bangalore. The licensee is working on development of the commercial scale process with IMTECH's advice.

Know-how for the production of alpha-amylase and alkaline protease was transferred to Celestial Labs, Hyderabad.

IMTECH's idyllic campus

Public Functions & Official Orations

- The Institute of Microbial Technology celebrated the **Technology Day** on May 11, 2005 in its campus by organizing several programmes for the general public and studentÝ. The Institute had declared this day as an 'Open day' and a number of students, teachers and lay visitors visited the Institute to acquaint themselves about the ongoing work. The Institute organized a public lecture by Dr. P.K. Ghosh, President, Biocare SBU, Cadila Pharmaceuticals Ltd., Ahmedabad on the topic **“Planning by Indian Biotech R&D Institutions: Post-2005 Era”.**

- The Institute celebrated the **CSIR Foundation Day** on September 26, 2005. On this day the general public was sensitized on the advancements which have taken place in the upcoming area of Biotechnology. On this day the Institute remained open to visitors from 10 am to 12.30 pm. Highlight of the day was a special lecture by Dr. Kanury V.S. Rao, Head, Immunology Group, International Centre for Genetic Engineering and Biotechnology, New Delhi on the topic **“The Dynamics of Receptor-Mediated Signaling: Elegance in Chaos”.**

- **IMTECH Foundation Day Lecture** was delivered by Dr. R.A. Mashelkar, Director General, Council of Scientific & Industrial Research, New Delhi on January 25, 2006 on the topic "**Making High Technology Work for the Poor**".

- To commemorate **CSIR Foundation Day**, the Institute organized a special lecture on September 26, 2006 which was delivered by Prof. G. Padmanaban, Former Director & Honorary Professor, Deptt. of Biochemistry, Indian Institute of Science, Bangalore on the topic “**Drugs and Drug Targets against Malaria: Old and New”.**

Participants of the workshop on Bioinformatics Resources on miRNA and siRNA

- The seventh **Prof. B.K. Bachhawat Memorial Lecture** was delivered on Dec. 19, 2006 by Prof. M. Vijayan, Distinguished Professor, Indian Institute of Science, Bangalore on the topic "**Structural Diversity and Carbohydrate Specificity of Plant Lectins**".

- The Institute celebrated its 23rd **Foundation Day** on January 24, 2007. The Foundation Day Lecture was delivered by Prof. H.S. Savithri, Deptt. of Biochemistry, Indian Institute of Science, Bangalore on the topic **"Studies in Protein Science: Two Decades of Fun and Adventure".**

The BSL - 3 facility being readied for inauguration

CSIR Finance Officers meet

Honoring Prof. H.S. Savithri before the IMTECH Foundation Day Lecture

Honoring Prof. M. Vijayan before the Prof. B.K. Bachhawat Memorial Lecture

Honoring Dr. V.C. Vora before the IMTECH Foundation Day Celebrations

Honoring Dr. Kanury S. Rao after the CSIR Foundation Day Lecture

Honoring Prof. Asis Datta before the IMTECH Foundation Day Celebrations

Honoring Dr. B.N. Ganguly after the CPYLS Programme

Dr. R. A. Mashelkar releasing Dr. V.C. Vora's
Book on "The Making of IMTECH"

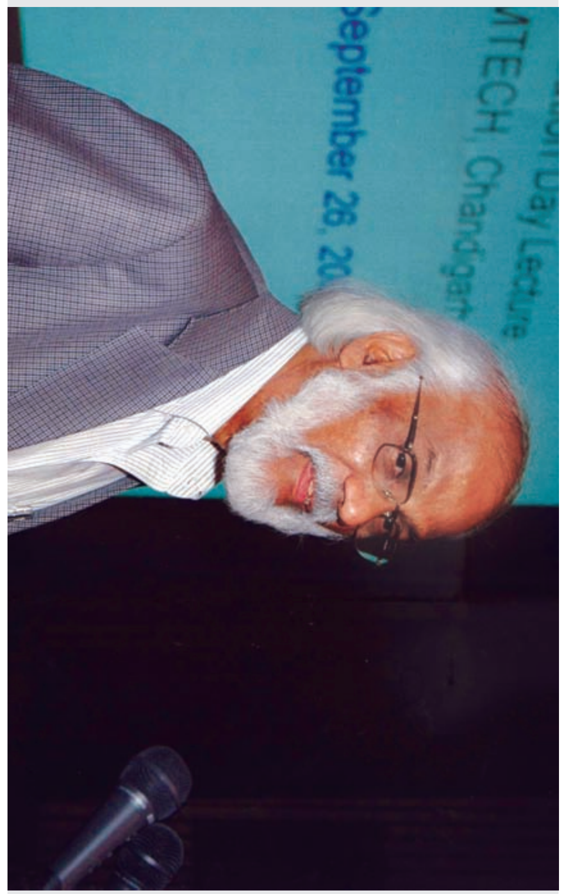
Dr. R. A. Mashelkar delivering the
IMTECH Foundation Day Lecture

Honoring Dr. N.K. Ganguly
after the Technology Day Celebrations

Prof. M. Vijayan delivering the
Prof. B.K. Bachhawat Memorial Lecture.

Prof. Courvalin, Scientist,
Institute Pasteur, Paris, speaking to the
IMTECH audience.

Prof. G. Padmanaban delivering the
CSIR Foundation Day Lecture



Prof. K.K. Talwar speaking to the IMTECH audience on CSIR Foundation Day Celebrations

Prof. H.S. Savithri delivering the IMTECH Foundation Day Lecture

Dr. P.K. Ghosh speaking at the Technology Day Celebrations

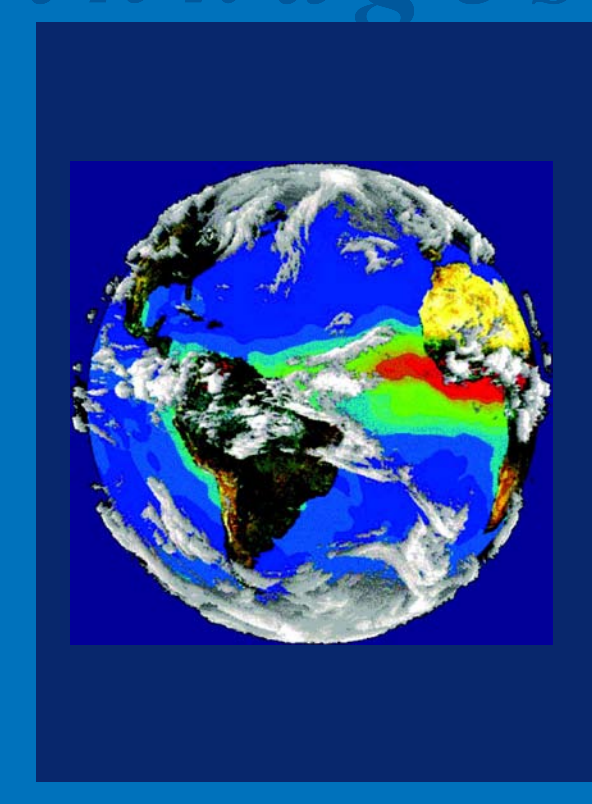
A view of the audience during IMTECH's Foundation Day Lecture

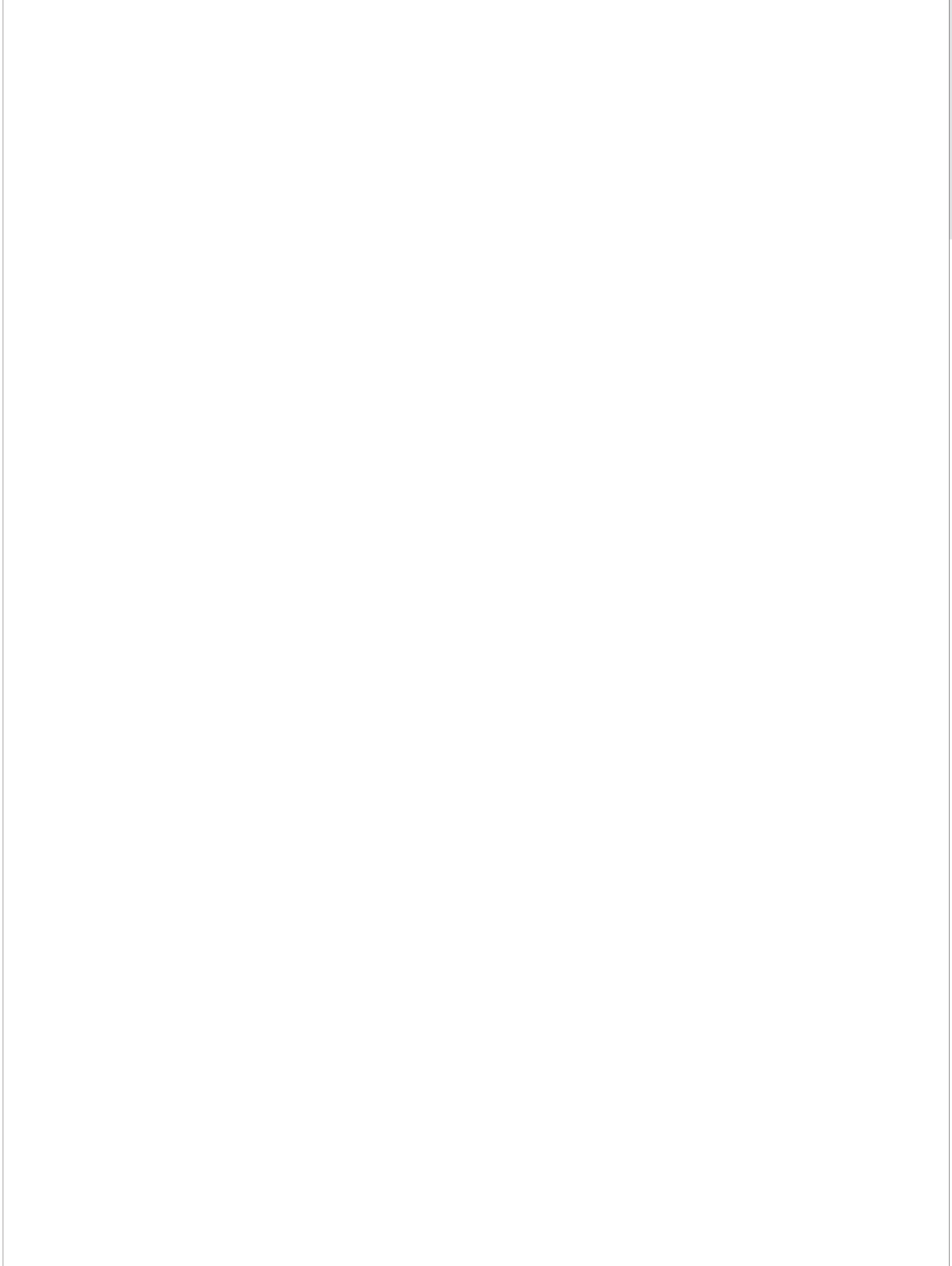
Dr. R.A. Mashelkar delivering the IMTECH Foundation Day Lecture

Participants of the Finance Officers meet

External Linkages

External Linkages





External Linkages

As in the previous years IMTECH scientists maintained a close cooperation with universities, other academic and r&d institutions and industries, both for the development of interactive programmes and also manpower training. Several of them were successful in obtaining financial support from various sources for the following projects:

- Mechanism of action of Anaphase promoting complex in gene silencing fission yeast (Funded by Deptt. of Science and Technology).
- Chemo-toxic and biodegradation of nitroaromatic compounds (Ministry of Environment and Forests).
- Establishment of Indian Bioresource Information Network (IBIN) (Funded by Deptt. of Biotechnology).
- Identification and investigation into the components of a recently identified 'alternate pathway' for glutathione degradation in the yeast *Saccharomyces cerevisiae*. (Funded by Deptt. of Science and Technology).
- Second-generation cytokine biogenerics (Funded by Deptt. of Biotechnology).
- Second-generation thrombolytic biogenerics (Funded by Deptt. of Biotechnology).
- Therapeutic humanized monoclonal antibodies (Funded by Deptt. of Biotechnology).
- The design, chemical synthesis and characterization of self-assembling B-turn like motifs (Funded by Deptt. of Science and Technology).
- Identification of potential antigens and antigenic regions for subunit vaccine design (Funded by Deptt. of Biotechnology).
- Potent role of pro-memory cytokines in the protection and generation and sustenance of memory responses in animals immunized with vaccine prepared from macrophages infected with live *M. tuberculosis* (Funded by Indian Council of Medical Research).
- Role of GAPDH as a multifunctional protein in dendritic cells (Funded by Deptt. of Biotechnology).
- An investigation into the presumed rate limiting steps & substrates of glutathione biosynthesis in the yeast *Saccharomyces cerevisiae* (Funded by Deptt. of Biotechnology).

- Comparative analysis of *luxO,* the Quorum Sensing Master Regulator among 01, 0139 and non-01, non-0139 *V. cholerae* strains (Funded by Deptt. of Biotechnology).

- Potent Role of Vaccine prepared from macrophages infected with live bacteria in the protection and generation of long-lasting memory cell against *Mycobacterium tuberculosis* (Funded by Deptt. of Biotechnology).

- Characterization of a novel membrane expressed GAPDH and its role in macrophage function (Funded by Deptt. of Science and Technology).

- Molecular mechanism of *Vitreoscilla* hemoglobin (VHb) function: Identification of cellular partner of VHb and studies on protein-protein interaction to explore novel function(s) of bacterial hemoglobins (Funded by Deptt. of Science and Technology).

- Structural analysis of FAD synthetase from yeast: Potential antifungal target for rational drug design (Funded by Deptt. of Science and Technology).

- Deciphering and Studies of Redox Network of *Mycobacterium Tuberculosis* H37Rv (Funded by Deptt. of Biotechnology).

- Studies on molecular mechanism of protein-protein interactions in hemoglobins of *Mybacterium tuberculosis:* Characterization of novel interacting partners of mycobacterial hemoglobins and their evaluation as potential drug targets (Funded by Deptt. of Biotechnology).

- Studies on peptide deformylase enzyme for development of a screening system to identify mycobacteria specific inhibitors (Funded by Deptt. of Biotechnology).

- Development of technology for recombinant staphylokinase (Licenced to Strides Arcolab Ltd., Bangalore).

- Characterization of the pathogen infecting the silkworm insect (Funded by Directorate of Sericulture, Uttaranchal).

- Development of technology for clot-specific streptokinase (Licenced to Nostrum Pharmaceuticals Inc., USA).

- Comparative studies on soil ecosystems in Bt and non-Bt corn crops (Funded by Monsanto India Ltd., Mumbai).

NETWORKED PROJECTS

During the period under report the Institute worked on the following ongoing networked programmes by CSIR:

- COR0023: Discovery, development and commercialization of new bioactive and traditional preparations

- COR0018: Advanced facility for safety evaluation of genetically engineered / modified drugs
- COR0011: Infectious disease handling, storage and research facilities
- SMM002: Exploration & exploitation of microbial wealth of India for novel compounds & biotransformation processes
- SMM003: Molecular biology of selected pathogens for developing drug targets
- CMM0016: Predictive medicine using repeat and single nucleotide polymorphisms
- CMM0017: Drug target development using in-silico biology
- CMM0018: Animals models and substitute technologies
- CMM0021: Technology standardisation of bioresources for and from leather

NMITLI Projects

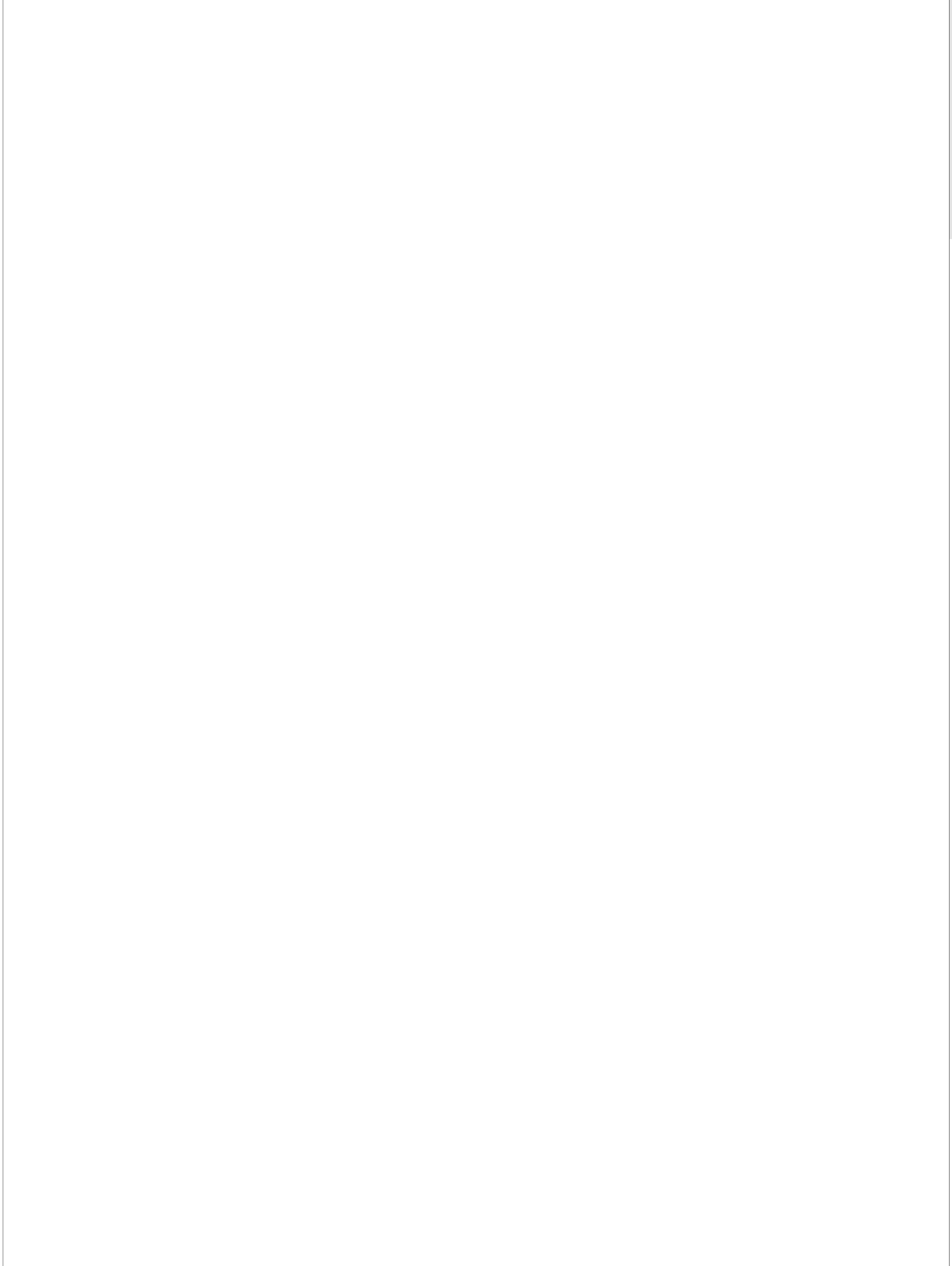
During the period under report the Institute worked on the following ongoing NMITLI projects sanctioned by CSIR:

