BIOLOGICAL SCIENCES CLUSTER

Scientific Excellence

Mutational variations buffered by chemical chaperons

Hidden genetic variations have the potential to lead to the evolution of new traits. Molecular chaperones, which assist protein folding, may conceal genetic variations in protein-coding regions. CSIR-IGIB has investigated the potential of chemical milieu of cells to affect protein folding. It was found that osmolyte trimethylamine N-oxide (TMAO) can buffer mutations that impose kinetic traps in the folding pathways of two model proteins. This study has provided a new insight into understanding of an important phenomenon.

Tuberculosis Drug Streptomycin as a Potential Cancer Therapeutic

CSIR-IGIB has reported that microRNAs (miRNAs) fine-tune gene expression, deregulation which has been causally associated with a number of debilitating conditions. Streptomycin, a well-known aminoglycoside drug, binds to RNA secondary structures and is shown to inhibit miR-21 function by direct binding to its precursor, thus presumably interfering with the processing by the Dicer enzyme.

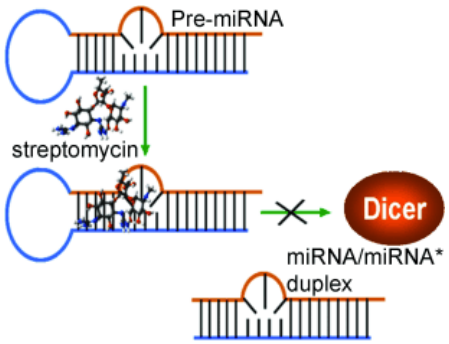

Fig. 1.1 Scheme

Nonprocessive [2 + 2]e- off-loading reductase domains from mycobacterial nonribosomal peptide synthetases

In mycobacteria, polyketide synthases and nonribosomal peptide synthetases (NRPSs) produce complex lipidic metabolites by using a thio-template mechanism of catalysis. CSIR-IGIB has demonstrated that the off-loading reductase (R) domain of mycobacterial NRPSs performs two consecutive [2 + 2]e(-) reductions to release corresponding alcohols. The first crystal structure of an R domain from *Mycobacterium tuberculosis* NRPS provides strong support to this mechanistic model. This study presents an elegant example of the recruitment of a canonical short-chain dehydrogenase/ reductase family member as an off-loading domain in the context of assembly-line enzymology.

Common Variants of IL6, LEPR, and PBEF1 are associated with obesity in Indian Children

The increasing prevalence of obesity in urban Indian children is indicative of an impending crisis of metabolic disorders. Although perturbations in the secretion of adipokines and inflammatory molecules in childhood obesity are well documented, the contribution of common variants of genes encoding them is not well investigated. CSIR-IGIB has assessed the association of 125 common variants from 21 genes, encoding adipocytokines and inflammatory markers in 1,325 urban Indian children. Associations of rs2069845, rs1137100, and rs3801266 were replicated (P = 7.9 ×

10(-4), 8.3 × 10(-3), and 0.036, respectively) and corroborated in meta-analysis (P = 2.3 × 10(-6), 3.9 × 10(-5), and 4.3 × 10(-4), respectively) that remained significant after multiple testing corrections. This is a unique study where the association of the common variants of IL6, LEPR,and PBEF1 with obesity studied in Indian children.

Metastases suppressor NME2 associates with telomere ends and telomerase and reduces telomerase activity within cells

Analysis of chromatin-immunoprecipitation followed by sequencing (ChIP-seq) usually disregards sequence reads that do not map within binding positions (peaks). Using an unbiased approach, CSIR-IGIB has analysed all reads for ChIP-seq experiments for human lung adenocarcinoma and fibrosarcoma cells for the metastasis suppressor non-metastatic 2 (NME2). It was found that NME2 associates with telomerase and reduces telomerase activity *in vitro* and *in vivo*, and resulted in reduced telomere length in aggressive human cancer cells. These findings reported a novel role for NME2 as a telomere binding protein that can alter telomerase function and telomere length. This presents an opportunity to investigate telomere-related interactions in metastasis suppression.

Genome-wide analysis reveals distinct patterns of epigenetic features in long non-coding RNA loci

A major fraction of the transcriptome of higher organisms is comprised of an extensive repertoire of long non-coding RNA (lncRNA). lncRNAs are proven component of epigenetic gene expression modulation, though their regulation remains poorly understood. CSIR-IGIB has analysed pan-genomic DNA methylation and histone modification marks associated with transcription start site (TSS) of lncRNA in four different cell types and three different tissue types representing various cellular stages. It has been observed that histone marks associated with active transcription have similar distribution pattern around TSS irrespective of cell types. Also, the density of these marks correlates well with expression of protein-coding and lncRNA genes. In contrast, the lncRNA genes harbour higher methylation density around TSS than protein-coding genes regardless of their expression status. These observations suggest that epigenetic regulation of lncRNA common features with mRNA except the role of DNA methylation which is markedly dissimilar.

Translational applications of hologenome sequencing: using next-generation sequencing on a sample containing mixed population of genomes from an epidemic with appropriate processing and enrichment

CSIR-IGIB has developed the hologenome sequencing approach which can accurately identify causative pathogens from cell culture hologenome samples containing mixed population of genomes and in principle can be applied to patient hologenome samples without any background information. This methodology could be widely applied to identify and isolate pathogen genomes and understand their genomic variability during outbreaks.

The data has been analyzed by CSIR-IGIB using an extensive computational pipeline involving mapping to reference genome sets and de-novo assembly. In depth analysis of the data generated revealed the presence of sequences corresponding

to Japanese encephalitis virus. The genome of the virus was also independently de-novo assembled. The presence of the virus was in addition, verified using standard molecular biology techniques.

New diagnostic method for detecting airway diseases

CSIR-IGIB has developed a new diagnostic method for detecting airway diseases. More than hundred patients with asthma or COPD have already been studied using this technique. It holds potential for early detection of bronchiolitis obliterans in post-transplant patients, a life threatening disease that can be suppressed if detected early. By using standard impulse oscillometry data and physiologic time series analysis, the study identifies cyclical and stochastic fluctuations in respiratory resistance during normal tidal breathing. Exaggeration of cyclical changes due to peripheral airway closure during exhalation is measurable as a novel index of lung function that provides exquisite detection of peripheral airway disease. Medical organizations worldwide have shown interest in adopting and testing this method. This study offers an entirely new direction in evaluation and monitoring of asthma and other airway diseases and points to the importance of application of mathematical tools to biological data for developing sensitive noninvasive markers in diseases.

A Molecule from Herbal Origin for the Treatment of Bronchial Asthma

Asthma is a non-infectious chronic inflammatory disease of the respiratory system characterized by a reversible airways obstruction. Acute airway obstruction, bronchial hyper-responsiveness and inflammatory state of the bronchial mucosa with increase levels of inflammatory mediators, are the most evident phenomenon which characterizes this pathology. Despite the increase in the prescribed anti-asthmatic treatments, the current trends indicate asthma is set to be the most chronic disease in industrialized countries, affecting mostly the children (15%) than the adults (10%). Jointly CSIR-IICB, CSIR-IGIB, CSIR-IIIM, CSIR-IITR and CSIR-IICT scientists have described a novel heterocyclic compound belonging to ‘triazine-aryl-bis-indoles’, useful for the treatment of asthma. The compound prepared is useful as phosphodiesterase-4 (PDE-4) enzyme inhibitor, inhibitor for signalling molecule for the treatment of asthma / COPD and asthma related diseases.

Environmental Contaminants: New Screening Technologies and Effect on Human Health

Alpha-1 antitrypsin (AAT) deficiency is an inherited disorder that causes low levels of, or no alpha-1 antitrypsin in the blood. The most common illness in adults with alpha 1 antitrypsin deficiency is lung disease during the third and fourth decades of life. Most commonly it is associated with chronic obstructive pulmonary disease (COPD). Mutations in the PI gene, located on chromosome 14, are associated with this genetic disorder. The Z protein is due to a single amino acid substitution of 342 glutamineto lysine. Although cigarette smoking is the main environmental risk factor, only about 15% of smokers develop clinically significant disease suggesting other influences on disease expression. The study carried out by CSIR-NEIST included hospital based age and sex matched 100 cases of COPD and 100 controls without COPD recruited from nearby places of Assam. The study reveals that other genes

besides A1AT (ZZ type) could be responsible for the prevalence of COPD. PCR amplification showed characteristic 179bp band indicating the presence of homozygous 'MM' type in all the samples. The sequences obtained were aligned with the mRNA of normal (MM type) Alpha-1-Antitrypsin gene. No difference was found in position Glu342 GAG Lys AAG in COPD smoker, COPD Non-Smoker, Non-COPD Smoker and Non-COPD Non-smoker respectively. It is observed that smoking was the prior cause of COPD. A1AT deficiency is not prevalent in our population subset but certain other genes could be the attributable factor for COPD.

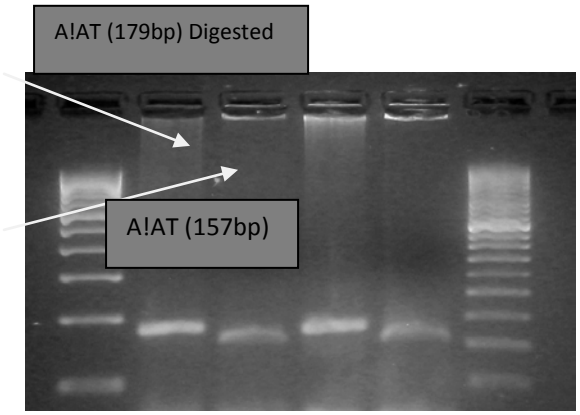

Fig:1.2 Detection of A1AT gene by site directed mutagenesis PCR method. Lanes 1,2 A!AT gene179bp normal (MM type). The primers used to amplify the sequence that included the Z mutation site yielded a product of the corrected size (179 bp) in all cases.

Multistress-protective molecules in bacteria

Evidences in the literature reveal that bacteria in the natural environments are most often challenged with more than one stress condition (extremities of temperature, pH, high pressure, high oxidative stress, high osmolarity, uv-radiation and exposure to toxic chemicals). Hence they evolve with some molecules (proteins, lipids) which confer multistress-tolerance in them. These multistress-protective molecules interlink mechanisms involved in adaptation of bacteria to different types of stress conditions. They could be useful for creating genetically engineered bacterial strains suitable for bioremediation in harsh environments. Their inhibitors could be useful for safe preservation of food materials.

No information was available on the tolerance of microorganisms that occur in the extreme cold climate of Antarctica to environmental stress factors. Database created by CSIR-CCMB on the stress tolerance of Antarctic bacteria fills up the gap. It has been demonstrated that the Antarctic bacteria are predominantly tolerant to alkali besides low temperature and sensitive to acid and ultraviolet- radiation. Some multistress-sensitive mutants have been obtained by Tn-5 transposon mutagenesis of two Antarctic organisms belonging to the genus *Pseudomonas*. By inverse PCR, it is found that the mutated gene in a potential multistress-sensitive mutant encodes citrate synthase. Other genes identified from similar mutants encode a putative oxidoreductase, a putative ABC transporter mannitol binding protein, a phosphotransferase and a general stress protein. Studies are in progress to detect the molecules that are found to be overproduced in some multistress-adapted

variants of several Antarctic and mesophilic organisms, by analyzing their protein and lipid profile.

Importance in bioremediation of pollutants: The multistress-protective molecules isolated and characterized are useful for various purposes. Petroleum products and other man-made garbage that are accumulating in the harsh environments (polar regions, Siachen glacier) due to human activities (science camp, military camp, expedition) pose a serious threat to the environment. Removal of the contaminated soil by air-lifting being prohibitively costly, bioremediation using degrader bacteria strain appears to be a suitable alternative. However, bioremediation in harsh environments is not possible because of the very slow growth of bacteria in non-permissive environments. The growth rate of degrader bacteria could be improved by cloning and expressing the genes which encode the multistress protective proteins. Evidences in the literature support this idea. It was possible to increase the growth rate of the mesophilic bacterium *E coli* by 141-times at 8^{o}C by recruiting two chaperonin proteins from an Antarctic bacteria through genetic engineering.

Importance in food preservation: The multistress-protective molecules confer multistress-tolerance to food-borne bacteria. When food materials are exposed to several stressors (cold-water, organic acids, high salt solution, hydrogen peroxide) during processing and storage to render them safe for use, a substantial portion of the bacteria present in the food material are annihilated but the survivors get more tolerant to the stress factors they are exposed to. They also develop cross tolerance to some other stress factors they are not exposed to. Stress hardness and cross-tolerance are two major problems encountered in the food industry. If the chemical nature of the multistress protective molecules is known, their inhibitors could be designed for the safe preservation of food materials.

RNA binding proteins and gene regulation

In understanding role of RNA binding proteins in gene regulation, CSIR-CCMB has solved solution structures of dsRNA binding domains of RDE-4 and CRC. Based on the studies, laboratory hypothesizes that two consecutive lysine mutations in dsRBD2 of RDE-4 impairs RNA recognition and hence disable RNAi pathway in *C. elegans*. Structural data on CRC suggests its divergent evolution from A Pendonuclease family of proteins and adoption to recognize RNA for carrying our regulatory roles in Pseudomonas.

The RNAi pathway of several organisms requires presence of double stranded RNA binding proteins for functioning of Dicer in gene regulation. In *C. elegans*, a double stranded RNA binding protein, RDE-4 (385 aa, 44 kDa) recognizes long exogenous dsRNA and initiates the RNAi pathway. Complete backbone and stereospecific methyl sidechain Ile (d1), Leu and Val chemical shifts of first 243 amino acids of RDE-4, namely RDE-4DC, has been achieved.

Hepatitis C Virus NS5A Binds to the mRNA Cap-binding Eukaryotic Translation Initiation 4F (eIF4F) Complex

For the first time CSIR-CCMB has shown that HCV up-regulates host cap-dependent translation machinery in Huh7.5 cells through simultaneous activation of mTORC1

and eukaryotic translation initiation factor 4E (eIF4E) by NS5A. NS5A, interestingly, overexpressed and subsequently hyperphosphorylated 4EBP1. NS5A phosphorylated eIF4E through the p38 MAPK-MNK pathway. Both HCV infection and NS5A expression augmented eIF4F complex assembly, an indicator of cap-dependent translation efficiency. Global translation, however, was not altered by HCV NS5A. 4EBP1 phosphorylation, but not that of S6K1, was uniquely resistant to rapamycin in NS5A-Huh7.5 cells, indicative of an alternate phosphorylation mechanism of 4EBP1. Resistance of Ser-473, but not Thr-308, phosphorylation of AKT to PI3K inhibitors suggested an activation of mTORC2 by NS5A.NS5 Associated with eIF4F complex and polysomes, suggesting its active involvement in host translation. This is the first report that implicates an HCV protein in the up-regulation of host translation initiation apparatus through concomitant regulation of multiple pathways. Because both mTORC1 activation and eIF4E phosphorylation are involved in tumorigenesis, CSIR-CCMB has proposed that their simultaneous activation by NS5A might contribute significantly to the development of hepatocellular carcinoma.

Signal Transduction pathways in human health and disease

In understanding of molecular mechanisms in normal physiological and abnormal pathological conditions in particular several human malignancies prominent in Indian sub-continent and also the tuberculosis. The signal transduction pathways particularly focuses on the downstream and upstream of the small GTPase Rap1 and the tumour suppressor Ras association domain family 1A (RASSF1A). Rap1A is a small G protein implicated in a spectrum of biological processes including cell proliferation, adhesion, differentiation and embryogenesis. The downstream effectors through which Rap1A mediates its diverse effects are largely unknown. It is shown that Rap1A, but not the related G proteins Rap2 or Ras binds to the tumour suppressor RASSF1A in a phosphorylation-dependent manner. Interaction with Rap1A influences the effect of RASSF1A on microtubules. Previously, it was shown that RASSF1A is a novel substrate of Protein Kinase C (PKC) and that the PKC upstream control confers a peculiar constraint on some of the cellular functions of RASSF1A such as modulation of the microtubule network dynamics and vimentin filaments. In the view of this, it appears that PKC phosphorylation of RASSF1A could represent a tumour de-suppression pathway that is an alternate to its genetic/epigenetic (deletion, promoter methylation) suppression, this study is underway in the CSIR-CCMB.

Use of nanoscaffolds to differentiate mesenchymal stem cells into osteogenic cell types and their *in vivo* potencies

Reconstruction of critical sized bone injuries is a major problem that continues to inspire the design of new materials and grafts. Natural ceramics [hydroxyapatite (HA) coralline HA, or synthetic HA] and b-tricalcium phosphate (b-TCP) have been explored for use as scaffolds in bone tissue engineering, among several other materials. CSIR-CCMB has evaluated the bone forming capacity of nanosize bioceramics synthesized in situ in poly-vinyl alcohol (PVA) with different ratios of HA and b-TCP; the Ca/P ratio was 1.62 for bioceramic P1, 1.60 for P2 and 1.58 for P3.

Further osteogenesis *in vitro* with mesenchymal stem cells (MSC) acquired from different sources and their bone healing properties *in vivo* were also evaluated. MSCs isolated from human placenta, Wharton's jelly from umbilical cord, fetal bone marrow and adipose tissue, cultured in presence of nanosize bioceramic particles, were monitored for osteogenic differentiation. Placental cells showed the best osteogenic potential of the different MSCs studied on the basis of expression of osteogenic markers. Complete regeneration of the damaged region was observed *in vivo* when MSC derived from placenta were used with nanoceramic (Ca/P ratio 1.58) in the experimental defect created in the femur of Wistar rats. Even small variation in the Ca/P ratio can alter the outcome of tissue constructs. Similar studies carried out with biphasic ceramics in ratios from 10:90 to 90:10 were evaluated to assess the role of each component in bone wound healing in animals.

Immune modulatory responses of Mesenchymal stem cells from different sources in cultures and *in vivo*

Immune-properties of Mesenchymal Stem Cells (MSC) isolated from human placenta, umbilical cord matrix, adipose and bone marrow have been studied by CSIR-CCMB. MSC collected from all these sources possess low levels of MHC- a comparison I and lacked MHC-II. These cells inhibited the mixed lymphocytic reaction in culture conditions when incubated with human allogeneic peripheral blood mono nuclear cell (PBMNC) population. Even after differentiation into different cell lineages, these MSCs are able to suppress the mixed lymphocytic reaction when cultured with stimulated allogeneic PBMNC. The cytokines profiles were determined for all types of MSCs when co-cultured with PBMNC. IFNγ and IL-1β increased in supernatants of all co-cultures and whereas TNF-α, I-1α-, IL2 levels diminished. The low immunogenicity *in vitro* suggests that these cells can be used for allogeneic transplantation.

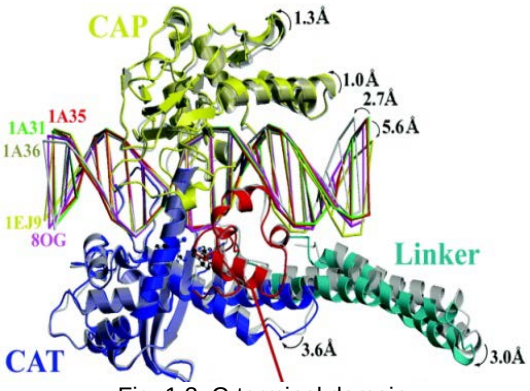

Fig. 1.3 C-terminal domain

A single infusion of MSC into immunocompetent BALB/c mouse resulted in immune suppressive changes in lymph nodes and spleen. Bone marrow and thymus remained largely unchanged. These findings suggest that MSC from AD, PL, and WJ could be substituted for BMSC where it is difficult to get the required number of autologous mesenchymal stem cells of bone marrow.

These studies are important for use in cell therapy for any disorder. CSIR-CCMB has compared Mesenchymal stem cells derived from different sources and most of these sources are medical waste and the cells have been obtained without any invasive procedure and stored. These studies prove the potential of these cells in bone healing injuries and their immunological impact.

An Insight into the Functional Mechanism of Type IB Topoisomerase

Most type IB topoisomerases do not require ATP. However, this enzyme is highly stimulated by ATP. The mechanism underlying this stimulation and involvement of different amino acids of the enzyme in binding with ATP and controlling the activity of the enzyme have been explored by CSIR-IICB scientists.

Visceral Leishmaniasis Immunotherapy

TLR4 stimulation by β-(1-4)-galactose terminal glycoconjugates induce expression of IFN-γ. CSIR-IICB has shown that immunotherapy with the β-(1-4)-galactose terminal glycosphingophospholipid (GSPL) antigen from *L.donovani* induces IFN-γ through the cooperative action of TLR4 and NKT-cells to cure murine visceral leishmaniasis.

New Approach to Treat Drug Unresponsive Kala-azar

The kala-azar patients do not respond to the host-protective cytokine IFNγ at the active stage of the disease, the cause of which is unknown. This research is designed to understand how cell surface receptors for IFNγ respond under parasitized condition. CSIR-IICB scientists have clarified the underlying mechanism and demonstrated that supplementation of cholesterol together with IFNγ may be a new approach to treat drug unresponsive Kala-azar cases.

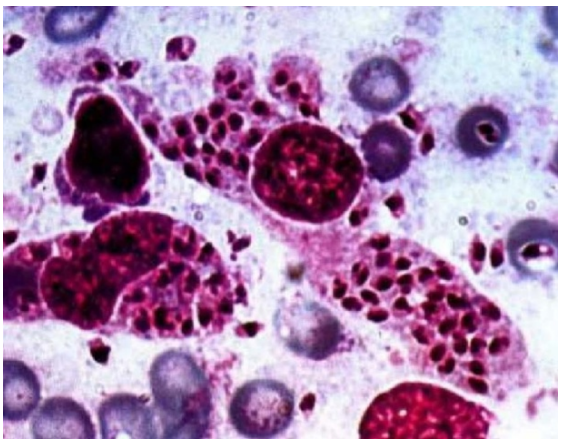

Fig.1.4 New approach to treat drug unresponsive Kala-azar

Easy Test for Visceral Leishmaniasis and Post–Kala-azar Dermal Leishmaniasis

CSIR-IICB has developed a dipstick assay for the serodiagnosis of visceral leishmaniasis (VL) and post-kala-azar dermal leishmaniasis (PKDL). The dipstick can even be used at primary health centers. For detection of the circulating anti-leishmanial IgG in the patient sera, leishmanial antigens prepared from the cultured promastigote membranes (LAg) is used as test antigen. The whole process takes about 2 hours. When stored at room temperature

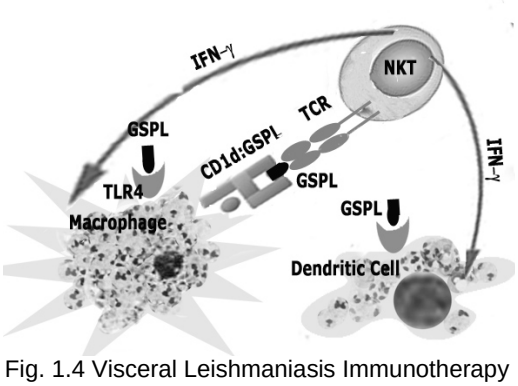

Fig. 1.4 Visceral Leishmaniasis Immunotherapy

without desiccation, dipsticks performs consistently for 12 months. CSIR-IICB dipsticks appeared equally sensitive and specific (100%) for VL from India and Brazil and for Indian PKDL. LAg dipsticks perform better than rK39 strip test for diagnosing VL patients from Brazil. This indigenously developed dipsticks strip test is approximately ten times more economical than rK39 strip test.

A novel quercetin analogue from a medicinal plant promotes peak bone mass achievement

CSIR-CDRI has reported that extracts made from the stem bark of *Ulmus wallichiana* promoted peak bone mass achievement in growing rats and preserved trabecular bone mass and cortical bone strength in ovariectomized (OVX) rats. Further, 6-C-β-D-glucopyranosyl-(2S,3S)-(+)-3',4',5,7-tetrahydroxyflavanol (GTDF), a novel flavonol-C-glucoside isolated from the extracts of *Ulmus wallichiana* had a non-estrogenic bone sparing effects on OVx rats. The effects of GTDF on osteoblast function and its mode of action and *in vivo* osteogenic effect was studied. GTDF stimulated osteoblast proliferation, survival and differentiation, but had no effect on osteoclastic or adipocytic differentiation. In cultured osteoblasts, GTDF transactivated aryl hydrocarbon receptor (AhR). Activation of AhR mediated the stimulatory effect of GTDF on osteoblast proliferation and differentiation. Furthermore, GTDF stimulated cAMP production, which mediated osteogenic gene expression. Together, data suggest that GTDF stimulates osteoblast growth and differentiation via AhR and promotes modeling-directed bone accrual, accelerates bone healing after injury and exerts anabolic effects on osteopenic rats by likely direct stimulatory effect on osteoprogenitors. Based on these preclinical data, clinical evaluation of GTDF as potential bone anabolic agent is warranted.