- Biotechnology for leather: Towards cleaner processing (Phase II)
- Development of novel fungicides

View of the BSL - 3 Facility

Lectures

Lectures



Lectures Delivered/Papers Presented

NAME	DATE	TOPIC	PLACE
G.S. Prasad	April 17, 2005	Role of MTCC in Conservation of Indian Microflora	International Conference of Microbial Diversity, Delhi University, Delhi
A.K. Bachhawat	April 18, 2005	Discovering Missing Links in Glutathione Metabolism : The Awesome Power of Yeast Genetics	Thapar Institute, Patiala
J.N. Agrewala	May 19-21, 2005	Regulatory role of M. tuberculosis Infected Macrophages in Tuberculosis	International Center for Genetic Engineering & Biotechnology, New Delhi
C.R. Suri	August 4, 2005	Immunosensors Based Monitoring	National workshop on Polymeric Sensors, Hindustan College of Engg. & Tech, Mathura
J.N. Agrewala	September 10-15,2005	Distinct Role of CD80 and CD86 in the Regulation of the Activation of B cell and B cell Lymphoma	First International Congress of Biochemistry & Molecular Biology, Tarbiat Modares University, Tehran, Iran
A.K. Bachhawat	September 24-26,2005	Regulation of the Yeast Glutathione Transporter, HGT1	Small Molecule Yeast Transporter Meeting of Europe, New Delhi
Jagmohan Singh	September 28, 2005	Understanding How Prions Cut the Silencing in Fission Yeast	Indian Institute of Science, Bangalore
A.K. Bachhawat	September 28-30,2005	An Alternative Pathway for Glutathione Degradation in the Yeast Saccharomyces Cerevisiae	Biology of Yeasts, Indian Institute of Science, Bangalore
Neeraj Khatri	October 7, 2005	1) Genetic Monitoring of Laboratory Animals and 2) Environment Enrichment for Laboratory Animals	Hisar Management and Healthcare of Laboratory animals, Hisar
Swaranjit Singh	October 7, 2005	Application and Role of Biosurfactants in Bioremediation	Kobe University, Japan
J.N. Agrewala	November 12-14,2005	Potent Role of Infected Macrophages in Eliciting Effective Immune Response Against M. tuberculosis	24th Biennial Conference of the Indian Association of Leprologists at Central JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra
Pradip Sen	November 24-27, 2005	Role of MerTK in Apoptotic cell Mediated Regulation of DC APC Function	PGIMER, Chandigarh

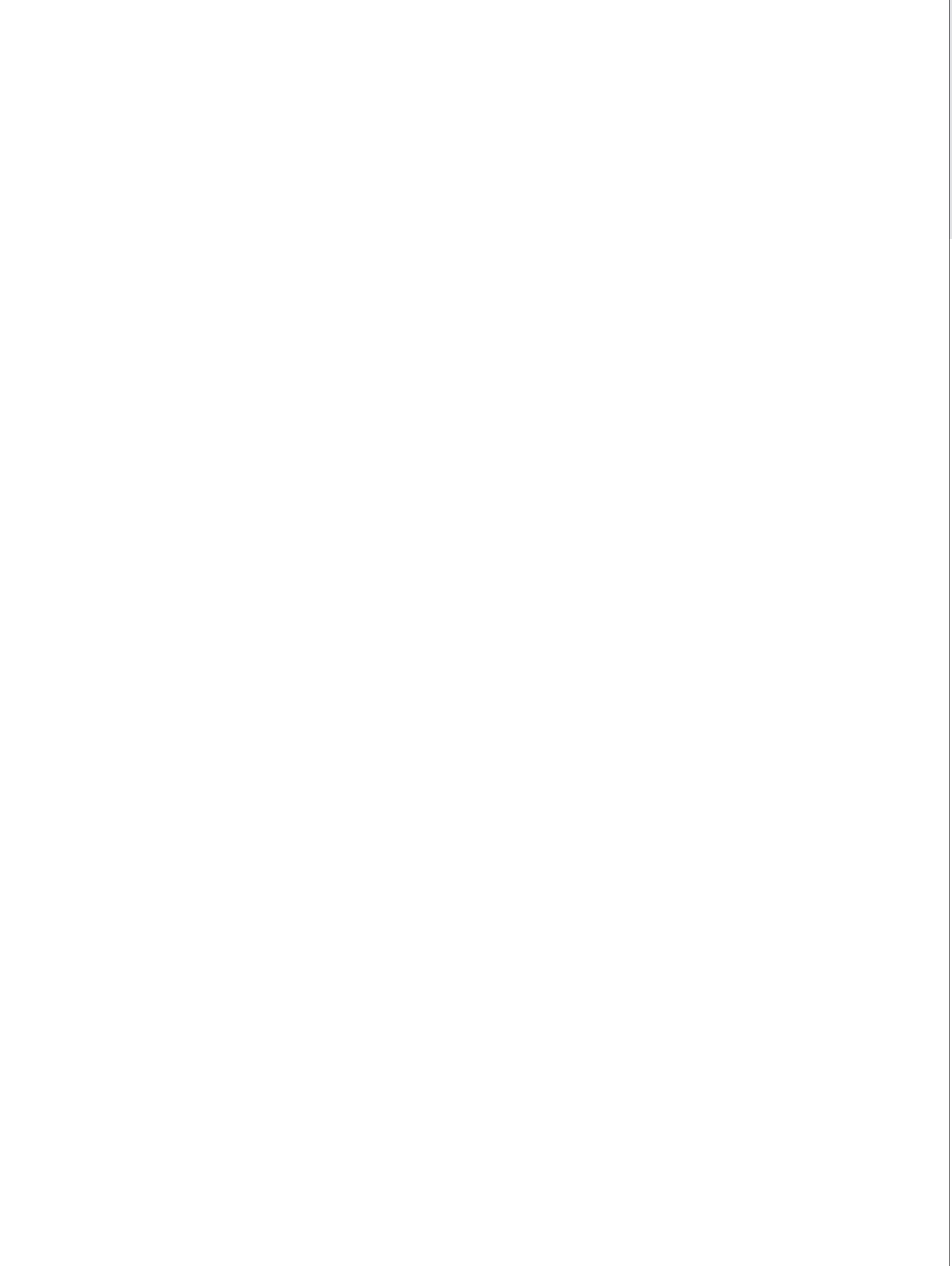
D.K. Sahoo	November 24, 2005	Perspective in Microbial and Food Technology	MCMDAV Collage, Chandigarh
J.N. Agrewala	November 28, 2005	Memory T cells and Antigen Presentation	12th Annual Symposium of Ranbaxy Science Foundation on "Immunotherapeutics & Disease Management: Recent Advances" at All India Institute of Medical Sciences, New Delhi
A.K. Bachhawat	November 25-29,2005	Glutathione Transporters of Fungi	Indian Society for Mycology and Plant Pathology, Udaipur
G.S. Prasad	December 9, 2005	Sequence Analysis of Internal Transcribed Spacer Region for Phylogenetic Assessment of Yeasts	Association of Microbiologists of India, Osmania University, Hyderabad
J.N. Agrewala	December 23, 2005	Influence of Costimulatory Molecules in Modulating the Activity of Antigen Presenting Cells	3rd conference of the Biotechnology Society of India "BIOTECH-2005". The Heritage Village, Manesar, Gurgaon
J.N. Agrewala	December 28, 2005	Deferential Effect of CD80 and CD86 in Regulating the Activity of B cells and B cell Lymphomas	National AIDS Research Institute, Pune
Pradip Sen	January, 2006	Role of MerTK in Apoptotic Cell Mediated Immunoregulation of Dendritic Cell Function	Molecular Immunology Forum, Bhubaneswar
C.R. Suri	January 21, 2006	Micromechanical Catilever Based Immunosensors	National Workshop on Sensors & Instrumentations, CFTRI, Mysore
D. Ananthapadmanaban	February 2-3, 2006	Fungal Resources of Biological and Medical Importance-The World Scenario	Sri. Bhagwan Mahaveer Jain Collage, Bangalore
K. Ganesan	February 22, 2006	Microarrays and their Applications, Advanced Applications of Biotechnology	DAV Collage Chandigarh
J.N. Agrewala	March 1-5, 2006	Migratory Patterns of Naïve, Effectors and Memory T-Helper Cells	8th Meeting of Federation of Immunological Societies of Asia-Oceania (FIMSA). All India Institute of Medical Sciences, New Delhi
C.R. Suri	March 6, 2006	Fluorescence Based Immunodetection.	Management Development Program on Analytical Instruments, CSIO, Chandigarh
J.N. Agrewala	March 6-8, 2006	Bi-directional Costimulation of B Cells	3rd Indo-Australian Conference on Biotechnology. Centre for DNA-Fingerprinting and Diagnostics, Hyderabad
S. Karthikeyan	October 11, 2006	Introduction to Protein Structures by X-Ray Crystallography:	National Dairy Research Institute, Karnal

		Modeling and Refinement of Biomolecular Structures Using CCP4 Suite	
K.L. Dikshit	October 19, 2006	Gene Expression and Regulation in Escherichia Coli	Center of Biotechnology, Banaras Hindu University
Girish Sahni	September 16, 2006	Art and Science of Writing Grant Proposals	CME cum workshop on Drafting Skills in Medical Research at Govt. Medical College, Chandigarh
P.K. Chakraborti	November 9, 2006	Peptide deformylase from Mycobacterium tuberculosis: Can it Achieve the Status of a Drug Target?	Symposium on ‘Tuberculosis Research’ at the 74th meeting of the Society of Biologocal Chemists, Lucknow.
K.L. Dikshit	November 22, 2006	Novel Hemoglobins of MycobacteriumTuberculosis : Role in Cellular Metabolism and Pathogenicity	Department of Biology, Illinois Institute of Technology, Chicago, USA
Sekhar Majumdar	December 4-6, 2006	Differential Expression of NF- B in Human Monocytic Leukemic Cell Line THP-1 Determines the Apoptotic Fate of Mycobacteria Infected Cells	International Centre for Genetic Engineering and Biotechnology, New Delhi
Girish Sahni	December 11-12, 2006	New Trends in Life Sciences	National Seminar on New Trends in Life Sciences at Punjab University, Chandigarh
Jagmohan Singh	December 11-13, 2006	Role of Anaphse Promoting Complex in Gene Silencing in Fission Yeast	Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore
A.K. Bachhawat	December, 2006	Glutathione Transporters: The Importance of Cysteine	Society for Biological Chemists, New Delhi
D. Ananthapad-manaban	December 28-29, 2006	Message from the MTCC, Chandigarh	J.J. College, Pudukkottai, Tamilnadu
A.K. Bachhawat	January 4-8, 2007	An Alternative Pathway for Glutathione Degradation in the Yeast Saccharomyces Cerevisiae	Indian Science Congress Association Meeting, Annamalai
D.K. Sahoo	January 7, 2007	Bioprocessing of plasmid DNA for therapeutic applications	National Institute of Pharmaceutical Education and Research, Mohali
Swaranjit Singh	January 12, 2007	Biodiversity of Microbial Surfactants	Banaras Hindu University, Varanasi
G.C. Varshney	February 3, 2007	Therapeutic Monoclonal Antibodies: Search for Novel Targets in Infectious Diseases, Particularly in Malaria and Tuberculosis	“Recent Advances in Biotechnology” Symposium held in GGDSD College Chandigarh

G.C. Varshney	February , 2007	The Hybridoma Revolution: An Emerging Market for Therapeutic Human Monoclonal Antibodies	Refresher Course Workshop held in PGIMER, Chandigarh
J.N. Agrewala	February 15, 2007	Unique Ability of Activated CD4+ T Cells but not Rested Effectors to Migrate to Non-Lymphoid Sites in the Absence of Inflammation	Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh
Swaranjit Singh	February 20, 2007	Bioremediation of Xenobiotics	Kanpur University
P.K. Chakraborti	February 22, 2007	Cross-talk Between Signaling Pathways	Refresher course on 'Biotechnology for Postgraduate Medical Faculty' at PGIMER, Chandigarh
R. Kishore	February 23-25,2007	-Alanine: A Plausible Conformational Constraint	First Indian Peptide Symposium, Hyderabad
Girish Sahni	February 28, 2007	Exploitation of Microbial Diversity for Biotechnological Benefits	Institute of Engineering and Biotechnology Sahauran, Kharar, Mohali
Manoj Raje	February 28, 2007	Fluorescence Activated Cell Sorting & Cytometery, Confocal Microscopy	PGIMER, Chandigarh
S. Karthikeyan	March 3, 2007	Protein Structure Determination & its Importance in Biomedicine and Biotechnology	PGIMER, Chandigarh
J.N. Agrewala	March 8-10, 2007	Bidirectional Costimulation: A Two Street to Activation	National Conference of Shanti Swarup Bahtnagar Prize Winners. Devi Ahilya Vishwavidyalaya, Indore
Girish Sahni	March 14, 2007	Harvesting New Functions from 'Old' Protein Scaffolds: Opportunities for Proteomics era	Workshop held in National Institute of Pharmaceutical Education and Research, Mohali
G.C. Varshney	March 22, 2007	Prevention and Cure of Important Infectious Diseases	Snow and Avalanche Study Establishment Research and Development Center, DRDO, Chandigarh.
A.K. Bachhawat	March 29, 2007	An Alternative Pathway for Glutathione Degradation in the Yeast *Saccharomyces cerevisiae*	Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore

Distinguished Visitors

Distinguished Visitors



Distinguished Visitors

DATE	NAME
April 15, 2005	Prof. Gotthard Kunze Head, Yeast Genetics Institute of Plant Genetics and Crop Plant Research Gatersleben, Germany
May 11, 2005	Dr. P.K. Ghosh President, Biocare SBU, Cadila Pharmaceuticals Ltd., Ahmedabad
June 24, 2005	Prof. Ashok K. Srivastava Dept. of Biochemical Engg. & Biotechnology, Indian Institute of Technology, New Delhi
June 28, 2005	Prof. Sudha Bhattacharya School of Life Sciences Jawaharlal Nehru University, New Delhi
July 6, 2005	Dr. Pallavi Kshetrapal Centre for Cellular & Molecular Biology, Hyderabad
July 25, 2005	Dr. Satish Raina University of Geneva, Switzerland
August 4, 2005	Dr. Anil Gulati President, Chicago Labs Inc., Chicago, USA
August 9, 2005	Dr. D.K. Adhikari Indian Institute of Petroleum, Dehradun
August 9, 2005	Dr. B.N. Ganguly Consultant, IMTECH, Chandigarh
September 26, 2005	Dr. Kanury V.S. Rao Head, Immunology Group, International Centre for Genetic Engineering and Biotechnology, New Delhi
September 29, 2005	Dr. Samik Nanda Biotechnology Research Center, Toyama Prefectural University, Japan
October 6, 2005	Dr. Hans-Peter E. Kohler EAWAG, Switzerland
October 14, 2005	Dr. Bishwajit Kundu Asst. Prof. Dept. of Biochemical Engg. & Biotech., Indian Institute if Technology, Delhi
October 18, 2005	Dr. Utpal Tatu Indian Institute of Science, Bangalore