L-Proline catalysed multicomponent synthesis of 3-amino alkylated indoles via a Mannichtype reaction under solvent-free conditions

An efficient L-proline catalyzed one-pot synthesis of 3-amino-alkylated indoles has been developed by CSIR-CDRI via a three-component Mannich-type reaction viz. secondary amines, aldehyde and indoles under solvent-free conditions at room temperature. Several amino acids (acidic, basic and neutral) have been screened for the reaction but the best results were obtained with L-proline.

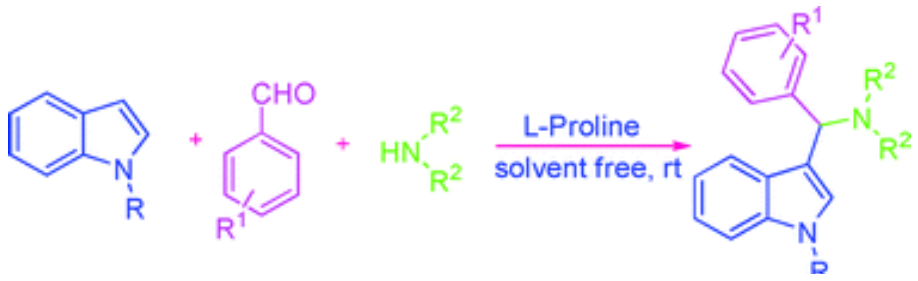

Fig.1.5 L-proline catalysed multicomponent synthesis of 3-amino alkylated indoles

β-Cyclodextrin catalysed synthesis of tryptanthrin

An efficient and green method has been developed by CSIR-CDRI for the synthesis of tryptanthrin employing β-cyclodextrin as a catalyst in aqueous media at room temperature from isatoic anhydride and isatin. The reactions were performed under mild conditions to afford biologically active natural product tryptanthrin in excellent yields.

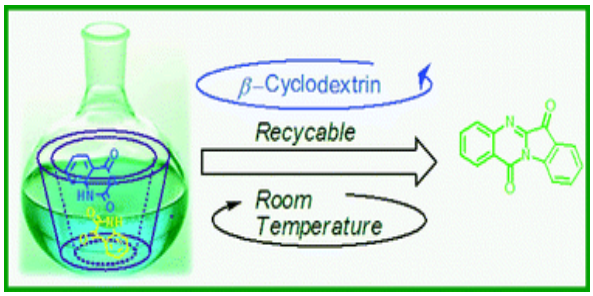

Fig.1.6 β-cyclo-dextrin catalysed synthesis of tryptanthrin

Partial biodistribution and pharmacokinetics of isoniazid and rifabutin following pulmonary delivery of inhalable microparticles to rhesus macaques

Dry powder inhalations (DPI) of microparticles containing isoniazid (INH) and rifabutin (RFB) are under preclinical development at CSIR-CDRI for use in pulmonary tuberculosis. Microparticles containing 0.25, 2.5, or 25 mg of each drug were administered daily for 90 days to rhesus macaques (n = 4/group). Single inhalations or intravenous (i.v.) doses were administered to separate groups. Drugs in serum, alveolar macrophages, and organ homogenates were assayed by high-performance liquid chromatography (HPLC). The RFB/INH in the lungs (101.10 ± 12.90/101.07 ± 8.09 µg/g of tissue) was twice that of the liver concentrations (60.22 ± 04.97/52.08 ± 4.62 µg/g) and four times that of the kidneys (22.89 ± 05.22/30.25 ± 3.71 µg/g). Pharmacokinetic parameters indicated the operation of flip-flop kinetics. Thus, the elimination half-life [t(1/2)] of RFB and INH was calculated as 8.01 ± 0.5 and 2.49 ± 0.23 h, respectively, upon intravenous (iv) administration, and as 13.8 ± 0.8 and 10.43 ± 0.77 h following a single inhalation; or 13.36 ± 3.51 and 10.13 ± 3.01 at a presumed steady state (day 60 of dosing). Targeted and sustained drug delivery to nonhuman primate lungs and alveolar macrophages was demonstrated. Flip-flop serum pharmacokinetics was observed, and nonlinearity in some pharmacokinetic parameters at logarithmic dose increments was indicated. The results suggest that human patients would benefit through improvement in biodistribution following DPI.

A synthetic S6 segment derived from KvAP channel self-assembles, permeabilizes lipid vesicles

KvAP is a voltage-gated tetrameric K(+) channel with six transmembrane (S1-S6) segments in each monomer from the archaeon Aeropyrum pernix. CSIR-CDRI has made an attempt to understand the plausible role of the S6 segment, which has been proposed to form the inner lining of the pore, in the membrane assembly and functional properties of KvAP channel. For this purpose, a 22-residue peptide, corresponding to the S6 transmembrane segment of KvAP (amino acids 218- 239), and a scrambled peptide (S6-SCR) with rearrangement of only hydrophobic amino acids but without changing its composition were synthesized and characterized

structurally and functionally. Although both peptides bound to the negatively charged phosphatidylcholine/phosphatidylglycerol model membrane with comparable affinity, significant differences were observed between these peptides in their localization, self-assembly and aggregation properties onto this membrane. S6- SCR also exhibited reduced helical structures in SDS micelles and phosphatidylcholine/ phosphatidylglycerol lipid vesicles as compared with the S6 peptide. Furthermore, the S6 peptide showed significant membrane-permeabilizing capability as evidenced by the release of calcein from the calcein-entrapped lipid vesicles, whereas S6-SCR showed much weaker efficacy. Interestingly, although the S6 peptide showed ion channel activity in the bilayer lipid membrane, despite having the same amino acid composition, S6-SCR was significantly inactive. The results demonstrated sequence specific structural and functional properties of the S6 wild type peptide. The selected S6 segment is probably an important structural element that could play an important role in the membrane interaction, membrane assembly, and functional property of the KvAP channel.

Anti-inflammmatory properties of melittin, venom antimikcrobial peptide

The bee venom antimicrobial peptide, melittin, besides showing versatile activity against microorganisms also neutralizes lipopolysaccharide (LPS)-induced proinflammatory responses in macrophage cells. However, how the amino acid sequence of melittin contributes in its anti-inflammatory properties is mostly unknown. To determine the importance of the leucine zipper sequence of melittin in its neutralization of LPS-induced inflammatory responses in macrophages and interaction with LPS, anti-inflammatory properties of melittin and its three analogues and their interactions with LPS were studied in detail by CSIR-CDRI. Two of these analogues, namely melittin Mut-1 (MM-1) and melittin Mut-2 (MM-2), possess leucine to alanine substitutions in the single and double heptadic leucine residue(s) of melittin, respectively, whereas the third analogue is a scrambled peptide (Mel-SCR) that contains the amino acid composition of melittin with minor rearrangement in its leucine zipper sequence. Although MM-1 partly inhibited the production of proinflammatory cytokines in RAW 264.7 and rat primary macrophage cells in the presence of LPS, MM-2 and Mel-SCR were negligibly active. A progressive decrease in interaction of melittin with LPS, aggregation in LPS, and dissociation of LPS aggregates with alteration in the leucine zipper sequence of melittin was observed. Furthermore, with alteration in the leucine zipper sequence of melittin, these analogues failed to exhibit cellular responses associated with neutralization of LPS-induced inflammatory responses in macrophage cells by melittin. The data indicated a probable important role of the leucine zipper sequence of melittin in neutralizing LPS-induced proinflammatory responses in macrophage cells as well as in its interaction with LPS.

Cascade intermolecular michael addition–intramolecular azide/internal alkyne 1,3-dipolar cycloaddition reaction in one pot

A rapid one-pot protocol for the synthesis of indole-based polyheterocycles via a sequential Lewis acid catalyzed intermolecular Michael addition and an intramolecular azide/internal alkyne 1,3-dipolar cycloaddition reaction has been

described by CSIR-CDRI. The generality of the method has been demonstrated by treating a series of aromatic/aliphatic 2-alkynyl indoles with substituted (E)-1-azido-2-(2-nitrovinyl) benzenes to furnish annulated tetracyclic indolo[2,3-c][1,2,3] triazolo [1,5-a][1]benzazepines in good yields.

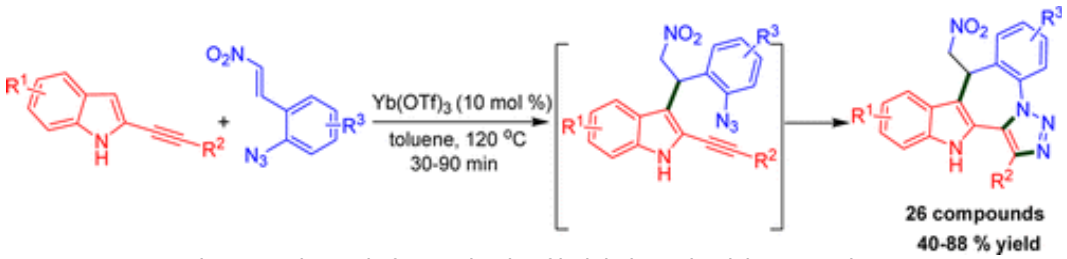

Fig. 1.7 Schematic for synthesis of indole based polyheterocycles

Candidate Drugs under Advance Stages of Development

CSIR-CDRI has developed following candidate drugs which are under advance stages of development as listed for specific disease/disorder:

Table 1.1 Candidate drugs under advance stage for development

Diseases / Disorders	Candidate Drug	Efficacy	Clinical Status	Licensees & Collaborators
Liver Disorder	**Picroliv**	Hepatoprotective	Phase III clinical trial completed at CSSMU, Lucknow and Seth GS Medical College & KEM Hospital Mumbai.	DIL, Mumbai
Dyslipidemia	**80-574 + Atorvastatin**	Antidyslipidemic	Extended phase III clinical trial	Cadila Pharmaceuticals Ltd., Ahmedabad
Malaria	**97-78**	Antimalarial	Phase-I single dose clinical study completed. Single dose pharmacokinetic study in healthy volunteers initiated.	IPCA Labs., Mumbai
Diabetes	**CDR 134D123**	Antihyperglycemic	Phase-I single & multiple dose studies completed. A quality document as per Ayurvedic Pharmacopoeia of India specifications, is being compiled for submission to CCRAS for expedited inclusion of *Xylocarpus granatum* in the Extra Ayurvedic Pharmacopeia to avail marketing permission from AYUSH. Additional Quality Monograph including additional Botanical & Chemical information submitted to DGCCRAS.	TVC Sky Shop Ltd., Mumbai
Diabetes & Dyslipidemia	**CDR 134F194**	Antihyperglycemic	DCG(I) permission to conduct Phase 1 Clinical Trial received in May 2011 and preparation for the same has been initiated. The preparations for the drug formulation to be used in Phase-I Single Dose and Multiple Dose Clinical Trial studies from a GMP certified company is in progress and the clinical trial would commence soon	TVC Sky Shop Ltd., Mumbai
Osteoporosis	**99-373**	Anti-osteoporotic	Plan and Protocol of phase I clinical trial has been approved by DCG(I).	Negotiations in progress with MDRI
Malaria	**99-411**	Antimalarial	Pre-clinical data is under compilation for IND submission.	IPCA Labs., Mumbai
Stroke	**Herbal Medicament**	Neuroprotective	Draft IND received from Themis Medicare is under review at CDRI for preparation of final IND document	Themis Medicare Ltd., Mumbai

Potential New Leads

CSIR-CDRI has developed the following potential new leads and are ready for licencing.

Table 1.2 Potential New leads

Diseases / Disorders	Lead	Efficacy	Status	Licensees & Collaborators
Osteoporosis	**CDR1020F147** OsteoJuvenate	Optimum bone health	Completed requisite preclinical development; Process to obtain approval for clinical trial has been initiated	Natural Remedies Pvt., Ltd., Bangalore
Osteoporosis	**S-007-1500**	Fracture healing	Compound found safe in single dose toxicity study by oral route in rat and mice and by IM route in rat. One year stability study completed	Under negotiation
Osteoporosis	**CDR914K058**	Osteogenic	Efficacy established in animal models. Synthesis of compound in progress Licensing agreement with Kemxtree, USA is in the final stage for developing it as a rapid bone fracture healing anabolic agents	Under negotiation
Thrombosis	**S-007-867**	Anti-thrombotic	Compound found safe in single dose toxicity study by oral route in rat and mice and by IM route in rat.	Open for licensing
	S-002-333	Anti-thrombotic	Compound found safe in single dose toxicity study by oral route in rat	Open for licensing
Diabetes & Dyslipidemia	**CDR267F018**	Anti-dyslipidemic	28 day repeat dose toxicity study in Rh monkey: No significant toxic effect observed up to dose of 250 mg/kg po.	Open for licensing
Contraception	**S-010-1255**	Spermicidal & Anti-trichomonal	Potent spermicidal and anti-trichomonal (against both metronidazole susceptible and resistant strains) activity established with much higher safety index compared with Nonox-9	Open for licensing
Cancer	**S-009-131**	Anti-cancer	As per the studies in mice bearing cervical cancer (HeLa), activity is better than that of standard drug Adriamycin	Open for licensing
Tuberculosis	**S-006-830**	Anti-TB	MIC<3 µg/mL for Mtb H37Rv. Efficacy established *in vitro* & *ex vivo.* Large scale synthesis completed. QC analysis of pure compound in progress.	Being developed under OSDD

Demonstration of Process Know-how of CDR-134- D123 (antihyperglycemic)

CDR134D123 is a standardized fraction isolated from a marine source. The product has shown promising antihyperglycemic activity. CSIR-CDRI has licensed the product to M/s TVC Skyshop Ltd., Mumbai for further development and commercialization. Phase I single and multiple dose study have been completed. Efforts are in place to avail marketing permission for the product in herbal mode. The process Know-how for preparation of CDR134D123 has been successfully demonstrated to the M/s TVC Skyshop Ltd during year.

Novel Path of Curing Ulcer by Indian Traditional Healer, the Turmeric

Fig.1.8 Indian Traditional Healer, the Turmeric

Gastric ulcer relates injury to mucosal layer with disrupted blood vessels, collagen matrices and vascular architecture in stomach. CSIR-IICB has found that curcumin (major component of turmeric) significantly accelerates healing of gastric ulceration caused by excessive intake of pain killer. The novel path is the induction of angiogenesis (new blood vessel formation) via upregulation of matrix metalloproteinase-2 during healing phase.

Antioxidant Enzymes from Mangrove Fungi & Actinomycetes: A Novel Tool for Industrial Applications

Mangrove microbes are ascertained to be an important source of new bioactive compounds. Marine actinomycetes and fungi are efficient producers of new secondary metabolites that show a range of biological activities including antibacterial, antifungal, anticancer, antioxidant producers etc. CSIR-IMMT has isolated actinomycetes and fungi from sediments of three different mangroves areas such as Bhitarakanika, Dhamra and Mahanadi areas of Odisha. The physico-chemical parameters have direct effect on the microbial diversity of the study areas. Isolated strains were screened for the presence of antioxidant enzyme i.e. catalase. Out of these isolates, the strains showing maximum effervescence during screening were studied for assay purpose. Fungal and actinobacterial strains such as MGF-3 and MGA-6 were showing maximum catalase activity such as 101U and 13U of enzyme activity respectively. Strains were identified morphologically, biochemically and microscopically as *Streptomyces sampsonii* and *Trichoderma viride*. It is concluded that the mangrove microbes are the potential source of antioxidant enzymes and can be further exploited for various industrial purposes.

New insecticidal proteins from plants

CSIR-NBRI has isolated six insecticidal proteins, three from ferns, two from mosses and one from salad onion. All of them were effective against whiteflies (LC50 ranging from 175 µg to 10 µg/ml), while three were also found effective against aphids. All toxin encoding genes were cloned from the source plants. Three of these insecticidal genes were cloned at the downstream of four promoters separately (CaMV35S, two insect specific promoters and one phloem specific promoter) in pBI based binary vector. In this way, a total of 12 plant transformation vectors were developed.

Natural Strawberry Red Color from Plants

CSIR-NBRI has developed technology for Natural Strawberry Red color for cosmetic use as lip balm. The isolated color is stable, lipophilic and non-toxic. The product has antioxidant and antimicrobial properties.

Fig.1.9 Lip Balm Natural Strawberry red color

Genomic and expressed SSRs for levant cotton *(Gossypium herbaceum L.)*

Four microsatellite-enriched genomic libraries for CA(15), GA(15), AAG(8) and ATG(8) repeats and transcriptome sequences of five cDNA libraries of *Gossypium herbaceum* were explored to develop simple sequence repeat (SSR) markers by CSIR-NBRI. A total of 428 unique clones from repeat enriched genomic libraries were mined for 584 genomic SSRs (gSSRs). In addition, 99780 unigenes from transcriptome sequencing were explored for 8900 SSR containing sequences with 12471 expressed SSRs. The study resulted in addition of 1970 expressed SSRs and 263 gSSRs to the public domain for the use of genetic studies of cotton. When 150 gSSRs and 50 expressed SSRs were tested on a panel of four species of cotton, 68 gSSRs and 12 expressed SSRs revealed polymorphism. These 200 SSRs were further deployed on 15 genotypes of levant cotton for the genetic diversity assessment. This is the first report on the successful use of repeat enriched genomic library and expressed sequence database for microsatellite markers development in *G. herbaceum*.

Response of stomata to environmental determinants and ABA in *Selaginella bryopteris*, a resurrection spike moss species

Experimental studies carried out by CSIR-NBRI demonstrated that *S. bryopteris* plants show a poor mechanism for its stomatal regulation in response to high light, high temperature, high VPD, high CO_2 and to ABA treatment. At the same time the plants show a high stomatal conductance leading to unrestricted rates of transpiration and a lack of capacity to optimize water use efficiency (WUE). Apparently the plant survives under desiccation by down regulation of metabolic activity and inward curling of its fronds which reduced the transpiration by reducing effective leaf area.

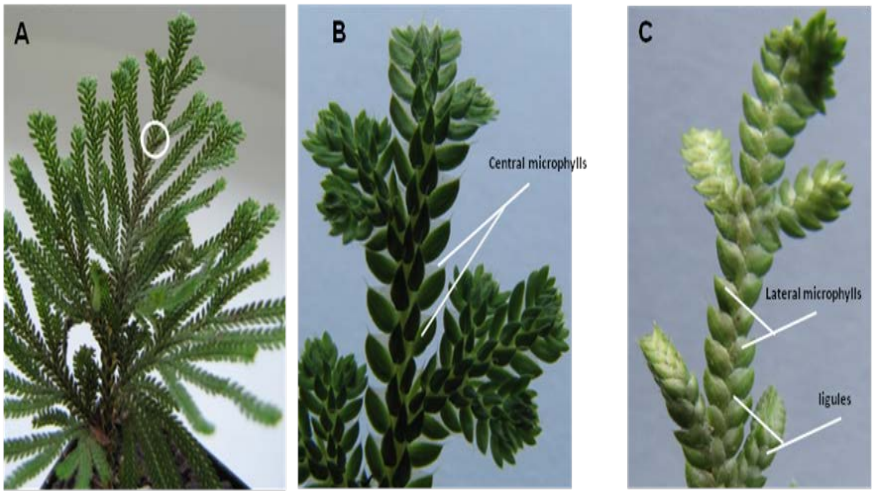

Fig. 1.10 Fronds of *S. bryopteris* growing in a pot (A). Close dorsal view of the frond showing the alternate arrangement of the microphylls on the axis (B). Ventral view of the fronds showing the lateral microphylls and the transparent ligule at its base (C)

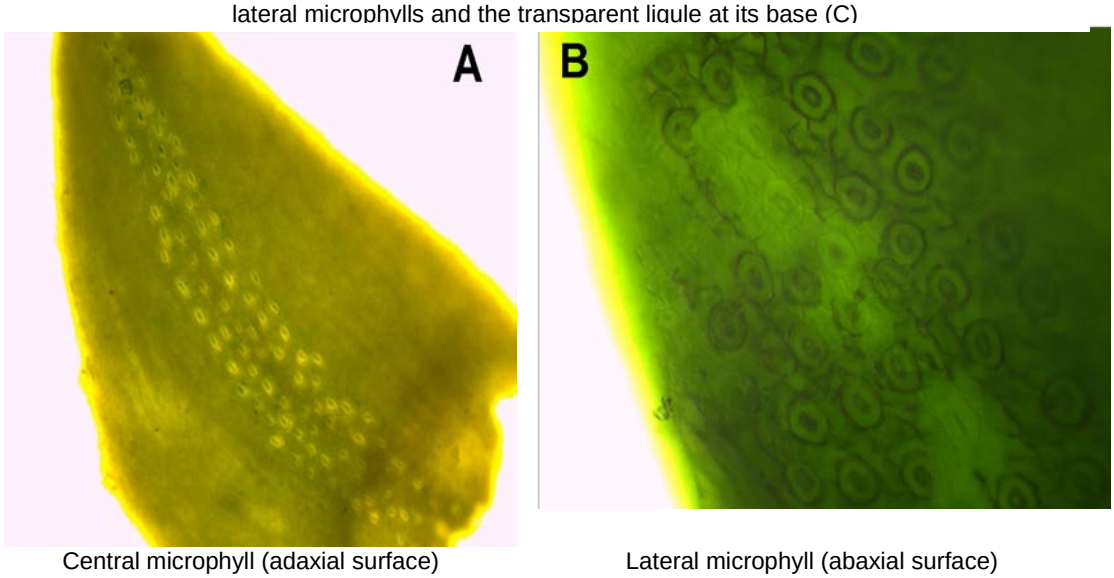

Fig. 1.11 Morphometric study of the stomata of *S. bryopteris*. Adaxial surface view of the central microphyll showing the presence of stomata in a regular row pattern (A). Abaxial surface of the lateral microphyll showing the scattered stomata (B)

Differential effects of Arsenic (As) on essential and non-essential amino acids in rice grains

CSIR-NBRI has analyzed the amino acids (AA) profile in rice grain grown in As affected areas. The sixteen rice genotypes differing in grain As accumulation, grown at three sites with different soil As concentrations, in ascending order, Chinsurah<Purbosthali<Birnagar were analyzed for the amino acid (AA) profile. Grain As accumulation negatively correlated with essential amino acids (EAAs) which were more prominent in high As accumulating rice genotypes (HAARGs). Conversely, non-essential amino acids (NEAAs) showed an increase in low As accumulating rice genotypes (LAARGs) but a decrease in HAARGs. EAAs like isoleucine, leucine, valine, phenylalanine, and tyrosine also decreased in most of the genotypes. NEAAs like glutamic acid, glycine, proline, and histidine showed an increase in all LAARGs. Likewise, sulfur containing AAs (methionine and cysteine) increased in LAARGs but decreased in HAARGs. Among NEAAs in HAARGs, only arginine and serine showed

some induction in most of the genotypes. At the highest As site (Birnagar) total EAAs and NEAAs showed significant reduction in HAARGs compared to LAARGs. The study concluded that As accumulation in rice grain alters EAAs and NEAAs differentially, and reduction was more pronounced in HAARGs than in LAARGs. Thus, As tainted rice limits required levels of AAs in rice based diets and therefore cannot alone fulfill the recommended daily intake (RDI) of AAs.

Pathway engineering and system biology approach towards homologous and heterologous expression of high-value phytoceuticals

The molecular basis of metabolite production in *Podophyllum hexandrum* and *Picrorrhiza kurrooa* has been elucidated by CSIR-IHBT. Improved protocol for the isolation of RNA from rhizome tissue of *P. hexandrum* was developed and transcriptome data were generated. De novo assembling to obtain a total of 60,089 assembled transcript sequences, with an average coverage of 88.34 and average length of 543.11bp was carried out. A total of 11 full-length genes associated with podophyllotoxin biosynthesis were cloned and analyzed. These were Phenylalanine ammonialyase (PAL), p-CoumaroylCoA ligase (4CL), Cinnamic acid 4-hydroxylase (C4H), Hydroxycinnamoyl transferase (HCT), Caffeic acid 3-O-methyltransferases (COMT), Cinnamoyl CoA NADPH oxidoreductase (CCR), Pinoresinol lariciresinol reductase (PLR) and Secoisolariciresinol dehydrogenase (SLD), P450s (of 3 different types). Detailed expression analysis was carried out to identify the possible regulatory genes of the pathway. The functionality of SLD was validated. Transcriptome data of leaf tissue of *P. kurrooa* were generated. A total of 74,336 assembled transcript sequences were obtained, with an average coverage of 76.6 and average length of 439.5. A regeneration protocol was also standardized using leaf segments and aseptic shoot cultures. Successfully optimised the parameters for developing transgenic plants using GUS reporter gene.