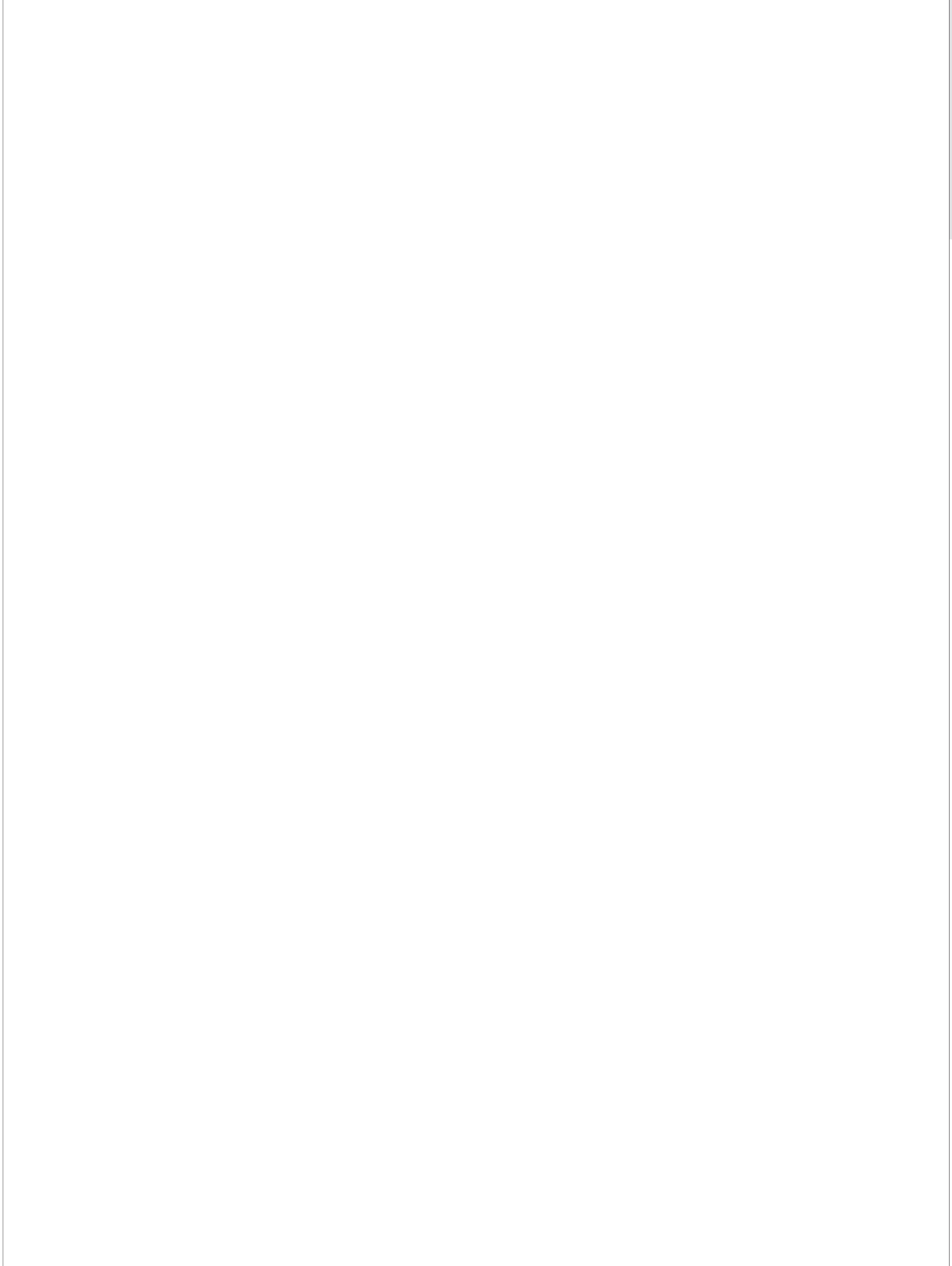
October 20, 2005	Prof. Surinder K Bata Department of Biochemistry and Molecular Biology, College of Medicine, Eppley Cancer Institute UNMC, Omaha, USA
October 24, 2005	Prof. Desirazu N. Rao Department of Biochemistry, Indian Institute of Science
October 28, 2005	Dr. D. P. Kasbekar Centre for Cellular & Molecular Biology, Hyderabad
November 9, 2005	Dr. Maneesh Jain UNMC, Omaha, USA
November 10, 2005	Dr. Nitya Nand Ex-Director, Central Drug Research Institute, Lucknow
November 24, 2005	Dr. Syamal Roy Indian Institute of Chemical Technology, Calcutta
December 13, 2005	Dr. Mukund V. Deshpande National Chemical Laboratory, Pune
January 23, 2006	Dr. Deba Prasad Nayak National Chemical Laboratory, Pune
January 25, 2006	Dr. R.A. Mashelkar Director General, Council of Scientific & Industrial Research, New Delhi
January 27, 2006	Dr. Sourav Mukherjee Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, USA
February 17, 2006	Dr. Deepak Nihalani Dept. Internal Medicine, University of Michigan, USA
February 18, 2006	Mr. Ian Dean Groman Consulting, South Africa
February 24, 2006	Dr. Vishwajeet Puri Asstt. Prof., University of Massachusetts Medical School Worcester, MA, USA
March 1, 2006	Dr. R.R. Hirwani Head, Unit for Research and Development of Information Products, Pune
March 14, 2006	Prof. Ben C. Stark Dept of Biological, Chemical & Physical Sciences Illinois Institute of Technology Chicago, USA
March 17, 2006	Prof. Surinder K. Batra Dept of Biochemistry and Molecular Biology College of Medicine, Eppley Cancer Institute UNMC, Omaha, USA
March 24, 2006	Dr. S. Pajni Underwood NCI-NIH, Frederick, USA

March 27, 2006	Prof. James Gomes Dept. of Biochemical Engineering & Biotechnology Indian Institute of Technology New Delhi
March 28, 2006	Prof. K. L. Chopra Ex-Director, Indian Institute of Technology, Kharagpur
April 05, 2006	Dr. Parikshit Bansal Assistant Professor, Intellectual Property Management National Institute of Pharmaceutical Education & Research, Mohali
June 9, 2006	Dr. Shantanu Sengupta Institute of Genomics and Integrative Biology, New Delhi
June 19, 2006	Dr. Sujit K. Sikdar Molecular Biophysics Unit, Indian Institute of Science Bangalore
July 27, 2006	Dr. Ranjan Sen Staff Scientist Center For DNA Fingerprinting and Diagnostics, Hyderabad
Aug 02, 2006	Dr. Vinay Vyas Bioprocess Development Scientist SAIC, Frederick
Aug 24, 2006	Dr. S. Kumaran Department of Biochemistry and Molecular Biophysics Washington University School of Medicine Saint Louis, MO 63110, USA
September 25, 2006	Dr. Heather Holeman Principal Investigator, Functional Genomics Group, Sigma-Aldrich, USA
September 26, 2006	Prof. G. Padmanaban Former Director & Honorary Professor, Deptt. of Biochemistry, Indian Institute of Science, Bangalore
October 05, 2006	Dr. Kasthuri Venkatesswaran Principal Scientist, Jet Propalsion Laboratory California Institute of Technology, Pasadena, USA
October 7, 2006	Dr. Raj Bawa Virginia, New York, USA
October 31, 2006	Prof. Serge Delrot Director, Institute of Vine and Wine Sciences, Bordeaux, France
November 6, 2006	Dr. Ipsita Roy Department of Biotechnology National Institute of Pharmaceutical Education & Research, Mohali
November 13, 2006	Prof. Courvalin Scientist, Institute Pasteur, Paris
December 19, 2006	Prof. M. Vijayan Distinguished Professor, Indian Institute of Science, Bangalore

December 26, 2006	Prof. Umesh Varshney Department of Microbiology & Cell Biology, Indian Institute of Science, Bangalore.
January 11, 2007	Prof. V. Nagaraja Department of Microbiology and Cell Biology. Indian Institute of Science, Bangalore
January 11, 2007	Prof. M. A. Vijayalakshmi Vellore Institute of Technology, Vellore, Tamil Nadu
January 16, 2007	Mr. W. M. Busing Life Sciences Product Manager TEM, M/s FEI Europe B. V. Netherlands
January 24, 2007	Prof. H.S. Savithri Deptt. of Biochemistry, Indian Institute of Science, Bangalore
February 08, 2007	Dr. Debasish Bhattacharyya Indian Institute of Chemical Biology, Kolkata
February 14, 2007	Prof. D. Balasubramanian Director Research LV Prasad Institute, Banjara Hills, Hyderabad
February 20, 2007	Dr. K.K. Pandey Institute of Molecular Virology St. Louis University Health Science Center St. Louis, MO, USA
February 20, 2007	Dr. Pascal Bailon Bailon Consultants Florham Park, NJ 07932, USA
February 21, 2007	Geoffrey J. Barton, Ph.D Professor of Bioinformatics, University of Dundee, Scotland, UK
March 12, 2007	Dr. Seema Kalra Medical College of Wisconsin Dept of Medicine Milwaukee, Wisconsin, USA

Conferences / Visits Abroad

Conferences / Visits Abroad



Conferences/Seminars attended

Date	Name	Conference/Seminar	Venue
April 16-18, 2005	S. Mayilraj	International Conference of Microbial Diversity: Current Perspective and Potential Applications	Delhi University, New Delhi
April 16-18, 2005	G.S. Prasad	International Conference of Microbial Diversity: Current Perspective and Potential Applications	Delhi University, New Delhi
July 14, 2005	Neeraj Khatri	Innovative solutions for the research with Laboratory animals	NII, New Delhi
July 18-23, 2005	Neeraj Khatri	Induction Training Programme for Scientists	NPL, New Delhi
July 18-23, 2005	Kailash Bhamare	Induction Training Programme for Scientists	NPL, New Delhi
July 18-23, 2005	Manoj Kumar	Induction Training Programme for Scientists	NPL, New Delhi
Sept. 10-15, 2005	J.N. Agrewala	First International Congress of Biochemistry and Molecular Biology	Tarbiat Modares University, Tehran, Iran
Sept. 27-29, 2005	K. Ganesan	International Conference on the Biology of Yeasts	Indian Institute of Science, Bangalore
Sept.27-29, 2005	A.K. Bachhawat	International Conference on the Biology of Yeasts	Indian Institute of Science, Bangalore
Sept.27-29, 2005	Jagmohan Singh	International Conference on the Biology of Yeasts	Indian Institute of Science, Bangalore
Nov. 24-27, 2005	Pradip Sen	32nd Annual Conference Indian Immunology Society	PGI, Chandigarh
Nov. 25-29, 2005	A.K. Bachhawat	Indian Society for Mycology and Plant Pathology	Udaipur
Dec. 9, 2005	G.S. Prasad	Association of Microbiologists of India	Osmania University, Hyderabad
Dec.14-17, 2005	D.K. Sahoo	CHEMCON-2005 and 58th Annual session of Indian Institute of Chemical Engineers	New Delhi
Feb. 22-25, 2006	Manoj Kumar	2nd CSIR-NSFC Workshop on Genome Informatics	IGOB, New Delhi
Mar. 3-4, 2006	Suresh Korpole	International Workshop on Biotechnology of Anaerobic Bacteria and Archaea	Aids Research Institute, Pune
Mar. 7-9, 2006	Dibyendu Sarkar	Golden Jubilee Symposium (International Conference on Chemical Biology)	Indian Institute of Chemical Biology Kolkata
Apr. 11-12, 2006	K. Ganesan	Frontiers of Molecular Biology in the New Millennium	JNU, New Delhi
Aug. 31-Sept.1, 2006	Neeraj Khatri	Training Programme on Good Laboratory Practice and Regulatory Issues	HRDC, Ghaziabad
Sept. 28, 2006	Girish Sahni	4th Conclave on Emerging Technology ‘Emerge Tech 2006’	New Delhi
Oct. 27-28, 2006	S. Karthikeyan	Hitech-Pune Maharashtra-2006	Pune
Nov. 1-2, 2006	Neeraj Khatri	National Seminar on Rational use of Animals in Research	NIN, Hyderabad
Nov. 2-5, 2006	S. Mayilraj	7th Asia Pacific Marine Biotechnology Conference	Cochin, Kerala
Nov. 2-5, 2006	Sony Pandey	7th Asia Pacific Marine Biotechnology Conference	Cochin, Kerala
Nov. 11-12, 2006	Manoj Kumar	INDO-US Symposium on ‘Viral Diagnostics, Epidemiology and Pathogenesis’	AIIMS, New Delhi

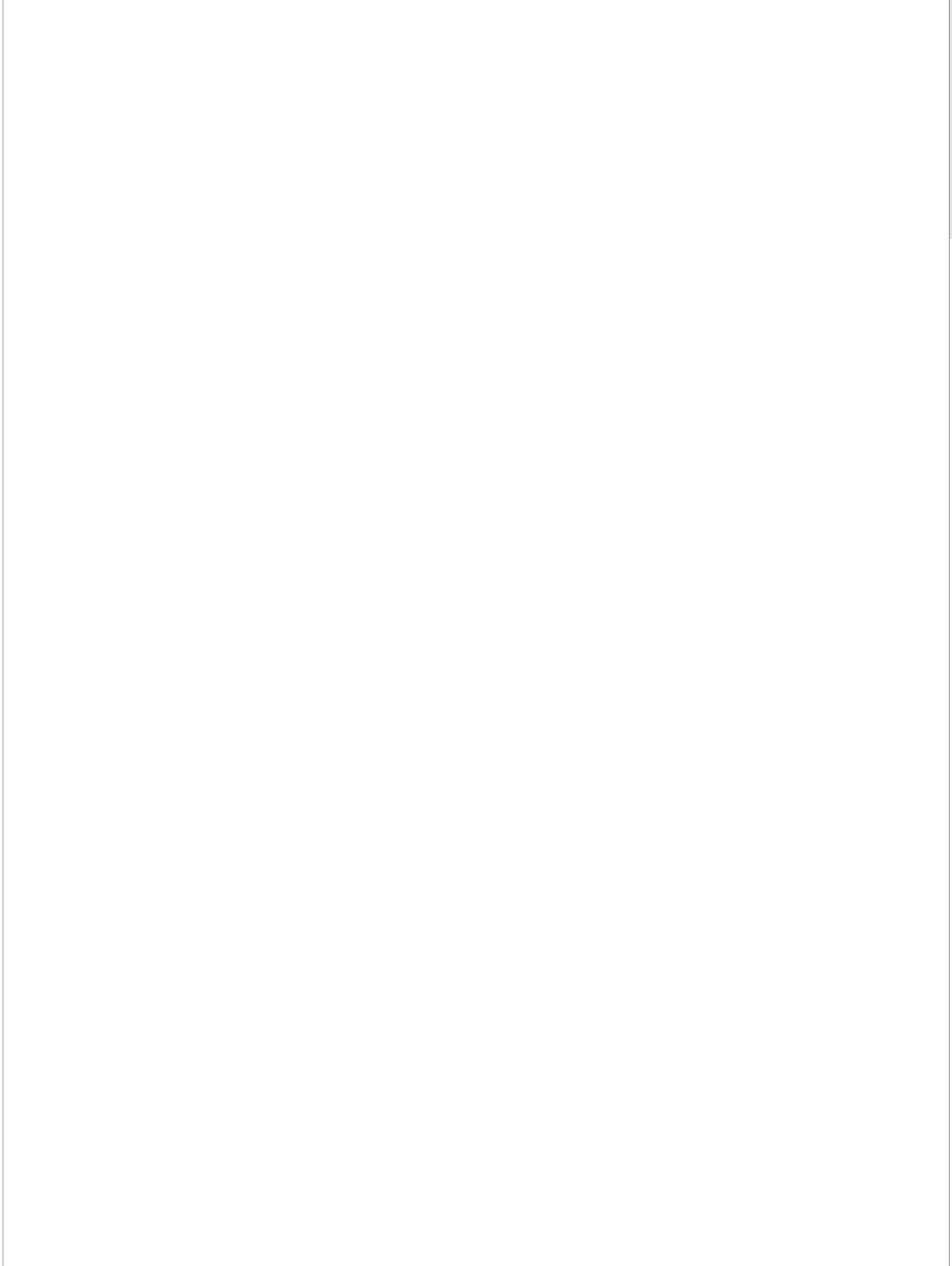
Nov. 13-15, 2006	Manoj Kumar	7th Asia Pacific conference of medical virology	AIIMS, New Delhi
Nov.21-23, 2006	R. Soni	Programme on Project Management Techniques and Practices	HRDC, Ghaziabad
Nov.21-23, 2006	Kailash Bhamare	Programme on Project Management Techniques and Practices	HRDC, Ghaziabad
Nov. 22-24, 2006	Pradip Sen	18th National Conference of Parasitology	IICB, Kolkata
Nov. 26-28, 2006	Girish Sahni	IV Annual Conference of Biotechnology Society of India 'Biotech 2006' Overexpession: Systems and Challenges	Centre for Cellular and Molecular Biology, Hyderabad
Dec. 4-6, 2006	J.N. Agrewala	International Symposium on New Frontiers in Tuberculosis Research	International Centre for Genetic Engineering and Biotechnology, New Delhi
Dec. 4-6, 2006	Sekhar Majumdar	International Symposium on New Frontiers in Tuberculosis Research	International Centre for Genetic Engineering and Biotechnology, New Delhi
Dec. 11-12, 2006	Girish Sahni	National Seminar on New Trends in Life Sciences	Punjab University, Chandigarh
Dec. 11-13, 2006	Jagmohan Singh	Nuclear Architecture: Chromosome – Chromatin Dynamics	JNCASR, Bangalore
Dec. 18-20, 2006	Manoj Kumar	International Conference in Bioinformatics InCoB	New Delhi
Dec. 27-30, 2006	D.K. Sahoo	CHEMCON 2006 and 59th Annual Session of Indian Institute of Chemical Engineers	Bharuch.
Jan. 10-12, 2007	D.K. Sahoo	Short course on 'Cell Culture Technology'	IIT Bombay, Mumbai
Feb. 3-4, 2007	Balvinder Singh	XVIIIth Conference of BTISnet Coordinators	Bioinformatics Centre at State Council of Science & Technology, Gangtok, Sikkim.
Feb. 3-4, 2007	Manoj Kumar	XVIIIth Conference of BTISnet Coordinators	Bioinformatics Centre at State Council of Science & Technology, Gangtok, Sikkim.
Feb. 15-17, 2007	K. Ganesan	Biology of Yeasts and Filamentous Fungi	NCL, Pune
Feb. 15-17, 2007	A.K. Bachhawat	Biology of Yeasts and Filamentous Fungi	NCL, Pune
Feb. 17-21, 2007	J.N. Agrewala	Current trends in Drug Discovery Research	Central Drug Research Institute, Lucknow
Feb. 23-25,2007	S. Karthikeyan	Advances in Structural Biology & Structure Prediction	CCMB, Hyderabad
Feb. 23-25,2007	R. Kishore	First Indian Peptide Symposium	Hyderabad
Mar. 1-2, 2007	G.C. Varshney	Molecular Immunology Forum	Indian Institute of Science, Bangalore
Mar. 7-9, 2007	Sekhar Majumdar	IICB, Golden JUBILEE International Symposium on Chemical Biology	Indian Institute of Chemical Biology, Kolkata
Mar. 22-25, 2007	Girish Sahni	US and European Patent Issues directed to Pharmaceuticals	HRDC, Ghaziabad