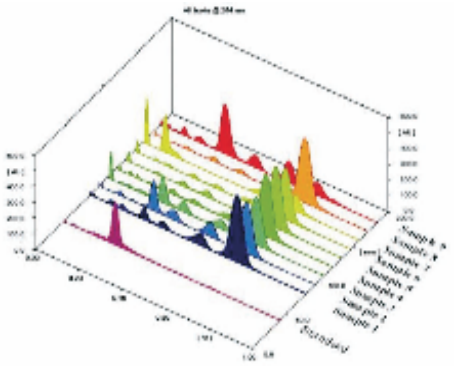

Fig. 1.12 HPTLC CCD image at 254 nm: standard tracks (A-E) of 1b at different concentrations, samples tracks 1-9 indicating formation of 1b

De-novo sequence assembly and transcriptome analysis of *Venturia inaequalis*, the deadly apple scab pathogen

Apple scab caused by *Venturia inaequalis* is one of the most deadly diseases of apple. Due to several distinct features, it has emerged as a model fungal pathogen to study various aspects of hemibiotrophic plant pathogen interactions. CSIR-IHBT has studied, de-novo assembling, annotation and characterization of the transcriptome of *V. inaequalis*.

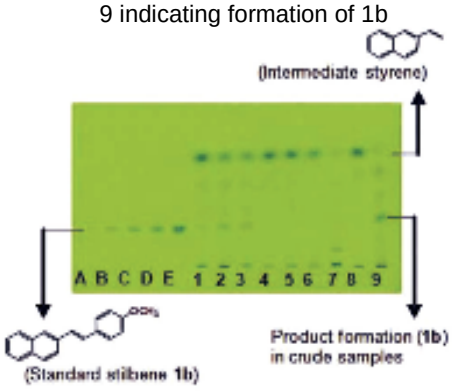

Fig.1.13 3D HPTLC densitogram of samples

In total, 94,350,055 reads specific to Venturia were obtained, and assembled into 62,061 contigs representing 24,571 unique genes. Biological process categories like metabolic process, response to stimulus, nucleic acid metabolism, cellular process and transport were among the highly represented GO terms. Also, they made effort to explore EC classification, KEGG pathway and those encoding kinases, proteases, glycoside hydrolases, cutinases, cytochrome P450 and transcription factors present in this scab pathogen. Several putative pathogenicity determinants and candidate effectors of *V. inaequalis* were identified. Large number of transcripts encoding membrane transporters has been identified and comparative analysis revealed that the number of transporters encoded by Venturia is significantly more as compared to that encoded by several other important plant fungal pathogens. Interestingly, RxLR motifs were detected in several of the secreted proteins of Venturia. Phylogenomics study unraveled that *V. inaequalis* is closely related to Pyrenophore tritici-repentis (the causal organism of tan spot of wheat). However, availability of complete genome sequence are required to augment this result. This work would further facilitate research towards understanding the intricacies of its interactions with host apple and innovating newer ways to control this disease.

Dehydrative-Heck olefination of 2° aryl alcohols in ionic liquid for stilbenoide synthesis

Alcohols being readily accessible have received great attention as precursors in various tandem oxidative /dehydrative cross coupling strategies. However, the direct use of 2° aryl alcohols as an *in situ* source of styrene (via dehydration) in Heck coupling has remained unexplored. The cross contamination of reagents/catalysts due to different media requirements in Heck and dehydration steps, limit the scope when these two steps are required to be done in one pot. Moreover, 2° aryl alcohols under Heck like conditions generally get converted into respective carbonyls via isomerization or oxidation processes. A tandem strategy has been developed by CSIR-IHBT wherein 2° aryl alcohols were directly coupled with aryl halides to provide stilbenoids through a dehydrative Heck sequence in the ionic liquid [hmim]Br and producing only water as byproduct under microwave irradiation. Classical methods do not permit this sequence to proceed in one pot, due to cross contamination of catalysts while other methods require multiple steps. This is the first report of its kind that utilizes HPTLC for the detection of the products formation which finally allowed to establish the optimum condition for formation of stilbenoids.

Nickel phthalocyanine assisted highly efficient and selective carbonyl reduction

A reusable green catalytic system based on nickel phthalocyanine with polyethylene glycol- 400 has been developed by CSIR-IHBT for highly chemo- and regioselective reduction of carbonyl compounds to corresponding alcohols at room temperature. The catalytic system tolerated various aromatic, hetero aromatic and aliphatic carbonyl compounds with high turnover number and frequency. This the first report on regioselective reduction of 1,3- and 1,4-benzenedicarbaldehydes to corresponding alcohols. The catalyst was reused up to seven times without any significance loss in activity. PEG-400, by making crown ether-type complex with

sodium borohydride, facilitated the attack of hydride on carbonyl group which was activated by NiPc through Lewis acid–base interaction.

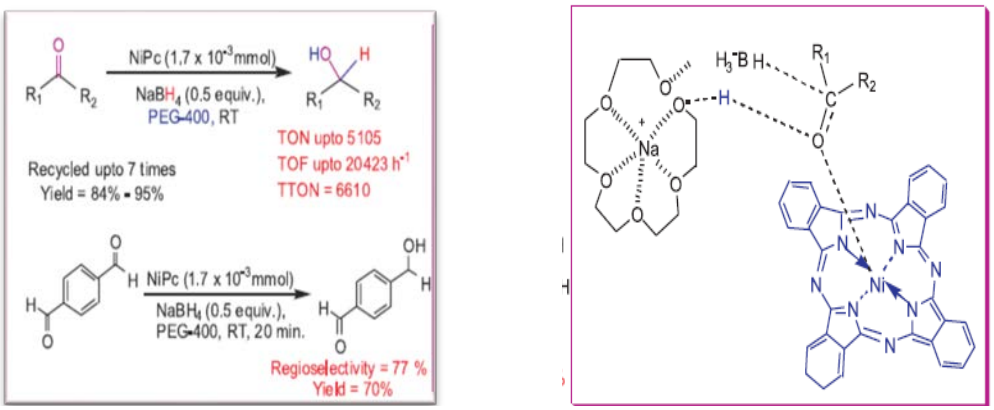

Fig: 1.14 Carbonyl reduction

SYBR green real time PCR based detection of *V. inaequalis*

Early detection of presence of the pathogens is crucial for timely detection of disease to take appropriate reassure to prevent yield losses due to several plant diseases. CSIR-IHBT has reported the robustness of PCR-RFLP based assay to detect and distinguish *Venturia inaequalis*, the causal of apple scab from other fungal pathogens of apple. Efficacy of the primers in SYBR green real-time PCR based assay to sensitively detect the Venturia was tested. DNA isolated from the pure culture of the fungal strains of *V. inaequalis*, *A. alternata*, *G. cingulata, C. acutatum, M. laxa and B. cinerea* were subjected to SYBR based RT-PCR assay. The melting curve analysis revealed distinct Tm for V. inaequalis when compared to other fungi tested (*V. inaequalis*: T_m =89.155; A. alternata: T_m=84.42: G. *cingulata:* T_m=86.77; C. acutatum: T_m=86.32; *M. laxa*: T_m=84.2 and B. cinerea: Tm=83.2; (Fig). The assay was able to detect the pathogen in the field infected leaf.

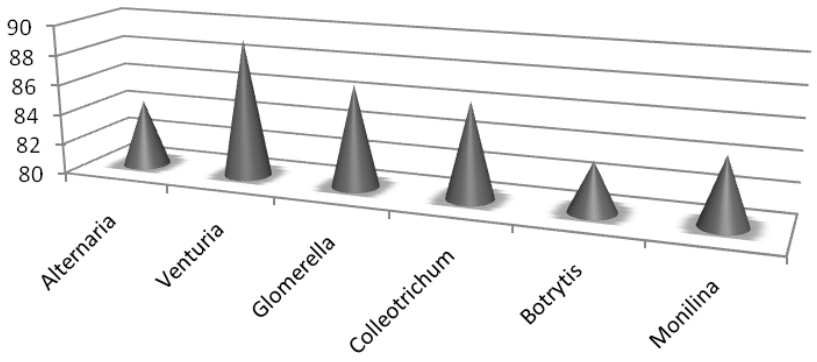

Fig. 1.15 SYBR green real time PCR based detection of *V. inaequalis*

Transgenic crop plants and genes for resistance to insect pests

Transgenic plants of chickpea (T_0 and T_1) expressing Cry1Ac and Cry1Ab endotoxins have been developed and selected promising plants showed complete resistance to *Helicoverpa armigera*. Transgenic T_5/T tomato plants expressing high level of Cry1Ab

endotoxin have shown complete protection against *H. armigera* and *Spodoptera litura* without any effect on yield and are ready for limited trial and commercialization. The Agrobacterium-mediated transformation of embryonic callus of chickpea has been documented and transgenic chickpea plants expressing 116 ng/mg soluble protein of Cry1Ac toxin has been developed via somatic embryogenesis route.

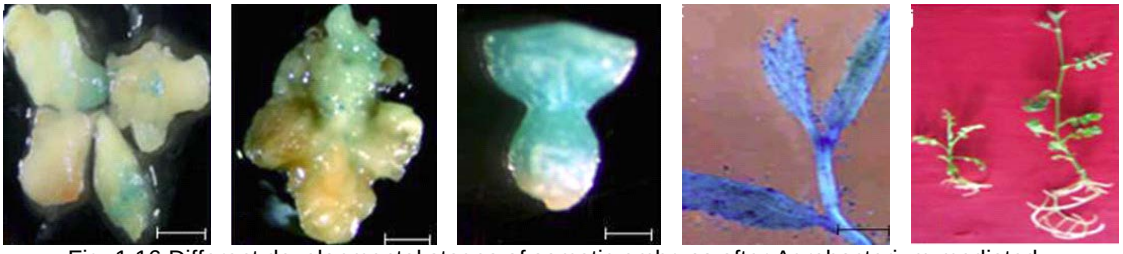

Fig. 1.16 Different developmental stages of somatic embryos after Agrobacterium-mediated transformation of MEA-derived embryogenic callus and molecular characterization of transgenic plants. A-histochemical GUS expression in embryogenic callus (x10), B-torpedo shaped embryo (x10), C-developing dicotyledonary embryo (x40), D-stable GUS expression in leaves of fully developed transgenic plant (x40)., E-complete plantlet regenerated via somatic embryogenesis after Agrobacterium-mediated transformation

Repression of DNA repair protein during rapid meiotic and mitotic division

CSIR-NBRI has studied molecular and functional diversity of microbes which included all agro climatic zones in India. Microarray analysis of *A. thaliana* plants colonized by plant growth promoting bacteria MTCC5279 have been studied to gain insight into MTCC5279 assisted plant growth promotion in *Arabidopsis thaliana*. The gene expression changes, represented by oligonucleotide array (24652 genes) were found to be involved in maintenance of genome integrity (At5g20850), growth hormone (At3g23890 and At4g36110), amino acid synthesis (At5g63890), abcissic acid (ABA) signaling and ethylene suppression (At2g29090, At5g17850), Ca+2 dependent signaling (At3g57530) and induction of induced systemic resistance (At2g46370, At2g44840). The genes At3g32920 and At2g15890 which are suggested to act early in petal, stamen and embryonic development are among the down-regulated genes. This is report of the first time MTCC5279 assisted repression of At3g32920, a putative DNA repair protein involved in recombination and DNA strand transfer in a process of rapid meiotic and mitotic division.

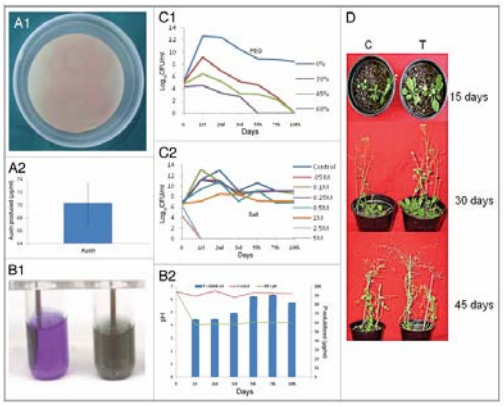

Fig. 1.17 Auxin production (A1, A2); P-solubilization (B1, B2); abiotic stress tolerance (C1, C2) and plant growth promotional attribute to *Pseudomonas putida* MTCC5279 on *Arabidopsis thaliana* (D)

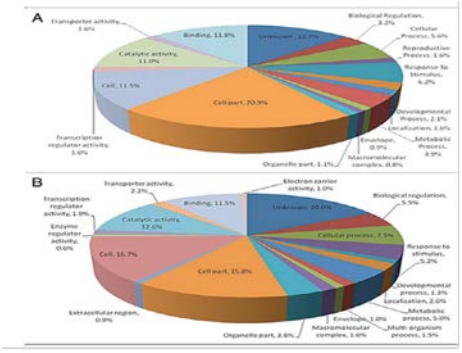

Fig. 1.18 Functional categorization of up-regulated (A) and down-regulated (B) Arabidopsis thaliana genes in terms of their gene ontology involved in lant growth promoion by the colonization of Pseudomonas putida MTCC5279

New report of phosphate solubilizing *Trichoderma* from India

Trichoderma species are usually considered soil organisms that colonize plant roots, sometimes forming a symbiotic relationship. CSIR-NBRI has isolated and identified a new phosphate solubilizing *Trichoderma* (*T. koningiopsis*) from rice plants based on morphological and molecular characterization. The BLASTn and *Tricho*BLAST results on ITS sequence showed 99-100% homology to *T. koningiopsis* accessions and in particular to vouchered culture *T. koningiopsis* GenBank accession no DQ379015. The GenBank accession for ITS sequence of *T. koningiopsis* NBRI-PR5 is JN375992 and it is the first report of *T. koningiopsis* from India.

New Varieties / Cultivars Chrysanthemum – NBRI-Pushpangadan

A new cultivar of chrysanthemum, named 'NBRI – Pushpangadan', in honour of the former Director of CSIR-NBRI, Dr. P Pushpangadan, was developed through bud sport selection. 'NBRI-Pushpangadan' is a yellow-flowered mutant of chrysanthemum cultivar 'A- 22', evolved as bud sport. Plant attains height up to 49 cm and produces more than 210 yellow anemone flower heads. Plant spread is 46.5 cm (N-S) and 42 cm (E-W). Flower head size (across) is 4.30 cm. Disc floret in the central region is pin cushion type and its colour is yellow (Yellow Group 6A, Fan-1). Ray florets are tubular and their number in a flower head is 21 and their colour is yellow (Yellow Group 6D, Fan-1). Morphological analysis of vegetative and floral characters of 'A-22' and its bud sports 'NBRI-Pushpangandan' revealed that all the characters of control and but sports are almost same except the flower head colour and shape. The cultivar is ideal for pot plant and bedding.

Fig: 1.19 NBRI – Pushpangadan: a new variety of Chrysanthemum developed by CSIR-NBRI

Antioxidant furofuran lignans from *Premna integrifolia*

Two new furofuran lignans, premnadimer and 4ß-hydroxyasarinin-1-O-ß-glucopyranoside were isolated by CSIR-CIMAP from the stem bark of *Premna integrifolia*. The compound was evaluated for radical scavenging and ferric reducing antioxidant power. Radical scavenging activity was found maximum in 4"-hdroxy-E-globularinin followed by 10-O-trans-p-coumaroylcatalpol and new dimer. In FRAP assay, premnosidic acid, 10-O-trans-p-coumaroyl-6-O-α-L-rhamnopyranosyl catalpol exhibited maximum ferric reducing ability supported by high reducing power.

Withania wet rot caused by *Choanephora cucurbitarum*

A new disease initially produced water soaked lesions on leaves and stems that progressed to a wet rot appeared in Withania fields during monsoon at Lucknow and in adjoining areas of northern India. Based on cultural, morphological and molecular characteristics fungus was identified by CSIR-CIMAP as *Choanephora cucurbitarum* (Berk. & Ravenel) Thaxt. Its sequence was submitted to NCBI GenBank with accession no. JN639861.

Efficient genetic transformation of *Withania coagulans* mediated by *Agrobacterium tumefaciens*

An efficient *Agrobacterium*-mediated transformation of *Withania coagulans* has been achieved by CSIR-IGIB using leaf explants of multiple shoot culture. The *Agrobacterium* strain LBA4404 harbouring the binary vector pIG121Hm containing β-glucuronidase gene (gusA) under the control of a CaMV35S promoter was used in the development of transformation protocol. 100% frequency of transient GUS expression was achieved with 5% stable transformation efficiency using developed and optimized conditions. PCR analysis of T0 transgenic plants showed the presence of gusA and nptII genes confirming the transgenic event. Histochemical GUS expression was observed in the putative transgenic *W. coagulans* plants. Metabolite analysis showed the presence of similar type of withanolides in the transgenic and non-transgenic regenerated plants.

Agrobacterium tumefaciens mediated transformation system via leaf explants developed by CSIR-CIMAP will be useful for pathway manipulation using metabolic engineering for bioactive withanolides in *Withania.*

Longevity-promoting effects of 4-hydroxy-E-globularinin in *Caenorhabditis elegans*

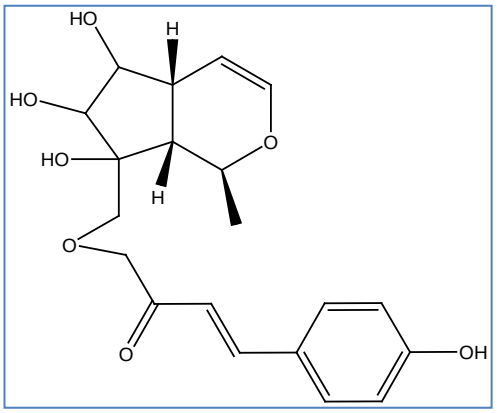

Fig. 1.20 4-Hydroxy-E-globularinin

CSIR-CIMAP reported that 4-Hydroxy-E-globularinin (4-HEG) from *Premna integrifolia* exhibited the longevity-promoting activity of 4-HEG in the animal model *Caenorhabditis elegans*. 4-HEG (20 mM) enhanced the mean life span of worms by over 18.8% under normal culture conditions and also enhanced their survival under oxidative stress. The longevity-promoting activity was associated with reduced reactive oxygen species (ROS) levels and fat accumulation in the worms. Gene-specific mutant studies verified the role of ROS detoxification pathways and simultaneous nuclear translocation of DAF-16 in the 4-HEG-mediated effects. Quantitative real-time PCR estimations and observations of transcriptional reporters indicated that 4-HEG was able to upregulate stress-inducible genes, viz., hsp-16.2 and sod-3. It is inferred that 4-HEG may serve as a lead compound of plant origin for the development of important nutraceuticals superseding the aging process.

The results demonstrate that 4-HEG has the potential to significantly increase the life span of worms under standard laboratory conditions of 20^{o}C and paraquat-induced oxidative stress as well. It was observed that 4-HEG also has the ability to attenuate ROS and fat accumulation inside worms, leading to improved physiological parameters. Finally, it was demonstrated that an endogenous ROS detoxification pathway, nuclear localization of the major transcription factor DAF-16, and upregulation of several DAF-16-regulated stress-inducible genes, viz., hsp-16.2 and sod-3, are responsible for the 4-HEG-mediated longevity in *C. elegans*. This study for the first time reports the longevity promoting activity of 4-HEG from *P. integrifolia* in the *C. elegans* model system.

Exploration and Screening of Bacterial Diversity in North-East India and Its Potential Application in Biocontrol

Phylogenetic analysis of alkaline proteinase producing fluorescent pseudomonads are associated with green gram (*Vigna radiata L.)* rhizosphere.

Fifty fluorescent pseudomonads were isolated by CSIR-NEIST from rhizospheric soil of green gram from nearby area of Kaziranga, Assam, and assayed for their extracellular proteinase production. Out of these isolates 20 were found to be prominent in proteinase production. Genetic diversity of the 20 isolates were analyzed through BOX-PCR fingerprinting and 16S rDNA-RFLP along with three reference strains, viz., *Pseudomonas fluorescens* (NCIM2099T), *P. aureofaciens* (NCIM2026T) and *P. aeruginosa* (MTCC2582T). Based on phenotypic characters and 16S rDNA sequence similarity all the 8 highly proteinase producing strains were affiliated with *P. aeruginosa*. The proteinase was extracted from two most prominent strains (KFP1 and KFP2), purified by a three-step process involving (NH4)2SO4 precipitation, gel filtration and ion exchange chromatography. The enzyme had an optimal pH of 8.0 and exhibit highest activity at 60^{o}C and 37^{o}C by KFP1 and KFP2 respectively. The purified enzyme was migrated as a single band on native and SDS-PAGE with a molecular mass of 32 kDa. $Zn^{2+,}$ Cu^{2+} and Ni^{2+} ion inhibited the enzyme activity. Enzyme activity was also inhibited by EDTA established as their metallo-proteinase nature.

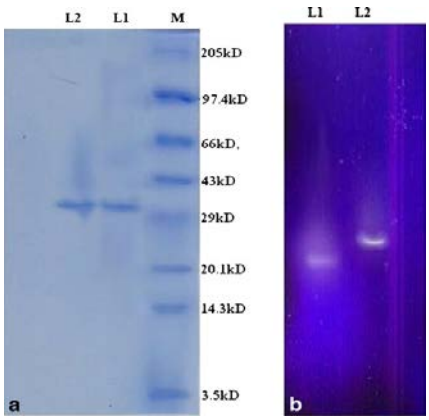

Fig:1.21 SDS-PAGE (15%) of the purified proteinase from *Pseudomonas aeruginosa* KFP1 and *P. aeruginosa* KFP2; M, molecular mass standard; L1, purified proteinase of KFP1; and L2, purified proteinase from KFP2 showing 32 kDa molar mass. b. Zymography of purified proteinase; L1, zymography of KFP1 purified proteinase and L2, zymography of KFP2-purified proteinase

DNA fingerprinting of fluorescent pseudomonads associated with rhizospheric soil of green gram - role in plant growth promotion under water stress

A total of 130 fluorescent pseudomonads from green gram rhizospheric soil of Jorhat district of Assam were isolated and characterized by CSIR-NEIST for multiple plant growth promoting traits, such as the production of indole acetic acid (IAA), nitrogen

fixation, phosphorus (P) solubilization, siderophore production, production of ammonia (NH_3) and production of different enzymes viz., protease, chitinase, pectinase and cellulase. On the basis of PGPR characteristics 8 isolates were tested for 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity. Further *in vitro* studies of role of two potential bacterial isolates on water stress resistance were examined. *In vivo* study of these two isolates in green gram plant revealed increase in root length of 10-20% and dry weight of between 22 to 35% in comparison to the positive controls under water stress condition. Chlorophyll content as well as accumulation of proline in the bacterized plant was compared with control one under stress condition. Proline accumulation and increase in chlorophyll concentration was higher in bacterized plant as compared to control. 16S rDNA-RFLP and BOX–PCR fingerprinting revealed huge genetic diversity among the isolates with different PGPR traits.

Diversity and proteinase-producing bacteria isolated from rhino's dung

Twenty four bacteria were isolated by CSIR-NEIST in four different media. Based on the sequence similarity of 16S rDNA and phylogenetic analysis, isolates belonged to nine genera, *Providencia sp*. (9 nos*.), Bacillus sp.* (5 nos.), *Pseudomonas sp.* (3 nos.), *Achromobacter sp.* (2 no.), *Brevibacillus sp.* (1 no.), *Sphingobacterium sp*. (1 no.), *Proteus sp.* (1 no.), *Caryophanon sp.* (1 no.) and *Acinetobacter sp*. (1 no.). The study revealed high degree of genetic variability among different genera of bacteria and Providencia is the predominant bacteria in rhino dung in this area. Only two strains *Achromobacter sp.* KRD9 and *Providencia sp.* KRD23 were found potential for proteinase production with molecular mass of 41kDa. The broad range temperature, pH dependency, dehairing ability, detergent stability and compatibility with consistent activity and excellent storage stability make the enzyme potentially useful for application in the detergent and leather industry. This is perhaps the first report on genetic diversity and isolation of proteinase producing bacteria from rhino dung.