Staff Visits Abroad

Name	Period	Place
P. Guptasarma	May 17-28, 2005	Deptt. of Life Sciences, Aalbag, University of Denmark, Denmark
Amit Ghosh	May 25-June 3, 2005	Montreal, Canada
S. Mayilraj	July 21-October 28, 2005	Braunschweig, Germany
R.K. Jain	July 3-8, 2005	Delft, Netherland
D. Ananthapadmanaban	July 23-28, 2005	San Francisco, California, USA
K.L. Dikshit	August 1-September 15, 2005	Illinois Institute of Technology, Chicago, USA
R.K. Jain	August 21-24, 2005	Copenhagen, Denmark
C.R. Suri	September 1- October 15, 2005	North Western University, USA
J.N. Agrewala	September 10-15, 2005	First International Congress of Biochemistry and Molecular Biology, Tarbiat Modares University, Tehran, Iran
Swaranjit Singh	September 10-December 10, 2005	Kobe University, Kobe, Japan
G.P.S. Raghava	October 26-30, 2005	Tokyo, Japan
Girish Sahni	November 7-10, 2005	Washington, Chicago, New Jersey USA
G.P.S. Raghava	March 15-September 15, 2006	Little Rock, USA
C.R. Suri	August 2 – September 1, 2006	EPFL, Lausanne, Switzerland
R.K. Jain	August 19-September 2, 2006	EPFL, Lausanne, Switzerland
K.L. Dikshit	November 1-December 15, 2006	Illinois Institute of Technology, Chicago, USA
R.K. Jain	November 13-15, 2006	Penang, Malaysia

Ph.D. Degrees Awarded

Ph.D. Degrees Awarded



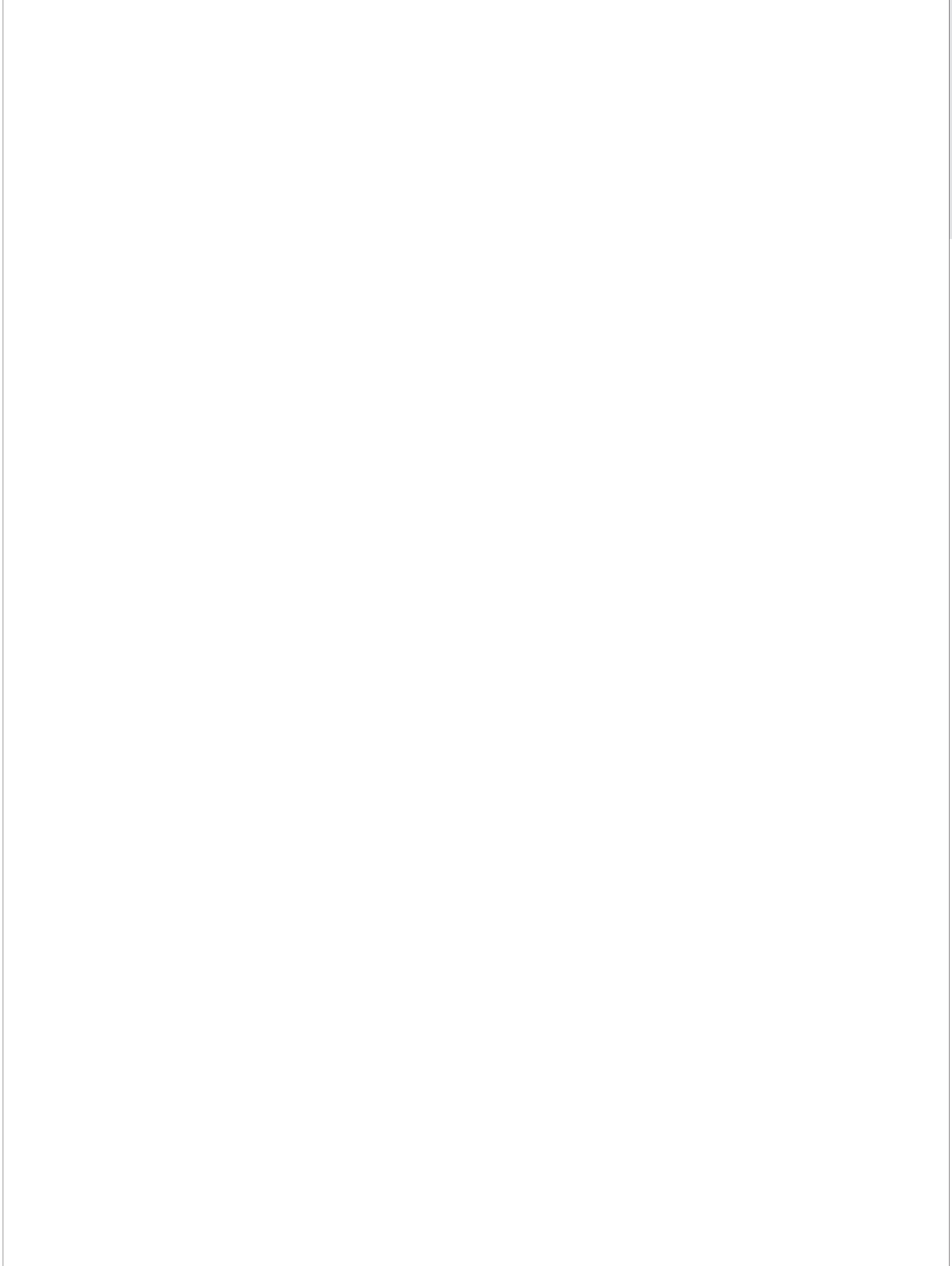
Ph.D. Degrees Awarded

Name of Student	Year	Title of Thesis	Guide
Monika Dahiya	2005	Structure function studies of staphylokinase	Dr. K.L. Dikshit
Harpreet Kaur	2005	Development of bioinformatics tools for predicting of -turns in proteins and their role in bioactive peptides	Dr. G.P.S. Raghava
C. V. Srikanth	2005	Studies on the glutathione transporters of the yeast *Saccharomyces cerevisiae*	Dr. A.K. Bachhawat
Vandana Gambhir	2005	Mycobacteria and infected macrophages: Isolation and characterization of the antigens	Dr. G.C. Varshney
Biju Issac	2005	Prediction of genes and repetitive elements in eukaryotic genome using artificial intelligence techniques.	Dr. G.P.S. Raghava
Saurabh Garg	2005	Structure Function studies of WhiB1 of *Mycobacterium tuberculosis* H37Rv	Dr. Pushpa Agrawal
K. Lakshmipathi	2005	Characterization of integrase gene of the phage PIS 136 and its use as a molecular tool	Dr. Pushpa Agrawal
Debarati Paul	2005	Molecular and biochemical studies on biodegradation of p-nitrophenol and bacterial chemotaxis toward this compound	Dr. R.K. Jain
Pradipta Saha	2005	Bacterial diversity of gorompani warmspring water sample collected from Assam, India	Dr. Tapan Chakrabarti
Amit Sharma	2005	Structural studies of proteolyzed intermediates: Insights into protein folding through complimentary fragments	Dr. K.V. Radhakishan
Jagpreet Singh	2005	Mechanistic studies on plasminogen activation by streptokinase	Dr. Girish Sahni
Shranjot Saini	2005	Interaction of DNA polymerase alfa and Rhp6 with heterochromatin components in *Schizosaccharomyces pombe*	Dr. Jagmohan Singh
Sumit Arora	2005	Studies on the mechanism of DNA replication and RNAi machinery in heterochromatin assembly in fission yeast	Dr. Jagmohan Singh

Rekha Puria	2005	Studies on genes involved in stress tolerance of yeast during ethanolic fermentation	Dr. K. Ganesan
Chitranshu Kumar	2005	Studies on the role of gamma glutamyl transpeptidase in glutathione homeostasis in yeast *Saccharomyces cerevisiae*	Dr. A.K. Bachhawat
Monika Aggarwal	2006	Studies on molecular and biochemical aspects of HAL2 homologue from a halotolerant yeast *Debaryomyces hansenii*	Dr. Alok Mondal
Dwaipayan Ganguly	2006	Studies on Glutathione degradation in the yeast *Saccharomyces Cerevisiae*	Dr. A.K. Bachhawat
Rahul Saxena	2007	Molecular and biochemical characterization of Mycobacterial peptide deformylase	Dr. P.K. Chakraborti
Swati Sharma	2007	Structural-biochemical studies of Protein (mis) folding and aggregation	Dr. P. Guptasarma
Sanjoy Paul	2007	Development of genetic tools to identify genes crucial for virulence in *Candida albicans*	Dr. K. Ganesan
Sudipto Saha	2007	Computer-aided prediction of potential vaccine candidates based on B-cell epitopes	Dr. G P S. Raghava

Institute Committees

Institute Committees



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Dr. K.V.S. Rao Head, Immunology Division International Centre for Genetic Engg. & Biotechnology (ICGEB) Aruna Asaf Ali Marg New Delhi – 110 067	Member

Dr. Kiran Mujumdar Chairman & Managing Director BICON (India) Ltd. 20th KM Hosur Road Electronic City P.O. Bangalore – 560 012.	Member
Shri Vara Prasad Reddy Managing Director Shanta Biotechnics Pvt. Ltd. 3rd Floor, Serene chamber Road No.7, Banjara Hills Hyderabad – 500 034	Member
Dr. Rajesh Kapoor Dept. of Biotechnology CGO Complex, Lodi Road New Delhi – 110 003	Director
Dr. Ch. Mohan Rao Centre for Cellular & Molecular Biology Uppal Road Hyderabad – 500007	Member
Dr. B.S. Srivastava Scientist Central Drug Research Institute Chattar Manzil Place Post Box No. 173 Lucknow – 226 001	DG CSIR's Nominee
Dr. Girish Sahni Director, IMTECH Chandigarh-160 036	Member Secretary

MANAGEMENT COUNCIL

Dr. Girish Sahni Director, IMTECH Chandigarh- 160 036	Chairman
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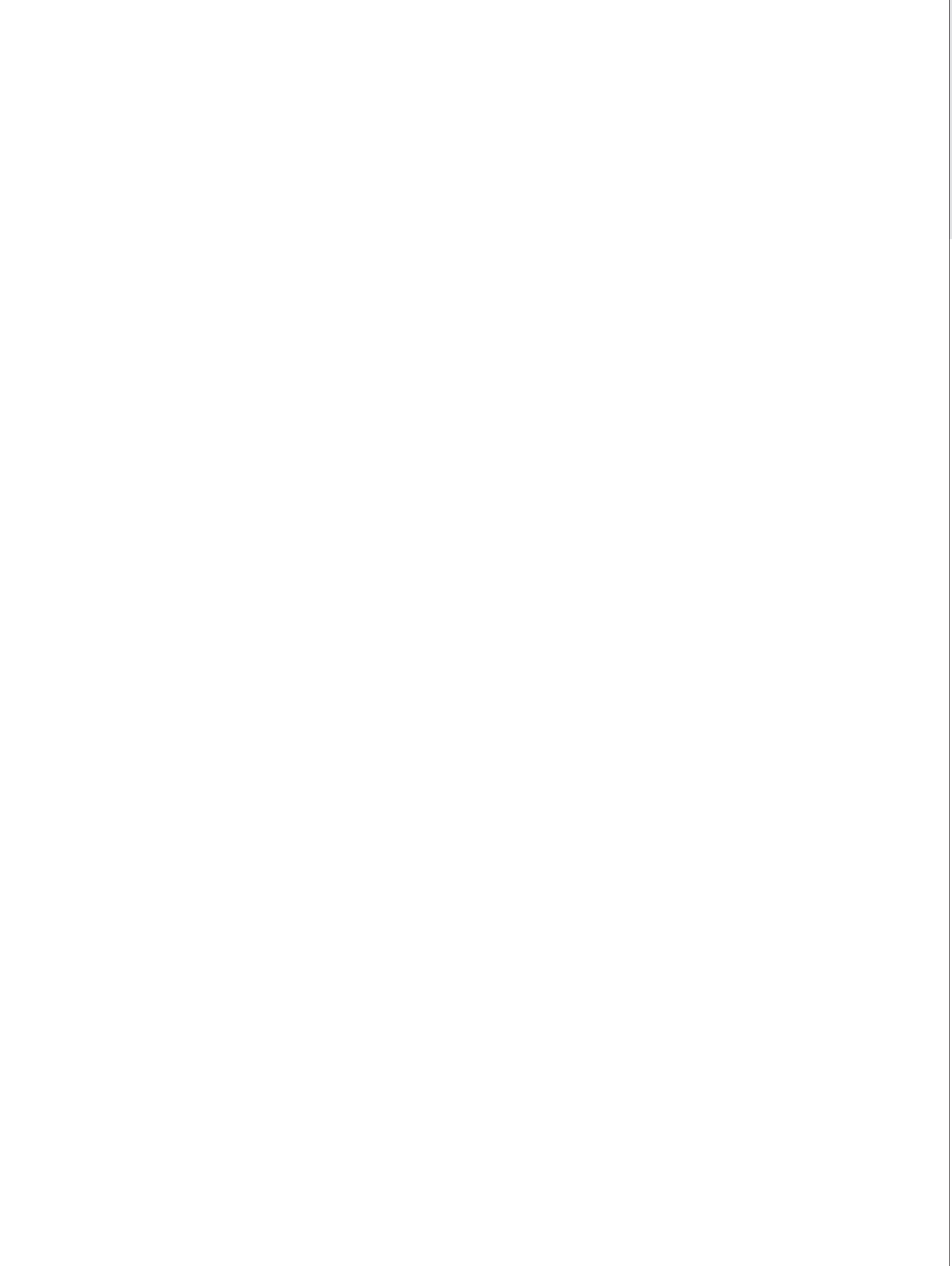
Mr. R. Soni Scientist, IMTECH Chandigarh-160 036	Member
Dr. A.K. Bachhawat Scientist, IMTECH Chandigarh-160 036	Member
Dr. P. Guptasarma Scientist, IMTECH Chandigarh-160 036	Member
Mr. A.K. Goel Member Ex. Engineer, IMTECH Chandigarh-160 036	Member
Mr. Ram Swaroop (till June 23, 2006) Finance & Account Officer IMTECH, Chandigarh-160 036	Member (Ex-Officio)
Mr. Brijesh Kumar (w.e.f. June 24, 2006) Finance & Account Officer IMTECH, Chandigarh-160 036	-do-
Mr. Hari Mohan Controller of Administration IMTECH, Chandigarh-160 036 (till Oct.14, 2005)	Member Secretary
Mr. M.R. Masan Controller of Administration IMTECH, Chandigarh-160 036 (w.e.f. Oct.17, 2005)	Member Secretary

Institute Staff

Institute Staff



Institute Staff

SCIENTIFIC STAFF

Name	Designation
Dr. Girish Sahni	Director
Dr. P.R. Patnaik	Scientist G
Dr. Tapan Chakrabarti	Scientist G
Mr. C.H. Premani	Scientist F
Mr. S.D. Bhatt	Scientist F
Dr. R.S. Jolly	Scientist F
Dr. R.K. Jain	Scientist F
Dr. P.K. Chakraborti	Scientist F
Dr. A.K. Bachhawat	Scientist F
Dr. Kanak Lata Dikshit	Scientist F
Dr. Jagmohan Singh	Scientist F
Dr. G.C. Varshney	Scientist F
Dr. Pushpa Agrawal	Scientist EII
Dr. C.R. Suri	Scientist EII
Dr. Manoj Raje	Scientist EII
Dr. D.A. Padmanaban	Scientist EII
Mr. Kuljeet Singh	Scientist EII
Dr. Swaranjit Singh	Scientist EII
Dr. Alok Mondal	Scientist EII
Dr. G.P.S. Raghava	Scientist EII
Dr. K. Ganesan	Scientist EII
Dr. Raghuvansh Kishore	Scientist EII
Dr. Gandham S. Prasad	Scientist EII
Dr. D.K. Sahoo	Scientist EII
Dr. Purnananda Guptasarma	Scientist EII
Dr. Sekhar Majumdar	Scientist EII
Dr. Javed Naim Agrewala	Scientist EII
Mr. Rajendra Soni	Scientist EI
Dr. Vijay C. Sonawane	Scientist EI
Mr. Mayil Raj Shanmugam	Scientist EI
Mr. G. Rajamohan	Scientist EI
Dr. Karthikeyan Subramanian	Scientist EI
Dr. K. Rajagopal	Scientist EI
Mr. Hemraj S. Nandanwar	Scientist C
Dr. Saumya Raychaudhuri	Scientist C
Dr. Balvinder Singh	Scientist C
Dr. Dibyendu Sarkar	Scientist C
Mr. Anirban Roy Choudhury	Scientist C
Dr. Pradip Sen	Scientist C
Dr. Neeraj Khatri	Scientist B
Dr. Suresh Korpole	Scientist B
Mr. E. Senthel Prasad	Scientist B
Dr. Manoj Kumar	Scientist B
Mr. Bhamare Kailash	Scientist B
Ms. Sony Pandey	Scientist B

ADMINISTRATIVE STAFF

Mr. M.R. Masan	Controller of Administration	Ms. Sudesh Sharma	Assistant (F&A) Gr.I
Mr. K K. Bharat	Store & Purchase Officer	Mr. Gurdeep Singh	Assistant (G) Gr.I
Mr. Brijesh Kumar	Finance & Account Officer	Mr. Raghu Nath	Assistant (G) Gr.I
Mr. J.N. Ahuja	Sr. Security Officer (S.G.)	Mr. Sanjeev Kumar	Assistant (G) Gr.I
Mr. Parag Saxena	Section Officer (G)	Mr. Raju Bansal	Assistant (F & A) Gr.I
Mr. S.D. Rishi	Section Officer (G)	Ms. Rani Devi	Assistant (F & A)Gr.II
Mr. Jai Prakash	Section Officer (F & A)	Mr. Bir Singh	Assistant (S & P) Gr.II
Mr. S.P. Singh	Section Officer (S & P)	Mr. Arun Kumar	Assistant (G) Gr.II
Mr. Sudhir Kumar	Private Secretary	Mr. Balvinder Singh	Assistant (G) Gr.II
Mr. R.S. Puri	Assistant (S & P) Gr.I	Ms. Kuldeep Kaur	Assistant (G) Gr.II
Mr. Kamal Kumar	Assistant (S & P) Gr.I	Mr. Rajinder Nautiyal	Assistant (G) Gr.III (Hindi)
Mr. Byomkesh Pandey	Astt. Gr-I (F&A)	Mr. Ashish Sharma	Assistant (G) Gr. III
Ms. Bimla	Assistant (F & A) Gr.I	Ms. Tajinder Kaur	Assistant (S & P) Gr. III
Ms. Shashi Batra	Sr. Stenographer	Mr. M.M. Parmanik	Guest Room Attendant
Ms. Satya Gupta	Sr. Stenographer	Mr. Gian Singh	Security Guard
Mr. Ashok Kumar	Jr. Stenographer (ACPS)	Mr. Dharminder Kumar	Staff-car Driver (NT)
Ms. Baljit Kaur	Jr. Stenographer (ACPS)	Mr. R.N. Manjhi	Peon
Mr. Sushil Kumar	Jr. Stenographer (ACPS)	Mr. Ralla Ram Dogra	Peon
Ms. Kavita Kumari	Jr. Stenographer	Mr. Rintu Bhattacharjee	Peon
Ms. Manoj Rani	Jr. Stenographer	Mr. Nand Lal	Peon
Mr. Ravinder Singh	Jr. Stenographer	Mr. Malkhan Singh	Wash Boy
Mr. Praveen Kumar	Jr. Stenographer	Mr. Suresh	Safaiwala
Ms. Navneet Anand	Sr. Translator Hindi	Mr. Braham Chand	Safaiwala
Mr. Parshotam Das	Assistant (G) Gr.I	Mr. Raj Pal	Safaiwala
Mr. Bhagirath	Assistant (G) Gr.I	Ms. Sheela Devi	Safaiwala
Mr. Gopal Krishan	Assistant (G) Gr.I		

TECHNICAL STAFF

Mr. Atul Kumar Goel	Superintendent Engineer
Dr. Lata Verma	Technical Officer 'B'
Mr. S.S. Bawa	Technical Officer 'B'
Mr. Rajendra Dass	Technical Officer 'B'
Mr. Dhan Prakash	Technical Officer 'A'
Mrs. Paramjeet Kaur	Technical Officer 'A'
Mr. Davinder Singh	Technical Officer 'A'
Dr. Raj Kumar Mehta	Technical Officer 'A'
Mr. Ram Nath Bansal	Library Officer
Mr. Harbhupinder Kumar	Asstt. Engineer
Mr. Ravinder K. Kanojia	Technical Officer 'A'
Mr. Samir Kumar Nath	Sr. Technical Assistant
Mr. Sagar N. Nimsadkar	Sr. Technical Assistant
Dr. K.P.S. Sengar	Library Assistant
Mr. Ashwani Kumar	Junior Eng. (Electrical)
Mr. S. Muthukrishnan	Technical Assistant
Mr. Harvinder	Technical Assistant
Mr. Dipak Bhatt	Technical Assistant
Mr. Chandrashekhar	Technical Assistant
Mr. Davinder Singh	Lab. Supervisor
Mr. Bhimi Ram	Library Assistant
Mr. Bhupinder Kumar	Electrical Assistant
Mr. Harinder Singh Bisht	Technician
Mr. Raj Kumar	Technician
Mr. Paramjit	Technician
Mr. Anil Theophilus	Technician
Mr. Upendra Singh	Technician
Mr. Maman Chand	Technician
Mr. Surinder Singh	Technician
Mr. Malkiat Singh	Technician
Mr. Ashok Kumar Yadav	Technician
Mr. Sharanjit Kaur	Technician
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Mr. Mohd. Mustafa	Technician
Mr. Raj Kumar Dass	Technician
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Mr. Rakesh K. Dhiman	Technician
Ms. Renu	Technician
Ms. Chetna	Technician
Mr. Chander Prakash	Technician
Ms. Ravneet Dhaliwal	Technician
Mr. Dinesh Kumar	Technician
Mr. Randeep Sharma	Technician
Ms. Preeti	Technician
Mr. D.R.Agnihotri	Technician
Mr. Rohtas Ranga	Technician
Mr. Jaideep Mehta	Technician
Mr. Gurubachan Singh	Driver
Mr. Malkit Singh	Driver
Mr. Ram Pal Singh	Driver
Mr. Jaswant S. Rawat	Driver
Mr. Mohd.Younus	Lab. Attendant
Mr. Mohinder Kumar	Lab. Attendant
Mr. Ramesh Singh	Lab. Attendant
Mr. Prem Chand	Lab. Attendant
Mr. Anil Kumar Sharma	Cataloguer
Mr. Kewal Krishan	Plumber
Mr. Vinod Kumar	Helper

RESEARCH ASSOCIATES/FELLOWS

Ms. Meghna Thakur	SRF	Mr. Janamjay Pandey	SRF
Mr. S. Krishnamurthi	SRF	Ms. Archana Chauhan	SRF
Ms. Sudesh Pawaria	SRF	Ms. Richa Bajpai	SRF
Ms. Suman Yadav	SRF	Ms. Neetu Saxena	SRF
Ms. Sukhwinder Kaur	SRF	Mr. Vijay Kumar	SRF
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Mr. Narender K. Sharma	SRF	Mr. Manish Kumar	SRF
Ms. Anuradha Ghosh	SRF	Ms. Sneh Lata	SRF
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Mr. Shubbir Amhed	SRF	Mr. Mitesh Dongre	SRF
Mr. Vibhu Jain	SRF	Mr. Robin Chandra	SRF
Mr. Kumar Rajesh	SRF	Ms. Puja Saluja	SRF
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Mr. Kamlesh K. Bisht	SRF	Mr. Manzoor Ahmad Mir	SRF
Mr. Ashok Kumar	SRF	Mr. Nigam Kumar	SRF
Ms. Shilpy Srivastava	SRF	Mr. Anuj Pathak	JRF
Mr. Mohd. Suhail Alam	SRF	Mr. Akhilesh Kumar	JRF
Ms. Swati Haldar	SRF	Ms. Anu Priya	JRF
Mr. Haider Hussen Dar	SRF	Ms. Dipawuita Biswas	JRF
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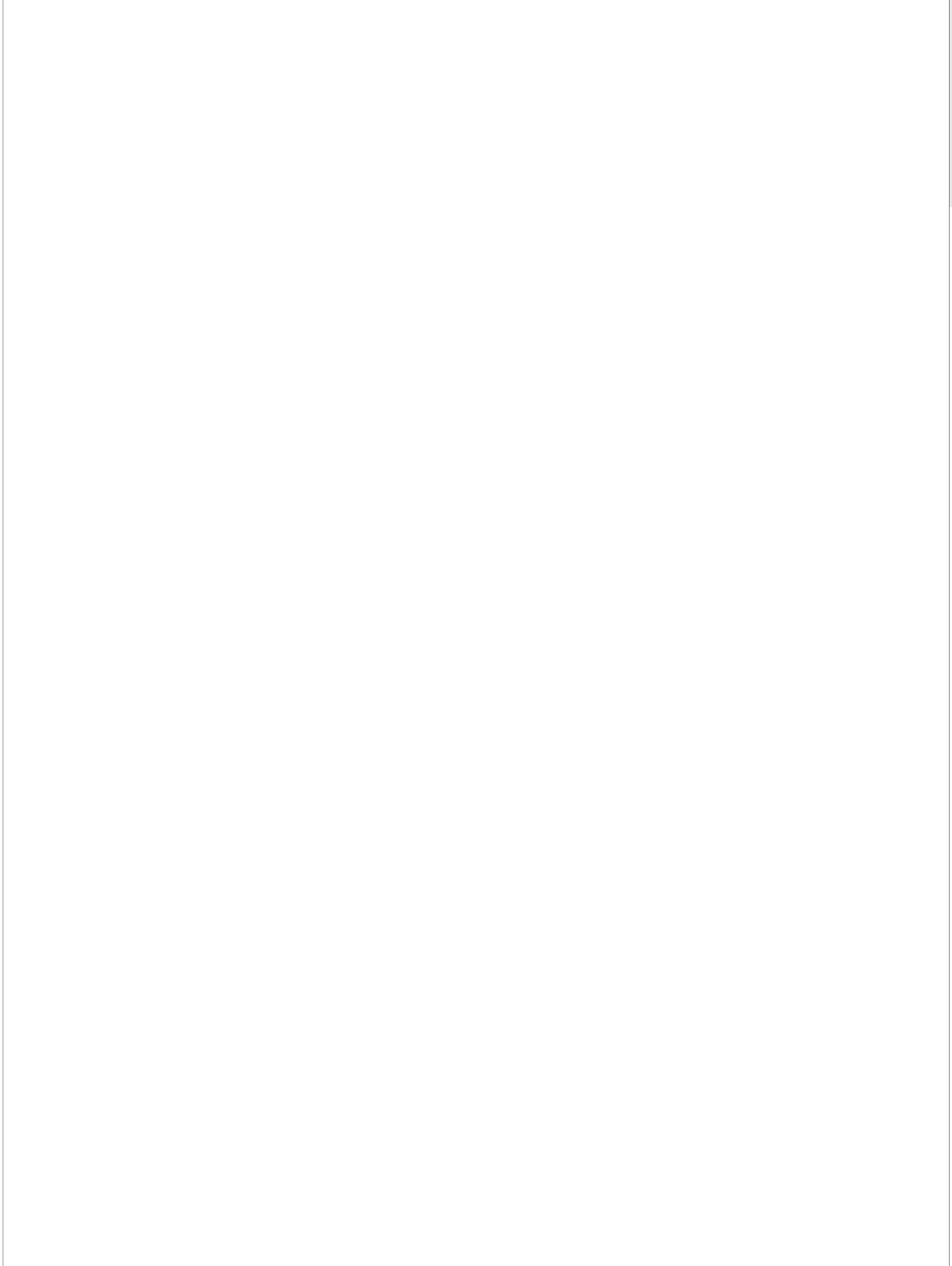
Mr. Amit K. Yadav	JRF

गतिविधियां

गतिविधियां

वैज्ञानिक/राजभाषा



अनुसंधान एवं विकास कार्यक्रम

माइक्रोबैक्टीरियम ट्यूबरक्यूलोसिस में फॉस्टफेट समावेश के नियामक घटकों की पहचान व लक्षण चित्रण
परियोजना प्रमुख – **डॉ. देबेन्दु सरकार**

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माइक्रोबैक्टीरियम ट्यूबरक्यूलोसिस के डब्ल्यूएचआईबी जैसे जीनों का आणविक विश्लेषण परियोजना प्रमुख – **डॉ. पुष्पा अग्रवाल**

....

क्या ट्रांस्पोसेसिस के डीडीई मोटिक अनिवार्य है ? पीआईएस 136 इंटीग्रेस जीन का प्रकार्यात्मक विश्लेषण – एक संभावित ट्रास्पोसेस
परियोजना प्रमुख – **डॉ. पुष्पा अग्रवाल**

....

कॉस्टीम्यूलेटरी अणुओं द्वारा प्रतिजनी कोशिकाओं के उत्प्रेरण तथा विभेदन को नियमित करना
परियोजना प्रमुख – **डॉ. जावेद नइम आगरेवाला**

....

विब्रोकॉलेरा से एन्टामीवा हिस्टोलिटिका के संबंध के तंत्र का आणविक आधार
परियोजना प्रमुख – **डॉ. सौम्य राय चौधरी**

....

वी.कॉलेरा के नॉन 01, नॉन–0139 विभेदों में तृतीय श्रेणी स्राव यंत्र की खोज: टीटीएस तंत्र के नियामन में कोरम संवेदन की भूमिका
परियोजना प्रमुख – **डॉ. सौम्य राय चौधरी**

....

खमीर में विद्यमान ग्लुटाथियोन होमियोस्टेसिस में गामा – ग्लुटामिल ट्रांस्पेप्टीडेस की भूमिका का अध्ययन
परियोजना प्रमुख – **डॉ. आनंद कुमार बच्छावत**

....

गणितीय मॉडलिंग इकाई
परियोजना प्रमुख – **डॉ. पी.आर. पटनायक**

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विषाक्त तथा अविषाक्त माइको बैक्टीरिया द्वारा टीएनएफ प्रभावित मृत्यु संकेतन का अध्ययन
परियोजना प्रमुख – **डॉ. शेखर मजूमदार**

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प्रोटीनों में वलन, पुंजन और स्थिरता के कारकों को समझने के लिए प्रोटीनों की अभियांत्रिकी, तथा उनके एंज़ाइमी या चिकित्सीय प्रकार्य को बदलना
प्ररियोजना प्रमुख – **डॉ. पूर्णानंद गुप्तासरमा**

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बैक्टीरिया द्वारा नाइट्रोफिनोसलो तथा नाइट्रोबेनजोएटों का रसो – अनुचलन तथा निम्नीकरण
परियोजना प्रमुख – **डॉ.आर.के.जैन**

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विखंडन यीस्ट में हेट्रोक्रोमोटिन संग्रहण और आरएनएआई की कार्यप्रणाली में संबंध के आणविक तंत्र का विश्लेषण
परियोजना प्रमुख – **डॉ. जगमोहन सिंह**

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यीस्ट डिबारीओमाइसिस हाँसेनी में लवण सह्यता की आणविक अनुवांशिकता का अध्ययन
परियोजना प्रमुख – **डॉ. आलोक मंडल**

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माइक्रोबैक्टीरियल सेरीन/थ्रियोनिन काइनेसिस का लक्षण चित्रण
परियोजना प्रमुख – **डॉ. प्रदीप कुमार चक्रवर्ती**

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माइक्रोबैक्टीरियल ट्यूबरक्यूलोसिस के नवीन हीमोग्लोबिन का आणविक एवं जैवरासायनिक अध्ययनः माइकोबैक्टीरियल हीमोग्लोबिनों के कोशिकीय प्रकार्यों का अध्ययन और संभावित औषध लक्ष्य के तौर पर मूल्याकंन
परियोजना प्रमुख – **डॉ. कनक लता दीक्षित**
एवं सहयोगी – **डॉ. जी. राजामोहन**

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स्वसज्जीकृत बीटा वलित मोटिफों का अभिकल्पः जल विरगि परस्पर क्रियाओं द्वारा स्थिरीकरण
परियोजना प्रमुख – **डॉ. रघुवंश किशोर**

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टी कोशिका एपिटोप संभाव्यता आधारित टीका अभ्यर्थियों की पहचान के लिए जैवसूचनात्मक विधि
परियाजना प्रमुख – **डॉ. जी. पी. एस. राघव**

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जल नमूनों में मिथाइल पैराथियोन की उपस्थिति की सटीक एवं सुग्राही पहचान के लिए डिपस्टिक आधारित इम्यूनोसेंसर तंत्र का विकास
परियोजना प्रमुख – **डॉ. चन्द्र रमन सूरी**

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रिकम्बिनेंट जैव अणुओं के उत्पादन की जैव प्रक्रियाओं का विकास तथा संश्लेषण
परियोजना प्रमुख – **डॉ. डी.के.साहू**

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मलेरिया संक्रमित रक्ताणुओं में प्रतिजनी और आणविक लक्ष्य
परियोजना प्रमुख – **डॉ. जी.सी.वार्ष्णेय**

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प्रोटीन अभियांत्रिकी विधियों को अपनाते हुए थक्का घोलक कारकों का आधारभूत एवं प्रयोगपरक अध्ययन
परियोजना प्रमुख – **डॉ. गिरीश साहनी**