Survey, Isolation and Preliminary Characterization of Microbial (Bacteria) Populations of Southern Brahmaputra Corridor of Assam

Bacterial strain has been isolated and identified by CSIR-NEIST as Plant Growth Promoting Rhizobacteria (PGPR) which shows growth promotion on different vegetable crop plants. The Nutrient agar and Nutrient broth were used for culturing PGPR strains. A pure culture of rhizobacterial strain was maintained in NA slants. The bacterial culture was spread by spraying near the root region of the individual plants and a 20% dilution of bacterial bioformulation was sprayed on leaves, stems and near the collar region of the crop plants. Similarly, a bacterial consortium (RB1+RB4+RB5) bioformulation was prepared and spread in the crop plants. The strains were identified at CSIR-IMTECH as follows: RB1 (MTCC 8297) Bacillus cereus, RB4 (MTCC 8299) & RB5 (MTCC 8300) *Pseudomonas rhodesiae*.

The effect of PGPR on growth enhancement in crops, viz, chilli (*Capsicum annum*), brinjal (*Solanum melongena*), tomato (*Solanum lycopersicum*), cauliflower (*Brassica oleracea* var. botrytis), ladys finger (*Abelmoschus esculentus*), rice (*Oryza sativa*) and tea (*Camellia sinensis*) was carried out in experimental field. The treated crops have shown various morphological differences like enhanced height, change in leaf colour, numbers of leaves etc. and have shown significant results in increase biomass when compared to control group. Two sets of crops have been planted, one treated with bioformulation and another without any treatment. The measurements and number of leaves were taken at regular intervals. Treated crops have shown a wide range of differences like number of leaves, height early flowering compared to the control crops. The effects of the PGPR combinations in plants were also determined through estimation of biochemical parameters. Fruit samples of vegetable crops were collected after treatment with PGPR and were subjected for biochemical estimation. Estimation of Total carbohydrate content, total soluble protein and free amino acids analysis showed higher value in treated crops than the control.

Genetic diversity of antimicrobial agents producing Streptomyces isolated from protected forest area of Assam and Arunachal Pradesh

CSIR-NEIST has screened 200 nos. of *Streptomyces* strains for antimicrobial activity against fungal pathogens (*Candida albicans* MTCC3017, *Fusarium oxysporum* f. sp. *ciceri* NCIM1281, *Aspergillus niger*, *Rhizoctonia solani*) and bacterial pathogens, *viz., Staphylococcus aureus* MTCC96, Bacillus *subtilis* MTCC441, *E. coli* MTCC739) and *Pseudomonas aeruginosa* MTCC2458. Out of these strains, 8 nos. showed antifungal activity against *F. oxysporum, R. solani* and *C. albicans.* In case of bacteria, 16 nos. of strain showed antibacterial activity against *Staphylococcus aureus* MTCC96 and *Bacillus subtilis* MTCC441 and six antifungal strains could degrade colloidal chitin in amended media. Antimicrobial metabolite was extracted from active strains in ethyl acetate. Extracted metabolite was subjected to the fractionation and purified by preparative TLC (hexane and ethyl acetate, 1:1). Three fractions were collected from the metabolite of *Streptomyces roseochromogenus* TSR12 and tested against the pathogens. Two fractions exhibited antibacterial activity against the bacteria (Fig. 1.23). Further purification and chemical profiling of the bioactive compound(s) is in progress.

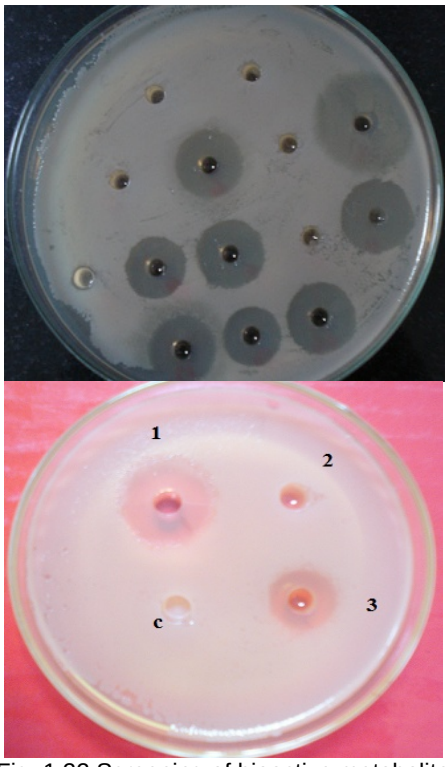

Fig: 1.22 Screening of bioactive metabolite of Streptomyces sp. against Candida albicans; b. Bioassay results of TLC purified compound (fraction 1, 2 and 3; c - control); extracted from S. roseochromogenus

Fifty nos. of *Streptomyces* spp. isolated from Tawang were investigated for antifungal activity against *Fusarium oxysporum* and 6 isolates were found to be positive. Sequencing of 16S rDNA of these Streptomyces was done and sequences to be submitted to NCBI-Gene Bank. Some isolates found to positive in proteinase and amylase production. Antifungal activity Streptomyces strains growing in colloidal chitin amended media were subsequently amplified gene coding for Family 18 chitinase A (ChiA). A real time qPCR comparative $\Delta\Delta C_T$ study among the six isolates showed glycosyl hydrolase (GTR25) to be the transcriptionally active. The six antimicrobial agent producing strains were identified as *Streptomyces virginiae*, S. s*poroverrucosus, S. roseochromogenus, S. kanamycelicus, S. purpeofuscus* and *S. indigoferus* which were exhibited great genetic diversity.

Better vaccines for tuberculosis

CSIR-IMTECH has proposed the use of novel vaccines called lipidated-promiscuous-peptide vaccines. These synthetic vaccines are safer than BCG because they do not contain infectious material. Moreover, they generate long-lasting, protective immune responses and are not influenced by pre-existing antibodies. This type of vaccine strategy has already proven to be successful in an animal model of tuberculosis and is being tested in human clinical trials for other infectious diseases and cancer. Lipidated-promiscuous-peptide vaccines have all the essential qualities that can make them successful in tuberculosis-endemic countries. Such vaccines can impart better protection than BCG and will have a long-reaching positive impact on millions of people.

Molecular mechanism of signal transduction and gene regulation by *M.tuberculosis* phop-phor system

The PhoP and PhoR proteins from *Mycobacterium tuberculosis* controls expression of genes involved in complex lipid biosynthesis and regulation of unknown virulence determinants in human and mouse macrophages or in mice.

CSIR-IMTECH has established cross-talk between *M. tuberculosis* PhoP and PhoR proteins; identified genetic determinants recognized by PhoP; characterized molecular mechanism of sequence-specific DNA binding; and identified strikingly novel structural features of the transcription factor that contributes to its expanded regulatory capability as a global regulator. To determine the mechanism, it has been shown that the protein recognizes a 23-bp sequence of the *phoP* upstream region comprising two adjacent direct repeat motifs believed to promote transcription regulation. Protein phosphorylation was not required for DNA binding; however, phosphorylation enhanced *in vitro* DNA binding through protein-protein interaction(s). Using biochemical probes, the institute has determined stoichiometry and a relatively unusual orientation of PhoP protomers within the DNA-protein binary complex- a model in which two PhoP protomers bind the duplex DNA with a symmetric head-to-head orientation.

Institute probed the molecular basis of sequence-specific PhoP-DNA interactions leading to identification of single amino acid of the protein and a specific base-pair of the target DNA as specificity-determinants. It is also shown that phosphorylation switch the transactivation domain to a different conformation, which specify additional

protein-protein contacts between PhoP protomers bound to adjacent cognate sites. These observations raise the possibility that PhoP, in the unphosphorylated and phosphorylated form may be capable of adopting different orientations as it binds to a vast array of genes to activate or repress transcription. In agreement with this view, CSIR-IMTECH has recently identified mechanism of dual mode of promoter recognition by PhoP-namely phosphorylation-independent and phosphorylation dependent. Together, the biochemical analyses on domain structure and inter-domain interactions of PhoP are central to understanding of how the DNA binding transcription factor may possibly function as a global regulator in *M. tuberculosis*. It has also been shown how PhoP activates complex lipid biosynthesis only upon phosphorylation, and thereby contributes to morphology of the bacilli.

Host nuclear receptors as combats and cohorts to *Mycobacterium tuberculosis* survival

Mycobacterium tuberculosis–macrophage interactions are key to pathogenesis and clearance of these bacteria. Although interactions between *M. tuberculosis*-associated lipids and TLRs, non-TLRs, and opsonic receptors have been investigated, interactions of these lipids and infected macrophage lipid repertoire with lipid-sensing nuclear receptors expressed in macrophages have not been addressed. CSIR-IMTECH has showed that *M. tuberculosis*–macrophage lipids can interact with host peroxisome proliferators activated receptor γ and testicular receptor 4 to ensure survival of the pathogen by modulating macrophage function. These two lipid-sensing nuclear receptors create a foamy niche within macrophage by modulating oxidized low-density lipoprotein receptor CD36, phagolysosomal maturation block by induction of IL-10, and a blunted innate response by alternative polarization of the macrophages, which leads to survival of *M. tuberculosis*. These results also suggest possible heterologous ligands for peroxisome proliferator-activated receptor γ and testicular receptor 4 and are suggestive of adaptive or coevolution of the host and pathogen. Relative mRNA expression levels of these receptors in PBMCs derived from clinical samples convincingly implicate them in tuberculosis susceptibility. These observations expose a novel paradigm in the pathogenesis of *M. tuberculosis* amenable for pharmacological modulation.

The possibility that host nuclear receptor crosstalks to *M. tuberculosis* lipid repertoire and may modulate antimicrobial responses have not been explored at all and is an original hypothesis, to which CSIR-IMTECH preliminary findings. There is less redundancy and pleiotrophy at level of the nuclear receptor as compared over signaling networks; with many of them being master regulators of certain phenotypes and as such offer themselves as novel therapeutic targets. Nuclear receptors are convenient for therapeutic intervention by agonist/antagonist good on ADMET. Institute has so far identified PPARγ and TR4 to be pro- *M. tuberculosis* and LXRα to be anti- *M. tuberculosis.*

Genomes of mango, pomegranate pathogens decoded

Fig.1.23 The genomes of pathogen affecting mango (left) and pomegranate have been decoded

CSIR-IMTECH has analyzed and reported the fully annotated genome of the Xanthomonas pathogens that cause serious diseases in high value fruit crops like mangoes, pomegranates and grape. Interestingly the team found that the genomes of these bacterial pathogens, that cause diseases in diverse fruits like mango pomegranate, grape and citrus, are highly identical suggesting their recent origin. Further the set of unique genes and markers present in their genomes will be valuable in epidemelogical and evolutionary studies of these fruit pathogens. These are the first plant pathogen genomes published from India. The study has also been covered as a featured article in Nature India (doi:10.1038/nindia.2012.70).

Extracellular polymeric substances from marine bacterium Vibrio parahaemolyticus

A marine bacterial strain identified as *Vibrio parahaemolyticus* by 16S rRNA gene (HM355955) sequencing and gas chromatography (GC) coupled with MIDI was selected by CSIR-CSMCRI from a natural biofilm by its capability to produce extracellular polymeric substances (EPS). The EPS had an average molecule size of 15.278 µm and exhibited characteristic diffraction peaks at 5.985°, 9.150° and 22.823°, with d-spacings of 14.76661, 9.29989 and 3.89650 Å, respectively. The Fourier-transform infrared spectroscopy (FTIR) spectrum revealed aliphatic methyl, primary amine, halide groups, uronic acid and saccharides. Gas chromatography mass spectrometry (GCMS) confirmed the presence of arabinose, galactose, glucose and mannose. 1HNMR (nuclear magnetic resonance) revealed functional groups characteristic of polysaccharides. The EPS were amorphous in nature (CIxrd 0.092), with a 67.37% emulsifying activity, thermostable up to 250°C and displayed pseudoplastic rheology. MALDI-TOF–TOF analysis revealed a series of masses, exhibiting low-mass peaks (m/z) corresponding to oligosaccharides and higher-mass peaks for polysaccharides consisting of different ratios of pentose and hexose moieties. This is the first report of a detailed characterisation of the EPS produced by *V. parahaemolyticus*, which could be further explored for biotechnological and industrial use.

Amide linker orientation profoundly influences serum compatibility and lung transfection properties of cationic amphiphile

Understanding the influence of structural parameters of cationic amphiphiles on their gene transfer properties is important for designing efficient liposomal gene delivery

reagents. In search for a serum compatible cationic amphiphile with circulation stable linker functionality, CSIR-IICT has designed a highly serum compatible novel cationic amphiphile which has shown remarkable selectivity in transfecting mouse lung. It has also been demonstrated that reversing orientation of amide linker functionality in the molecular structure adversely affects the serum compatibility and the lung selective gene transfer property of cationic amphiphiles. Importantly, these new lipoplexes (electrostatic complexes of cationic liposomes and plasmid DNA) exhibited remarkable mouse lung selective gene transfer properties. This work demonstrated for the first time that amide linker orientation profoundly influences the serum compatibility and lung transfection efficiencies of cationic amphiphiles.