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डीएनए पेप्टाइड/प्रोटीन डॉकिंग के नियम तय करने के लिए सामान्यतः प्रयुक्त बल क्षेत्रों का संश्लेषण एवं मूल्यांकन
परियोजना प्रमुख – **डॉ. बलविंदर सिंह**

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कैंडिडा अल्बाकेन्स का आनुवंशिक विश्लेषण तथा उसमें परिवर्तन करना
परियोजना प्रमुख – **डॉ. के.गणेशन**

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डेंडरिटिक कोशिका प्रकार्य की हिपेटोसाइट वृद्धि कारक प्रभावित संक्रमण नियामकता के आणविक तंत्र की व्याख्या
परियोजना प्रमुख – **डॉ. प्रदीप सेन**

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जीवाणुरोधी औषध की खोजः एक सम्भावित लक्ष्य के प्रति संरचनागत औषध अभिकल्प
परियोजना प्रमुख – **डॉ. कार्तिकेन सुब्रह्मणियम**

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एक झिल्ली आधारित बहु प्रकार्यात्मक ग्लाइकोलिटिक प्रोटीन की पहचान तथा मैक्रोफाज क्रिया में उसकी भूमिका
परियोजना प्रमुख – **डॉ. मनोज राजे**

प्रोटीन मॉडलिंग/अभियांत्रिकी का जैवसूचना केन्द्र
परियोजना प्रमुख – **डॉ. जी.पी.एस. राघव**

सूक्ष्मजीव प्रतिरूप संवर्ध संग्रह तथा जीन बैंक (एमटीसीसी) केन्द्र
परियोजना प्रमुख – **डॉ. तपन चक्रवर्ती**

जैव रासायनिक अभियांत्रिकी शोध तथा प्रक्रम विकास केन्द्र
परियोजना प्रमुख – **डॉ. डी.के.साहू**

राजभाषा गतिविधियाँ

सूक्ष्मजीव प्रौद्योगिकी संस्थान, चण्डीगढ़ में राजभाषा कार्यान्वयन के विशेष प्रयास किए जा रहे हैं। संस्थान का कार्य वैज्ञानिक प्रकृति का है अतः तकनीकी स्टॉफ को **<u>विज्ञान विषयों पर हिन्दी में सेमिनार</u>** देने को प्रेरित किया जाता है जिससे यह भ्रांति दूर हो सके कि तकनीकी विषयों पर हिन्दी में कार्य सम्भव नहीं। रिपोर्ट की अवधि के दौरान "उच्च रक्तचाप और हमारा स्वास्थ्य; उड़ान; रिमोट सेंसिंग; मधुमेह; जैविक हथियार; पृथ्वी पर जीवन की उत्पत्ति तथा विकास; मौसमी बीमारियाँ; स्वतंत्र भारत की वैज्ञानिक उपलब्धियाँ" आदि विषयों पर सेमिनार दिए गए। निदेशक, इमटैक द्वारा गठित निर्णायक मंडल के पैनल द्वारा इन सेमिनारों का मूल्यांकन किया जाता है तथा सर्वश्रेष्ठ तीन सेमिनारों के विजेताओं को हिन्दी दिवस समारोह में पुरस्कृत किया जाता है। संस्थान के स्वागत कक्ष में **<u>एक बोर्ड पर हिन्दी के प्रशासनिक/वैज्ञानिक शब्दों</u>** के अंग्रेज़ी पर्याय लगाए जाते हैं। जिनकी सूची संस्थान के सदस्यों में परिचालित की जाती है। इसके आधार पर हिन्दी पखवाड़े के दौरान **<u>हिन्दी शब्दज्ञान प्रतियोगिता</u>** आयोजित की जाती है।

<u>हिन्दी वाद–विवाद प्रतियोगिता</u>

हिन्दी वाद विवाद प्रतियोगिता में निदेशक, इमटैक एवं अन्य वरिष्ठ अधिकारीगण

राजभाषा गतिविधियों की कड़ी में दिनांक 20.06.05 को 3.00 बजे अपराह्न सेमिनार हाल में 'नारी सशक्तीकरण (Women empowerment) – कितना सही' विषय पर हिन्दी वाद–विवाद प्रतियोगिता आयोजित की गई।

कार्यक्रम का संचालन सुश्री नवनीत आनंद, वरिष्ठ अनुवादक ने किया।

11 प्रतियोगियों ने इस सामयिक विषय के पक्ष व विपक्ष में अपने विवेकपूर्ण एवं अकाट्य तर्क दिए तथा पक्ष ने विपक्ष पर विजय पाई। पक्ष के प्रतियोगियों ने नारी सशक्तीकरण को सही ठहराते हुए कहा कि सशक्तीकरण से तात्पर्य यह नहीं लेना चाहिए कि

हिन्दी वाद विवाद प्रतियोगिता में एक प्रतिभागी।

नारी किसी सहारे की मोहताज है, शक्तिहीन है अपितु जब तक भारतीय समाज अपनी हीन मानसिकता को बदलकर जन्म से ही बालक व बालिका को समान रूप से लालन पालन नहीं करता, समान अवसर नहीं देगा महिला आरक्षण व सशक्तीकरण की आवश्यकता पड़ती रहेगी। यह प्रतियोगिता संस्थान के सदस्यों में बहुत लोकप्रिय है तथा सभी वर्गों के सदस्यों ने इसमें भाग लिया ।

संस्थान के निदेशक डॉ. गिरीश साहनी, प्रशासन नियंत्रक श्री हरि मोहन व सुश्री अनुराग सचदेव, अनुभाग अधिकारी भी इस अवसर पर उपस्थित थीं। डॉ. पी.आर. पटनायक, वैज्ञानिक, डॉ. कनक लता दीक्षित, वैज्ञानिक व श्री रामस्वरूप, वित एवं लेखा अधिकारी ने हिन्दी वाद—विवाद प्रतियोगिता में निर्णायक मंडल के तौर पर अपनी सेवाएँ दीं।

अनुवाद कार्यशाला के अवसर पर डॉ. नरेश कुमार एवं अन्य वरिष्ठ अधिकारीगण तथा डॉ. सुरेन्द्र कुमार शर्मा, सहायक निदेशक, राभा. एवं सचिव नराकास बोलते हुए।

अनुवाद कार्यशाला

दिनांक 5—6 अप्रैल, 2005 को नराकास, चण्डीगढ़ के सहयोग से दो दिवसीय अनुवाद कार्यशाला आयोजित की गई जिसमें चण्डीगढ़ स्थित केन्द्रीय कार्यालयों के लगभग 25 अनुवादकों/हिन्दी पदधारियों ने भाग लिया। कार्यशाला में अनुवाद, हिन्दी साहित्य तथा पत्रकारिता जगत से विभिन्न विद्वानों ने प्रतिभागियों को संबोधित किया।

प्रशासनिक हिन्दी कार्यशाला

हिन्दी कार्यशालाओं के आयोजन की कड़ी में दिनांक 25 मई, 05 को हिन्दी कार्यशाला का आयोजन किया गया जिसमें संस्थान के प्रशासन नियंत्रक श्री हरिमोहन ने प्रशासन के विभिन्न पहलुओं पर एक विशेष वार्ता दी। कार्यशाला में संस्थान के सभी सदस्य आमंत्रित थे।

उन्होंने प्रशासन के क्षितिज के विस्तार को राष्ट्रीय स्तर पर व्याख्यायित किया तथा प्रशासन एवं सतर्कता संबंधी आयामों पर नवीन रोचक जानकारी दी।

लेखा कार्य संबंधी कार्यशाला

इसी कड़ी में दिनांक 12 जुलाई, 05 को श्री रामस्वरूप, वित एवं लेखा अधिकारी ने वैज्ञानिक एवं तकनीकी स्टाफ को लेखा

कार्यशाला में भाग लेते वरिष्ठ वैज्ञानिक एवं अन्य प्रतिभागी

संबंधी कार्य में आने वाली व्यावहारिक कठिनाइयों को दूर करने के उद्देश्य को लेकर 'लेखा एवं सेवा मामले' विषय पर हिन्दी में एक विशद वार्ता दी। उन्होंने छुट्टी नियमों, यात्रा भतों, पेंशन, उपदान, छुट्टी के बदले नकद भुगतान आदि सभी विषयों पर सरल बोलचाल की भाषा में जानकारी दी। संस्थान के सभी सदस्यों ने पूरी प्रतिभागिता से वार्ता में भाग लिया।

हिन्दी पखवाड़े का आयोजन वर्ष 2005

हिन्दी दिवस समारोह मे श्रोतागण

संस्थान में दिनांक 01.09.05 से 14.09.05 तक हिन्दी पखवाड़े का आयोजन किया गया।

इसके दौरान विभिन्न प्रतियोगिताएँ आयोजित की गई। दिनांक 14.9.05 को संस्थान के सम्मेलन कक्ष में मुख्य समारोह आयोजित किया गया।

संस्थान के निदेशक डॉ. गिरीश साहनी किसी विशेष प्रशासनिक बैठक के सिलसिले में दौरे पर होने के कारण कार्यक्रम में उपस्थित नहीं हो सके। अतः श्री हरि मोहन, प्रशासन नियंत्रक ने कार्यक्रम की अध्यक्षता की। वरिष्ठ वैज्ञानिक डॉ. नरेश कुमार तथा सुश्री अनुराग सचदेव, अनुभाग अधिकारी भी इस अवसर पर उपस्थित थीं। सर्वप्रथम इस समारोह का मुख्य आकर्षण 'हिन्दी प्रश्नोतरी में विविध विषयों के साथ–साथ हिन्दी साहित्य के क्षेत्र से लिए गए प्रश्न भी शामिल किए गए। श्री हरि मोहन, प्रशासन नियंत्रक ने इस अवसर पर संस्थान के सदस्यों को संदेश दिया कि प्रशासन के साथ–साथ हमें भारत की पहचान के तौर पर भी हिन्दी को स्थापित करना है। उन्होंने सभी भारतीयों भाषाओं को भी साथ लेकर चलने का अनुरोध किया जिससे हिन्दी के प्रति अहिन्दी भाषियों की भी स्वस्थ मानसिकता विकसित होगी। उन्होंने पखवाड़े के दौरान आयोजित विज्ञान पर हिन्दी में लेखन प्रतियोगिता, कम्प्यूटर पर हिन्दी टंकण हिन्दी शब्दावली, हिन्दी पत्र/आवेदन लेखन प्रतियोगिताओं के विजेताओं को तथा वर्षभर की गतिविधियों के विजेताओं को व हिन्दी में मूल कामकाज की प्रोत्साहन योजना में भाग लेने वाले कर्मचारियों को पुरस्कार वितरित किए। पखवाड़े के दौरान आयोजित हिन्दी कार्यशाला के प्रतिभागियों को भी इस अवसर पर उनके द्वारा प्रमाण पत्र प्रदान किए गए।

मूल कामकाज की प्रोत्साहन योजना के विजेता को पुरस्कार देते हुए श्री हरी मोहन, प्र.नि.

मूल कामकाज की प्रोत्साहन योजना के विजेता को पुरस्कार देते हुए श्री हरी मोहन, प्र.नि.

निर्णायक मंडल के रूप में डॉ. आर.किशोर, वै., डॉ. बलविंदर, वै. व सुश्री शशि बतरा, वरिष्ठ आशुलिपिक ने अपना योगदान दिया। हिन्दी पखवाड़े के आयोजन की समग्र परिकल्पना राजभाषा अनुभाग द्वारा की गई तथा श्री हरि मोहन, प्रशासन नियंत्रक व सुश्री अनुराग सचदेव, अनुभाग अधिकारी द्वारा समय–समय पर अमूल्य मार्गदर्शन प्रदान किया गया।

टेबल वर्कशॉप एवं हिन्दी कार्यशाला

कार्यशाला में भाग लेते प्रतिभागी

संस्थान के नए भर्ती सदस्यों का नाम राजभाषा रोस्टर में शामिल किया जाता है तथा प्रत्येक तिमाही के अंत में टेबल वर्कशॉप करके राजभाषा अधिनियमों की संक्षिप्त जानकारी दी जाती है एवं उनके कार्य की प्रकति के अनुसार नेमी कार्य हिन्दी में करने को प्रेरित किया जाता है। इसी कड़ी में हिन्दी पखवाड़े के दौरान गत दो वर्षों के दौरान संस्थान में भर्ती हुए वैज्ञानिकों, तकनीकी व गैर तकनीकी स्टॉफ के लगभग 30 सदस्यों के लिए दिनांक 09.09.05 को 4. 00 बजे अपराह्म संस्थान के सेमिनार हाल में हिन्दी कार्यशाला का आयोजन किया गया जिसमें सुश्री नीरू, व. हिन्दी अधिकारी, सीएसआईओ को प्रतिभागियों को संबोधित करने के लिए आमंत्रित किया गया। कार्यशाला का संचालन सुश्री नवनीत आनंद, व.अनुवादक ने किया व सुश्री अनुराग सचदेव, अनुभाग अधिकारी भी इस अवसर पर उपस्थित थीं। सुश्री नीरू ने सरल एवं धाराप्रवाह भाषा में राजभाषा अधिनियमों व हिन्दी वर्तनी संबंधी जानकारियाँ दी। प्रतिभागियों ने वर्तनी संबंधी कठिनाइयों के निवारण में उत्साहपूर्ण रूचि दिखाई।
इसके अतिरिक्त दिसम्बर, 05 में समाप्त तिमाही के दौरान वित्त एवं लेखा अनुभाग की तथा मार्च, 06 में समाप्त तिमाही के दौरान सामान्य, बिल, प्रेषण, अनुभागों की **टेबल वर्कशाप** आयोजित की गई। जिसमें सदस्यों की टेबल पर जाकर उनकी राजभाषा में कार्य करने संबंधी कठिनाइयों का निवारण किया गया तथा अभ्यास कराया गया।

''सीएसआईआर के विज्ञान में युवा नेतृत्व कार्यक्रम'' की तरह संस्थान के सदस्यों के बच्चों के लिए हिन्दी में विशेष योग्यता प्राप्त करने पर प्रोत्साहन योजना –वर्ष 2004–05

संस्थान में चलाई जा रही प्रोत्साहन योजनाओं यथा – 'हिन्दी में मूल कामकाज योजना' व 'विज्ञान पर हिन्दी में सेमिनार योजना' के अतिरिक्त जैसाकि समिति के समक्ष सुझाव रखा गया कि सीएसआईआर के 'विज्ञान में युवा नेतृत्व कार्यक्रम' की तरह इमटैक सदस्यों के बच्चों में राजभाषा के प्रति रूझान बढ़ाने के उद्देश्य से एक प्रोत्साहन योजना चलाई गई है जिसमें कक्षा 4 से 12 तक के छात्रों के हिन्दी में विशेष योग्यता (अधिकतम अंक

हिन्दी दिवस के अवसर पर हिन्दी विषय में विशेष योग्यता प्राप्त करने पर संस्थान के सदस्यों के बच्चों को पुस्कार देते डॉ. गिरीश साहनी

हिन्दी दिवस समारोह में पुरस्कार देते डॉ. गिरीश साहनी

निर्धारित नहीं किए गए हैं क्योंकि अलग – अलग स्कूलों में मूल्यांकन का स्तर भिन्न–भिन्न हो सकता है) प्राप्त करने पर हिन्दी दिवस के अवसर पर पुरस्कृत किया गया जाएगा। यह योजना वर्तमान शिक्षा वर्ष से लागू की गई।

धारा 3(3) का अनुपालन–इलेक्ट्रॉनिक तौर पर – संस्थान में धारा 3(3) के अनुपालन पर विशेष ध्यान दिया जाता है तथा सभी सामान्य आदेश द्विभाषी जारी किए जाते हैं तथा अब ई. मेल के माध्यम से इनके द्विभाषी अनुपालन के लिए संस्थान में ई.मेल सुविधा के द्विभाषीकरण की प्रक्रिया आरंभ की गई है।

विशेष उल्लेख

राजभाषा पुरस्कार – नगर राजभाषा कार्यान्वयन समिति, चण्डीगढ़ द्वारा वर्ष 2004–05 में राजभाषा कार्यान्वयन के विशेष प्रयासों के लिए संस्थान को नगर स्तर पर द्वितीय स्थान प्राप्त हुआ। **दिनांक 20.05.06 को चण्डीगढ़ में आयोजित एक समारोह में सुश्री एस.के.औलख, मुख्य आयकर आयुक्त एवं अध्यक्ष, नराकास ने संस्थान के निदेशक, डा. गिरीश साहनी के साथ–साथ राजभाषा अनुभाग को भी सम्मानित किया गया।** नराकास द्वारा नगर स्तर पर आयोजित की गई हिन्दी निबंध लेखन प्रतियोगिता में संस्थान के श्री जानकी प्रसाद, तकनीशियन को तृतीय पुरस्कार प्राप्त हुआ।

संसदीय राजभाषा समिति की दूसरी उपसमिति द्वारा संस्थान का राजभाषा संबंधी निरीक्षण दिनांक 07.06.07

संसदीय राजभाषा समिति के समक्ष मुख्यालय एवं इमटैक के अधिकारीगण

डॉ. लक्ष्मी नारायण पाण्डेय की अध्यक्षता में दिनांक 07.06.07 को संस्थान का राजभाषा संबंधी निरीक्षण किया गया। डॉ.वी.के.गुप्ता, निदेशक निस्केयर, नई दिल्ली ने परिषद् मुख्यालय का प्रतिनिधित्व किया तथा डॉ. पूरनपाल, व. हि. अधिकारी भी इस अवसर पर उपस्थित थे। संस्थान की ओर से निम्नलिखित अधिकारियों ने भाग लिया।

1.	डॉ. गिरीश साहनी	– निदेशक
2.	डॉ. पी.आर. पटनायक	– वैज्ञानिक
3.	डॉ. तपन चक्रवर्ती	– वैज्ञानिक
4.	श्री एस.डी. भट्ट	– वैज्ञानिक एवं राजभाषा अधिकारी
5.	डॉ. प्रदीप कुमार चक्रवर्ती	– वैज्ञानिक
6.	डॉ. आनंद कुमार बच्छावत	– वैज्ञानिक
7.	डॉ. आर.एस. जौली	– वैज्ञानिक
8.	श्री एम. आर. मसान	– प्रशासन नियंत्रक
9.	श्री राम स्वरूप	– वित एवं लेखा अधिकारी
10.	श्री के.के. भरत	– भंडार एवं क्रय अधिकारी
11.	श्री पराग सक्सेना	– अनुभाग अधिकारी
12.	सुश्री नवनीत आनंद	– व.अनुवादक

हिन्दी पखवाड़े का आयोजन –2006

सूक्ष्मजीव प्रौद्योगिकी संस्थान, चण्डीगढ़ में दिनांक 01.09.06 से 14.09.06 तक हिन्दी पखवाड़े का आयोजन किया गया। इसके दौरान विभिन्न प्रतियोगिताएँ आयोजित की गई। दिनांक 14.09.06 को संस्थात के सभागार में मुख्य समारोह आयोजित किया गया। संस्थान के निदेशक डॉ. गिरीश साहनी ने कार्यक्रम की अध्यक्षता की, श्री एम. आर. एसान, प्रशासन नियंत्रक किसी विशेष प्रशासनिक कार्य के सिलसिले में दौरे पर होने के कारण कार्यक्रम में उपस्थित नहीं हो सके। सर्वप्रथम इस समारोह का मुख्य आकर्षण 'हिन्दी वाद– विवाद प्रतियोगिता' आयोजित की गई मिसका विषय था ''अपनी देशभक्ति का सबूत देते रहना हर नागरिक का कर्तव्य है'' जिसमें संस्थान के सभी सदस्यों ने उत्साहपूर्ण भागीदारी की । यह प्रतियोगिता संस्थान के सभी वर्गों के सदस्यों में विशेष लोकप्रिय है। पक्ष व विपक्ष के सोलह प्रतियोगियों ने अपने विचारोत्तजक तथ्य रखे और अंत में विपक्ष का पलड़ा भारी रहा। डॉ. साहनी ने इस अवसर पर संदेश देते हुए संस्थान के सदस्यों को कर्तव्यनिष्ठा की ओर प्रेरित किया कि नियमों का पालन करना हमारा कर्तव्य है और राजभाषा हिन्दी को अपनाना हमारी संवैधानिक आवश्यकता है। उन्होंने वाद – विवाद में भाग लेने वाले प्रतिभागियों को बधाई देते हुए कहा कि रोचक विषयों पर हिन्दी में की गई बातचीत से संस्थान में हिन्दी के प्रयोग के लिए सकारात्मक वातावरण उत्पन्न होगा। उन्होंने वाद – विवाद के संबंध में भी अपने गहन विचार रखे और देशभक्ति की व्याख्या को एक नया आयाम दिया। कार्यक्रम का संचालन सुश्री नवनीत आनंद, वरिष्ठ अनुवादक ने किया तथा संस्थान में राजभाषा कार्यान्वयन संबंधी उपलब्धियों पर प्रकाश डाला कि संस्थान में समस्त कार्यकलाप द्विभाषी करने का प्रयास किया जाता है और इसी कड़ी में इस अवसर पर घोषणा की गई कि हिन्दी दिवस के अवसर पर संस्थान में प्रयोग में लाए जाने वाले फार्मों का द्विभाषी रूप संस्थान की वैब साइट पर ऑनलाइन उपलब्ध करा दिया गया है। सभागार में उपस्थित सभी सदस्यों

हिन्दी दिवस समारोह में निर्णायक मण्डल

हिन्दी दिवस समारोह में हिन्दी वादविवाद प्रतियोगिता

हिन्दी दिवस समारोह के अवसर पर श्रोतागण

ने इस सुविधा का करतल ध्वनि से स्वागत किया।

इसके पश्चात् निदेशक, इमटैक द्वारा पखवाड़े के दौरान आयोजित विज्ञान पर हिन्दी में स्लोगन लेखन प्रतियोगिता, कम्प्यूटर पर हिन्दी टंकण, हिन्दी शब्दावली, हिन्दी पत्र/आवेदन लेखन, तस्वीर क्या बोलती है ?, हिन्दी वाद विवाद प्रतियोगिता के विजेताओं को तथा वर्षभर की गतिविधियों के विजेताओं को व हिन्दी में मूल कामकाज की प्रोत्साहन योजना में भाग लेने वाले सदस्यों को पुरस्कार वितरित किए। इसके अतिरिक्त ''सीएसआईआर के विज्ञान में युवा नेतृत्व कार्यक्रम'' की तरह चलाई जा रही स्कूली छात्रों के लिए हिन्दी में विशेष योग्यता प्राप्त करने पर नकद पुरस्कार प्रदान करने की प्रोत्साहन योजना के तहत भी इमटैक सदस्यों के बच्चों को भी इस अवसर पर पुरस्कृत किया गया।

संस्थान में राजभाषा के प्रभावशाली कार्यान्वयन हेतु सभी क्षेत्रीय समन्वयकों/अनुभाग/प्रभागाध्यक्षों की बैठक

संसदीय राजभाषा समिति की दूसरी उपसमिति के द्वारा दिनांक 07.06.06 को हुए संस्थान के राजभाषा संबंधी निरीक्षण के संबंध में परिषद् मुख्यालय के दिनांक 08.08.06 के पत्रांक 20–24 (23)/2001–रा.भा. के माध्यम से प्राप्त रिपोर्ट (संस्थान की ओर से दिए गए आश्वासनों के संबंध में) की अनुवर्ती कार्रवाई के तौर पर सभी अनुभाग /प्रभागाध्यक्षों की बैठक दिनांक 05.09.06 को संस्थान के कमेटी कक्ष में आयोजित की गई जिसमें निम्नलिखित सदस्य उपस्थित थे –

1. डॉ. गिरीश साहनी, निदेशक
2. डॉ. पी.आर. पटनायक, वै.
3. श्री एस.डी.भट्ट, वै. एवं राजभाषा अधिकारी
4. डॉ. प्रदीप कुमार चक्रवर्ती, वै.
5. डॉ. आनंद कुमार बच्छावत, वै.
6. डॉ. आर.एस. जौली, वै.
7. डॉ. जी.सी. वार्ष्णेय, वै.
8. डॉ. डी.के.साहू, वै.
9. श्री अतुल कुमार गोयल, अधी.अभि.
10. श्री आर.सोनी, वै.
11. श्री एम.आर.मसान, प्र.नि.
12. श्री बृजेश कुमार, वि. एवं ले.अधि
13. श्री के.के.भरत, भं. एवं क्रय अधि.
14. श्री पराग सक्सेना, अ.अ.
15. श्री आर.एन. बंसल, पुस्तक.अधि.
16. सुश्री नवनीत आनंद, व. अनुवादक

संसदीय समिति को दिए गए आश्वासन पूरे करने के पश्चात् दिनांक 15.12.06 परि. मुख्या. को एतदर्थ रिपोर्ट भिजवाने हेतु आश्वासनों की पूर्ती की दिशा में बैठक में महत्वपूर्ण निर्णय लिए गए।

संस्थान में राजभाषा कार्यान्वयन संबंधी निरीक्षण ।

संसदीय राजभाषा समिति की दूसरी उपसमिति द्वारा संस्थान के राजभाषा संबंधी निरीक्षण की अनुवर्ती कार्रवाई के तौर पर संस्थान के विभिन्न अनुभागों का राजभाषा संबंधी निरीक्षण किया गया।
दिनांक 16.10.06 से 19.10.06 तक के दौरान निदेशक, इमटैक द्वारा एतदर्थ गठित समिति द्वारा निम्नवत निरीक्षण किया गया –

दिनांक	अनुभाग	समय
16.10.06	लेखा, बिल, कैश, सामान्य, प्रेषण	11.30 से 12.30 पूर्वाह्न
17.10.06	स्था – ।।, भर्ती, सुरक्षा, बागवानी, कैंटीन,	11.30 से 12.30 पूर्वाह्न
18.10.06	भण्डार एवं क्रय, इ.एस.डी. इन्स्ट्रूमेंटेशन	11.30 से 12.30 पूर्वाह्न
19.10.06	पुस्तकालय, पी.टी.एम.	11.30 से 12.30 पूर्वाह्न

परिषद् मुख्यालय द्वारा संस्थान का राजभाषा संबंधी निरीक्षण

हिन्दी कार्यशाला के उद्घाटन पर डॉ. पूरन पाल, व. हिन्दी अधिकारी, डॉ. गिरीश साहनी, निदेशक एवं श्री एम.आर. मसान, प्र.नि. (बाएं से)

दिनांक 02.11.06 का डॉ. पूरनपाल वरिष्ठ हिन्दी अधिकारी द्वारा संस्थान का राजभाषा संबंधी निरीक्षण किया गया। निरीक्षण के दौरान संस्थान के प्रशासनिक अनुभागों यथा– स्थापना, सामन्य, डिस्पैच, बिल व लेखा अनुभागों में सहायकों की टेबलों पर जाकर व. हिन्दी अधिकारी ने राजभाषा कार्यान्वयन की स्थिति की समीक्षा की तथा सहायकों की व्यावहारिक समस्याओं को भी सुना तथा उपयोगी सुझाव भी दिए।
निरीक्षण की रिपोर्ट प्राप्त हुई तथा दिनांक 14.11.06 को हुई राजभाषा कार्यान्वयन समिति की बैठक में उसपर बिन्दुवार चर्चा की गई तथा अनुवर्ती कार्रवाइयाँ की गई ।

हिन्दी का ज्ञान रखने वाल सदस्यों के लिए हिन्दी कार्यशाला का आयोजन

भारत सरकार के राजभाषा तथा परिषद् मुख्यालय द्वारा सरकारी कामकाज में हिन्दी के उपयोग को ज्यादा से ज्यादा बढ़ाने के उद्देश्य से समय–समय पर जारी निदेशों के अनुपालनार्थ संस्थान में विभिन्न गतिविधियाँ आयोजित की जाती हैं।

इसी क्रम में तथा संसदीय राजभाषा समिति की दूसरी उपसमिति द्वारा संस्थान के राजभाषा संबंधी निरीक्षण की अनुवर्ती कार्रवाई के तौर पर

कार्यशाला में भाग लेते निदेशक (दाएँ), वरिष्ठ वैज्ञानिक एवं अन्य प्रतिभागी

हिन्दी का ज्ञान रखने वाले संस्थान के सदस्यों को हिन्दी में कार्य करने के लिए प्रेरित करने के उद्देश्य से दिनांक 03.11.2006 को संस्थान के सेमिनार हाल में हिन्दी कार्यशाला का आयोजन किया गया। परिषद् मुख्यालय से डॉ. पूरनपाल, व हिन्दी अधिकारी को संस्थान के सदस्यों को संबोधित करने हेतु आमंत्रित किया गया।

कार्यशाला का उद्घाटन करते हुए निदेशक, इमटैक डॉ. गिरीश साहनी ने कहा कि सरकारी सेवा में कार्य करते हुए अन्य नियमों के पालन के साथ–साथ हिन्दी में कार्य करना हमारा परम कर्तव्य है हिन्दी हम सब बोलते हैं और हमें बोलचाल की भाषा का ही प्रयोग करना चाहिए। डॉ. साहनी ने डॉ. पूरनपाल को भी उनके द्वारा संस्थान को दिए गए मार्गदर्शन एवं सहयोग के लिए साधुवाद दिया। संस्थान के लगभग सभी सदस्यों को हिन्दी का ज्ञान प्राप्त है तथा उद्घाटन सत्र में संस्थान के सभी वरिष्ठ वैज्ञानिक अधिकारीगण एवं सदस्य उपस्थित थे। डॉ. पूरनपाल ने कार्यशाला की प्रस्तावना प्रस्तुत की तथा संसदीय राजभाषा समिति की दूसरी उपसमिति को दिए गए आश्वासनों की पूर्ती में संस्थान की तत्परता के लिए बधाई दी कि निदेशक एवं वरिष्ठ अधिकारियों की अभिरूचि एवं राजभाषा अनुभाग की प्रतिबद्धता से ही यह संभव हो पाया है। श्री एम.आर.मसान, प्रशासन नियंत्रक भी इस अवसर पर उपस्थित थे तथा उन्होंने धन्यवाद प्रस्ताव दिया तथा मुख्यालय के सतत् सहयोग एवं मार्गदर्शन के लिए आभार व्यक्त किया। कार्यक्रम का संचालन सुश्री नवनीत आनंद, वरिष्ठ अनुवादक ने किया। उन्होंने इस बात पर बल दिया कि भारत जैसे बहुभाषी देश में जहाँ सभी धर्मों, भावनाओं को सम्मान दिया जाता है हिन्दी के कार्यान्वयन का समाधान हमें स्वयं खोजना है।

एक दिवसीय कार्यशाला का आयोजन दो सत्रों में किया गया। प्रथम सत्र में 11.00 से 1. 00 बजे पूर्वाह्न हिन्दी का कार्यसाधक ज्ञान रखने वाले सदस्यों को नामित किया गया जिनकी संख्या 104 थी तथा अपराह्न 3.00 से 5.00 बजे आयोजित किए जाने वाले सत्र में हिन्दी में प्रवीणता प्राप्त सदस्यों (संख्या 63) ने भाग लिया। दोनों सत्रों में डॉ. पूरनपाल ने राजभाषा नीति के संक्षिप्त परिचय के साथ–साथ हिन्दी वर्तनी का भी परिचय दिया। सदस्यों ने पूरी रूचि से इसमें भाग लिया। चूंकि कार्यशाला में विविध कार्य प्रकार के सदस्यों ने भाग लिया था अतः विभिन्न अनुभागों से संबंधित कार्य के हिन्दी के नमूने प्रस्तुत किए। सदस्यों को प्रोत्साहित

हिन्दी कार्यशाला के उद्घाटन के अवसर पर बोलते हुए निदेशक डॉ. गिरीश साहनी

किया गया कि अपने कार्यक्षेत्र से संबंध में हिन्दी में कार्य करने संबंधी कठिनाइयों को सामने रखें । डॉ. पूरनपाल के धन्यवाद के साथ कार्यशाला सम्पन्न हुई।

इमटैक, चण्डीगढ़ में दिनांक 6.11.06 से 10.11.06 तक सतर्कता जागरूकता सप्ताह का आयोजन

भारत सरकार के केन्द्रीय सर्तकता आयोग से प्राप्त अनुदेशों के अनुसरण में संस्थान में दिनांक 6.11.06 को सतर्कता जागरूकता सप्ताह का आरंभ शपथ ग्रहण समारोह से किया गया। संस्थान के सेमिनार हाल में डॉ. गिरीश साहनी, निदेशक, इमटैक ने संस्थान के सदस्यों को कर्तव्य पालन व सत्यनिष्ठा की शपथ दिलाई। संस्थान के सतर्कता अधिकारी एवं प्रशासन नियंत्रक श्री एम.आर.मसान के साथ–साथ सभी वरिष्ठ वैज्ञानिक एवं वरिष्ठ अधिकारी इसमें उपस्थित थे।

इसके अतिरिक्त संस्थान के सदस्यों में सतर्कता के प्रति जागरूकता उत्पन्न करने के उद्देश्य से सप्ताह के दौरान इस विषय पर ''तस्वीर क्या बोलती है?'' हिन्दी में लेखन व स्लोगन लेखन प्रतियोगिताएँ आयोजित की गई जिनके विजेता इस प्रकार हैं।

स्लोगन लेखन प्रतियोगिता

श्री दविन्द्र सिंह, प्रयोगशाला पर्यवेक्षक – प्रथम पुरस्कार	सतर्कता है वो अभियान। मिटे भ्रष्टाचार, बने देश महान।।
श्री पंकज कुमार अरोड़ा, शोध छात्र – द्वितीय पुरस्कार	देश की उन्नति का है यही मंत्र। भष्ट्राचार मुक्त रहे हमारा गणतंत्र।।
श्री जानकी प्रसाद, तकनीशियन – तृतीय पुरस्कार	सतर्कता को अपनाना है। अपनी ज़िम्मेदारी निभाना है।।

सतर्कता जागरूकता सप्ताह के अवसर पर पुरस्कार देते निदेशक

हिन्दी लेखन प्रतियोगिता 'तस्वीर क्या बोलती है?'

श्री प्रवीण कुमार, क.आशु.– प्रथम पुरस्कार
सुश्री पूजा सलूजा, शो. छात्रा – द्वितीय पुरस्कार
श्री विभु जैन, शोध छात्र – तृतीय पुरस्कार

दिनांक 10.11.06 को समापन दिवस पर बोलते हुए डॉ. गिरीश साहनी, निदेशक, इमटैक ने संस्थान के सभी सदस्यों का आहवान किया कि नियमों की जानकारी आवश्यक है परन्तु मुख्य बात यह है कि हम सब सतर्कता को अपने मन से अपनाएँ, अपने विवेक से बड़ा कोई प्रहरी नहीं। यदि हम इसे अपने मन से अपना लेंगे तो इस तरह सतर्कता जागरूकता सप्ताह मनाने की कोई आवश्यकता नहीं होगी। इस अवसर पर एन.टी.टी.टी.आई.आर, सैक्टर 26, चण्डीगढ़ के व.प्रशासनिक अधिकारी एवं

सतर्कता अधिकारी श्री के.एल.सिंगला को संस्थान के सदस्यों को संबोधित करने के लिए विशेष रूप से आमंत्रित किया गया था । उन्होंने सतर्कता जागरूकता सप्ताह के आयोजन के महत्व पर प्रकाश डालते हुए बड़ी सरल शैली में सर्तकता संबंधी नियमों पर प्रकाश डाला। श्री एम.आर.मसान, प्र.नि. ने मुख्य वक्ता का धन्यवाद किया तथा संस्थान की कार्यप्रणाली के संदर्भ में सतर्कता नियमों की सटीक जानकारी दी। इसके पश्चात् सप्ताह के दौरान आयोजित प्रतियोगिताओं के विजेताओं को निदेशक, इमटैक द्वारा पुरस्कार प्रदान किए गए।

सतर्कता जागरूकता सप्ताह का आयोजन राजभाषा अनुभाग के सहयोग से किया गया। डॉ. जी.पी.एस. राघव, वैज्ञानिक, सुश्री नवनीत आनंद, वरिष्ठ अनुवादक तथा श्री एम.आर.मसान, प्रशासन नियंत्रक ने निर्णायक मंडल के तौर पर अपनी सेवाएँ दीं।

सतर्कता जागरूकता सप्ताह के अवसर पर अभिभाषण सुनते निदेशक, प्र.नि. एवं अन्य सदस्य

प्रशासनिक एवं सेवा अनुभागों में राजभाषा नियमों का अनुपालन सुनिश्चित करने हेतु प्रशासनिक प्रधान के साथ बैठक

संसदीय राजभाषा समिति की दूसरी उपसमिति को संस्थान की ओर से दिए गए आश्वासनों की पूर्ती के लिए प्रशासनिक व सेवा अनुभागों में राजभाषा नियमों का अनुपालन सुनिश्चित करने हेतु (धारा 3 (3) के अनुपालन हेतु व हिन्दी पत्राचार के प्रतिशत को बढ़ाने हेतु) दिनांक 22.02.07 प्रशासन नियंत्रक की अध्यक्षता में सभी सम्बद्ध प्रभारियों/अनुभाग अधिकारियों की बैठक आयोजित की गई जिसमें निम्नलिखित अधिकारियों ने भाग लिया।

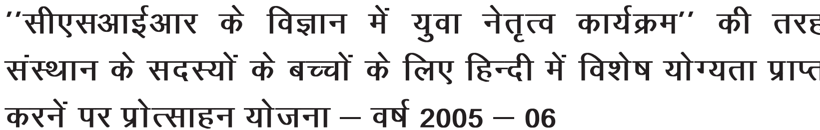
1. श्री एम.आर.मसान, प्र.नि.
2. श्री एस.डी. भट्ट, वै. एवं राजभाषा अधि.
3. श्री ए. के. गोयल, अधि. अभियंता
4. श्री के.के.भरत, भंडार एवं क्रय अधि
5. श्री जे.एन.अहूजा, व.सु.अधि. (च.ग्रे.)
6. श्री कुलजीत सिंह, वै (बागवाणी)
7. श्री जय प्रकाश, अ.अ. (वि एवं ले.)
8. श्री पराग सक्सेना, अ.अ.
9. श्री एस.डी. ऋषि, अ.अ.
10. श्री आर.एन.बंसल, पुस्त. अधि.
11. सुश्री नवनीत आनंद, व. अनु.

टेबल वर्कशाप एवं हिन्दी कार्यशाला

संस्थान के नए भर्ती सदस्यों का नाम राजभाषा रोस्टर में शामिल किया जाता है तथा प्रत्येक तिमाही के अंत में टेबल वर्कशॉप करके राजभाषा अधिनियमों की संक्षिप्त जानकारी दी जाती है एवं उनके कार्य की प्रकृति के अनुसार नेमी कार्य हिन्दी में करने को प्रेरित किया जाता है। इसी कड़ी में जनवरी, 07 मास में तीन नए भर्ती/स्थानान्तरित सदस्यों को राजभाषा नियमों की जानकारी दी गई तथा संस्थान में आयोजित की जा रहीं राजभाषा गतिविधियों की भी जानकारी दी गई।

वरिष्ठ अधिकारियों के लिए डिक्टेशन एवं कम्प्यूटर कार्यशाला

परिषद् मुख्यालय से प्राप्त निदेशों के क्रम में हिन्दी टंकण व आशुलिपि में प्रशिक्षित स्टाफ की सेवाओं के समुचित उपयोग की दृष्टि से तथा संस्थान में हिन्दी में पत्राचार के प्रतिशत में अपेक्षित सुधार हेतु सभी अनुभाग/प्रभागधिकारियों व क्षेत्रीय समन्वयकों को हिन्दी में डिक्टेशन देने का प्रशिक्षण देने/कम्प्यूटर पर हिन्दी में कार्य करने हेतु दिनांक 06 मार्च, 07 को कार्यशाला का आयोजन किया गया कार्यशाला में व्याख्यान देने हेतु श्री संतराम, सहायक निदेशक (टं. व आशु.) को आमंत्रित किया गया। संस्थान के सेमिनार हाल में आयोजित इस कार्यशाला में एक घंटे का व्यावहारिक सत्र भी रखा गया ।



''सीएसआईआर के विज्ञान में युवा नेतृत्व कार्यक्रम'' की तरह संस्थान के सदस्यों के बच्चों के लिए हिन्दी में विशेष योग्यता प्राप्त करनें पर प्रोत्साहन योजना – वर्ष 2005 – 06

संस्थान में चलाई जा रही प्रोत्साहन योजनाओं यथा – 'हिन्दी में मूल कामकाज योजना' व 'विज्ञान पर हिन्दी में सेमिनार योजना' के अतिरिक्त सीएसआईआर के 'विज्ञान में युवा नेतृत्व कार्यक्रम' की तरह इमटैक सदस्यों के बच्चों में राजभाषा के प्रति रुझाान बढ़ाने के उद्देश्य से चलाई गई प्रोत्साहन योजना के तहत कक्षा 4 से 12 तक के छात्रों (सं. 7 को) को हिन्दी में विशेष योग्यता प्राप्त करने पर हिन्दी दिवस के अवसर पर पुरस्कृत किया गया।

विशेष 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 दिल्ली के अ.शा. पत्र सं. 1/14013/03/94 –रा. भा. (नीति–1) दिनांक 8 नवम्बर, 2002 के अधीन प्राप्त माननीय प्रधानमंत्री के निदेशों के अनुसार सभी अधिकारियों/कर्मचारियों की वार्षिक गोपनीय रिपोर्टों में 'अन्य उपलब्धियों' के कॉलम में राजभाषा हिन्दी में अच्छा कार्य करने संबंधी उल्लेख करने हेतु 'राजभाषा हिन्दी में किया

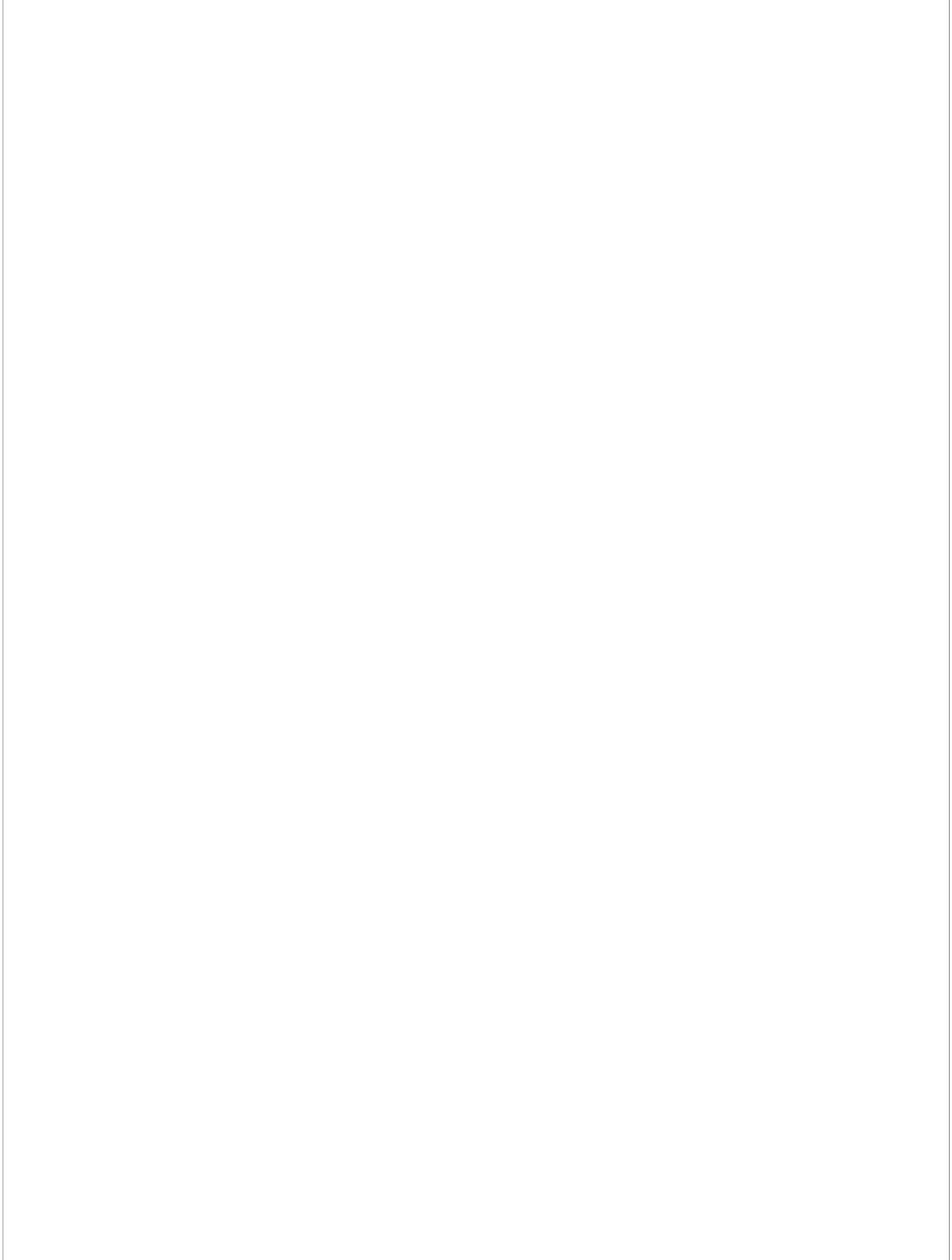
गया सराहनीय कार्य' की स्टैम्प बनाकर लगा दी गई है जिससे संस्थान में राजभाषा कार्यान्वयन को एक नई दिशा प्राप्त हुई है।

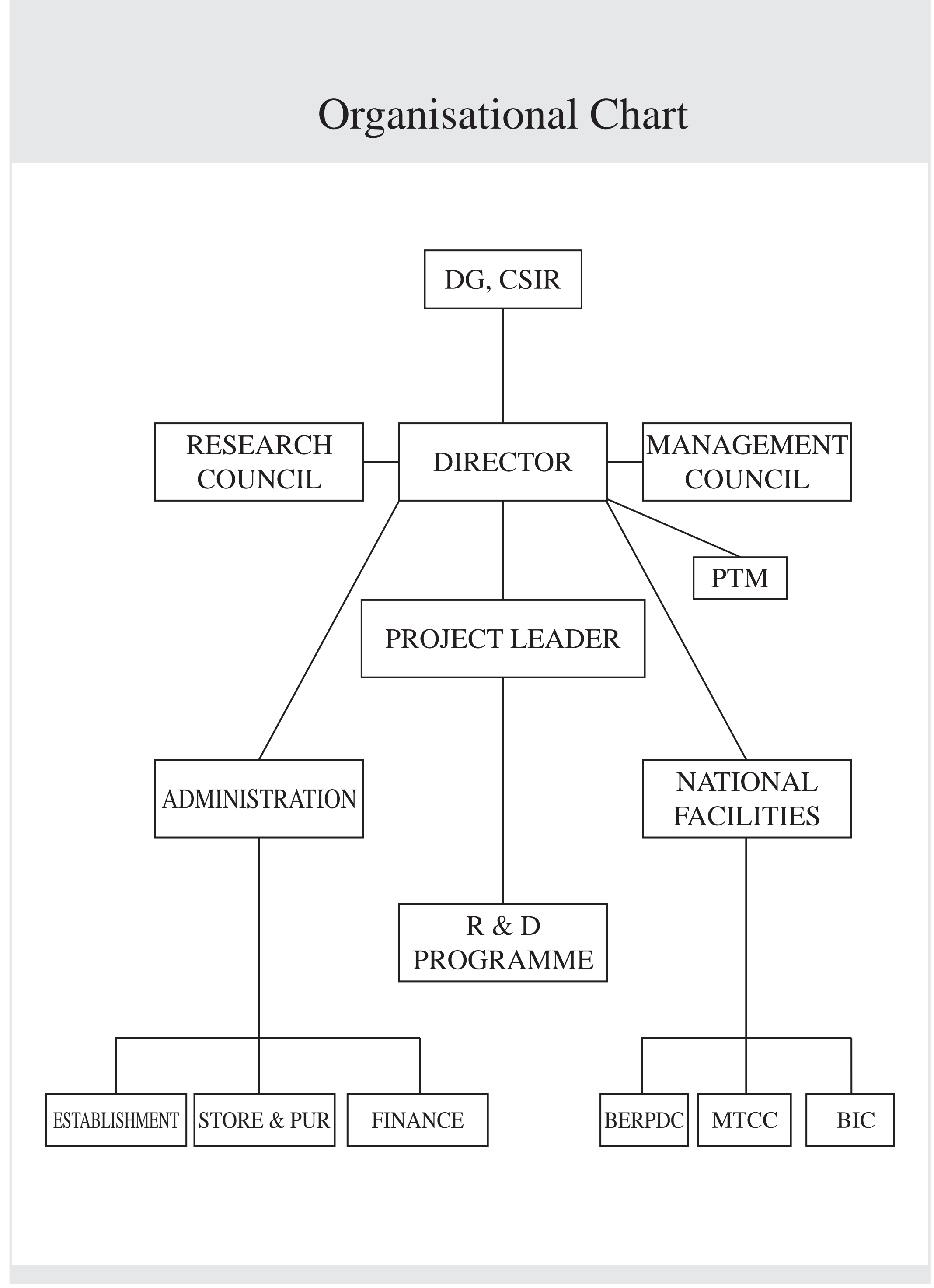
राजभाषा पुरस्कार – वर्ष 2006–07 में राजभाषा के प्रयोग को बढ़ाने हेतु किए गए प्रयासों के लिए नगर राजभाषा कार्यान्वयन समिति, चण्डीगढ़ द्वारा **संस्थान को द्वितीय पुरस्कार** प्रदान किया गया है। साथ ही श्री चन्द्र प्रकाश तकनीशियन को नगर स्तर पर आयोजित हिन्दी निबंध प्रतियोगिता में प्रोत्साहन पुरस्कार प्राप्त हुआ। यह पुरस्कार नराकास के वार्षिक समारोह में दिनांक 10.04.08 को सायं 3.00 बजे राष्ट्रीय तकनीकी प्रशिक्षण संस्थान (टी.टी.टी.आई), सैक्टर 26, चण्डीगढ़ में वार्षिक राजभाषा समारोह के दौरान प्रदान किया गया। श्री पी.के.चोपड़ा, मुख्य आयकर आयुक्त एवं अध्यक्ष, नराकास, चण्डीगढ़ द्वारा कार्यक्रम की अध्यक्षता की गई।

श्री पी.एस. चोपड़ा, मुख्य आयकर आयुक्त एवं श्री श्री डी.एस.सरा, मुख्य आयुक्त, केन्द्रीय उत्पाद शुल्क से इमटैक की ओर से राजभाषा पुरस्कार ग्रहण करते श्री एस.डी. ऋषि, अअ. एवं सुश्री नवनीत आनन्द, हिन्दी अधिकारी

निदेशक, इमटैक किन्हीं प्रशासनिक कारणोंवश समारोह में उपस्थित नहीं हो पाए। अतः श्री एम.आर.मसान, प्र.नि. ने संस्थान का प्रतिनिधित्व किया। इस अवसर पर राजभाषा अनुभाग से श्री एस.डी.ऋषि, अनुभाग अधिकारी तथा सुश्री नवनीत आनंद, हिन्दी अधिकारी को भी सम्मानित किया गया।

संस्थान की वार्षिक रिपोर्ट भी द्विभाषी तैयार की जाती है।





INSTITUTE OF MICROBIAL TECHNOLOGY

Sector 39-A,Chandigarh-160036, INDIA

Tel. : +91 172 2695215, 2690908, 2690173, 2690025

Fax : +91 172 2690585, 2690632

E-mail : root@imtech.res.in

Website : www.imtech.res.in

INSTITUTE OF MICROBIAL TECHNOLOGY

सूक्ष्मजीव प्रौद्योगिकी संस्थान

Sector 39-A,Chandigarh-160 036, INDIA सैक्टर 39–ए, चण्डीगढ़ – 160 036