Induction of ROS, mitochondrial damage and autophagy in lung epithelial cancer cells by iron oxide nanoparticles

Autophagy has attracted a great deal of research interest in tumor therapy in recent years. CSIR-IITR has reported that ironoxide NPs, synthesized by the institute selectively induces autophagy in cancercells (A549) and not in normal cells (IMR-90). It was also noteworthy that autophagy correlated with ROS production as well as mitochondrialdamage. Protection of NAC against ROS clearly suggested the implication of ROS in hyper-activation of autophagy and cell death. Pre-treatment of cancer cells with 3-MA also exhibited protection against autophagy and promote cellular viability. Results also showed involvement of classical mTOR pathway in autophagy induction by iron oxide NPs in A549 cells. The results had shown that bare ironoxide NPs are significantly cytotoxic to human cancercells (A549) but not to the normal human lung fibroblast cells (IMR-90). In other words nanoparticles, developed by the institute selectively kill cancerous cells. It is encouraging to conclude that ironoxide NPs bear the potential of its applications in biomedicine, such as tumor therapy specifically by inducing autophagy mediated cell death of cancercells.

Effect of Long term exposure of cypermethrin on adult rats

CSIR-IITR has investigated the effects of cypermethrin on biochemical, histopathological, and motor behavioral indices of the nigrostriataldopaminergic system in adult rats treated with or without cypermethrin (1/10 adult dose) during postnatal days 5–19. Spontaneous locomotor activity (SLA) and rotarod tests were performed to assess motor behavior. Levels of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum, and tyrosine hydroxylase (TH) immunoreactivity and 4′,6-

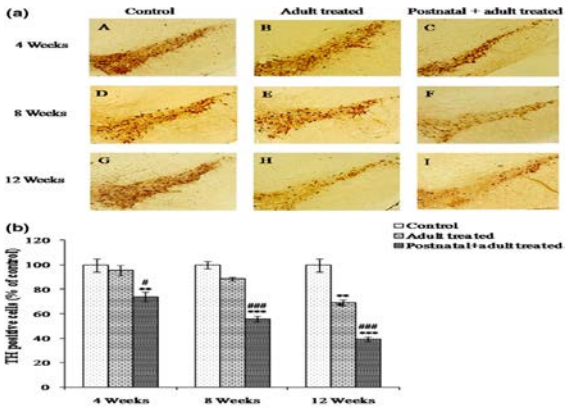

Fig.1.24 Tyrosine hydroxylase (TH) immunoreactivity of dopaminergic neurons in the substantia nigra pars compacta of the brain, as observed by bright-field microscopy at 10X and 4X magnifications, respectively. (a) The representative TH immunoreactivity in control (A, D, and G), cypermethrin adult alone-treated (B, E, and H), and cypermethrin-treated postnatal + adult rats (C, F, and I). (b) The counting of TH-positive neurons in control, cypermethrin-treated adult alone, and cypermethrin-treated postnatal + adult rats. The values are calculated as mean (±) standard error of the mean (n = 3). The data are expressed as percentage of control. Significant changes are expressed as ** ($p<0.01$), *** ($p<0.001$) in comparison with control and # ($p<0.05$), ### ($p<0.001$) in comparison with adulthood alone treated animals.

diamidino-2-phenylindole (DAPI)/ Fluoro-Jade B staining in the substantia nigra were measured to assess dopaminergic neurodegeneration. Postnatal treated animals did not exhibit significant changes in any measured parameters. The significant reduction in the time of stay on rotarod, spontaneous locomotor activity, dopamine, 3,4-dihydroxyphenylacetic acid, and tyrosine hydroxylase immunoreactivity while an increase in homovanillic acid level and Fluoro-Jade B-positive cells were observed in cypermethrin treated adult rats. These changes were more pronounced in the animals treated with cypermethrin during postnatal days followed by adulthood compared with adulthood alone. The results obtained thus demonstrate that exposure to cypermethrin during adulthood induces dopaminergic neurodegeneration in rats and postnatal exposure enhances the susceptibility of animals to dopaminergic neurodegeneration if rechallenged during adulthood.

Melatonin as a Neuroprotective Agent in the Rodent Models of Parkinson's disease: Is it All Set to Irrefutable Clinical Translation?

Parkinson's disease (PD), a neurodegenerative disorder, is characterized by the selective degeneration of the nigrostriatal dopaminergic neurons, continuing or permanent deficiency of dopamine, accretion of an abnormal form of alpha synuclein in the adjacent neurons, and dysregulation of ubiquitin proteasomal system, mitochondrial metabolism, permeability and integrity, and cellular apoptosis resulting in rigidity, bradykinesia, resting tremor, and postural instability. Melatonin, an indoleamine produced almost in all the organisms, has anti-inflammatory, anti-apoptotic, and anti-oxidant nature. Experimental studies employing 1-methyl 4-phenyl 1, 2, 3, 6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), methamphetamine, rotenone, and maneb and paraquat models have shown an enormous potential of melatonin in amelioration of the symptomatic features of PD. Although a few reviews published previously have described the multifaceted efficacy of melatonin against MPTP and 6-OHDA rodent models, due to development and validation of the newer models as well as the extensive studies on the usage of melatonin in entrenched PD models, it is worthwhile to bring up to date note on the usage of melatonin as a neuroprotective agent in PD. CSIR-IITR has presented an update on the usage and applications of melatonin in PD models along with incongruous observations.

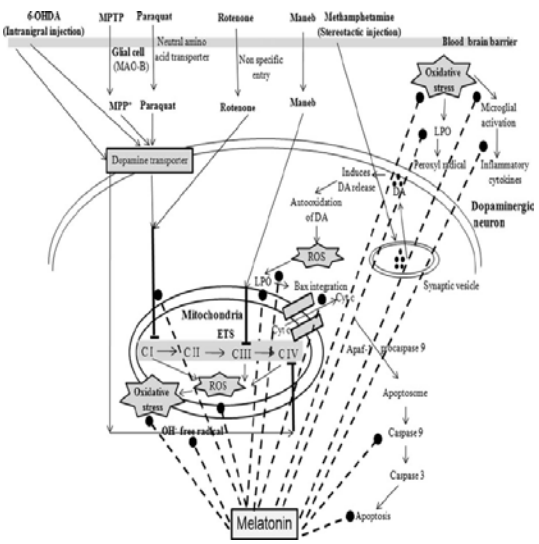

Fig. 1.25 Diagrammatic representation of the 6-OHDA, MPTP, paraquat, rotenone, maneb, and methamphetamine-induced neurodegeneration

Engineered ZnO and TiO_2 nanoparticles induce oxidative stress and DNA damage leading to reduced viability of *Escherichia coli*

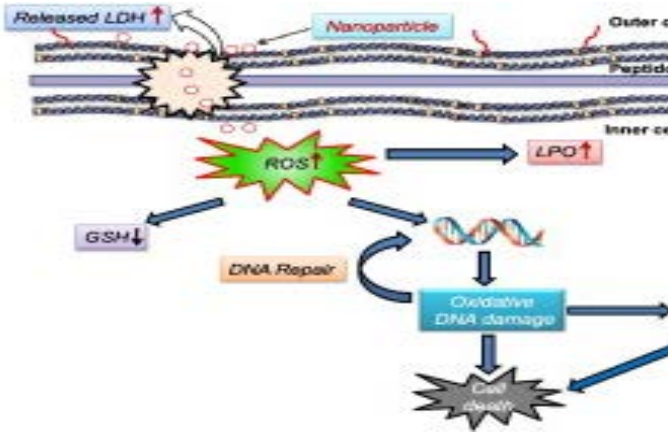

Fig:1.26 Possible mechanism of Zno and T_iO_2 ENP-Induce genotoxicity and cytotoxicity

Extensive use of engineered nanoparticle (ENP)-based consumer products and their release into the environment has raised a global concern pertaining to their adverse effects on human and environmental health. CSIR-IITR has studied the toxicity mechanism of ZnO and TiO_2 ENPs in a gram-negative bacterium, *Escherichia coli.* Internalization and uniform distribution of characterized bare ENPs in the nano range without agglomeration was observed in *E. coli* by electron microscopy and flow cytometry. The data showed a statistically significant concentration-dependent decrease in *E. coli* cell viability by both conventional plate count method and flow cytometric live–dead discrimination assay. Significant ($p < 0.05$) DNA damage in *E. coli* cells was also observed after ENP treatment. Glutathione depletion with a concomitant increase in hydroperoxide ions, malondialdehyde levels, reactive oxygen species, and lactate dehydrogenase activity demonstrates that ZnO and TiO_2 ENPs induce oxidative stress leading to genotoxicity and cytotoxicity in *E. coli.* The study substantiates the need for reassessment of the safety/toxicity of metal oxide ENPs.

Biodegradable Poly(vinyl alcohol)-polyethylenimine Nano composites for Enhanced Gene Expression *in vitro* and *in vivo*

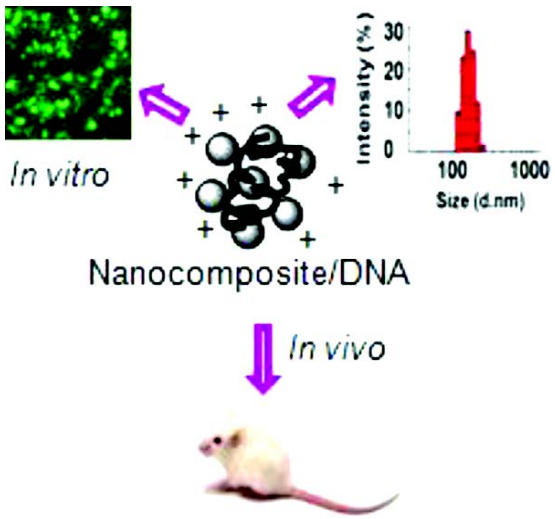

Fig:1.27 Biodegradable Poly (vinyl alcohol)-polyethylenimine Nanocomposites for enhanced gene expression in vitro and in vivo

Use of cationic polymers as nonviral gene vectors has several limitations such as low transfection efficiency, high toxicity, and inactivation by serum. In a study carried out by CSIR-IITR, varying amounts of low molecular weight branched polyethylenimine 1.8 kDa (bPEI 1.8) were introduced on to a neutral polymer, poly(vinyl alcohol) (PVA), to bring in cationic charge on the resulting PVA-PEI (PP) nanocomposites. Institute has rationalized that by introducing bPEI 1.8, buffering and condensation properties of the proposed nanocomposites would result in improved gene transfer capability. A series

of PVA-PEI (PP) nanocomposites was synthesized using well-established epoxide chemistry and characterized by IR and NMR. Particle size of the PP/DNA complexes ranged between 120 to 135 nm, as determined by dynamic light scattering (DLS), and DNA retardation assay revealed efficient binding capability of PP nanocomposites to negatively charged nucleic acids. *In vitro* transfection of PP/DNA complexes in HEK293, HeLa, and CHO cells revealed that the best working formulation in the synthesized series, PP-3/DNA complex, displayed ~2–50-fold higher transfection efficiency than bPEIs (1.8 and 25 kDa) and commercial transfection reagents. More importantly, the PP/DNA complexes were stable over a period of time, along with their superior transfection efficiency in the presence of serum compared to serum-free conditions, retaining the nontoxic property of low molecular weight bPEI. The *in vivo* administration of PP-3/DNA complex in Balb/c mice showed maximum gene expression in their spleen. The study demonstrates the potential of PP nanocomposites as promising nonviral gene vectors for *in vivo* applications.

miR-497 and miR-302b Regulate Ethanol-induced Neuronal Cell Death through BCL2 Protein and Cyclin D2

In chronic alcoholism, brain shrinkage and cognitive defects because of neuronal death are well established, although the sequence of molecular events has not been fully explored yet. CSIR-IITR has explored the role of microRNAs (miRNAs) in ethanol-induced apoptosis of neuronal cells. Ethanol-sensitive miRNAs in SH-SY5Y, a human neuroblastoma cell line, were identified using real-time PCR-based TaqMan low-density arrays. Long-term exposure to ethanol (0.5% v/v for 72 h) produced a maximum increase in expression of miR-497 (474-fold) and miR-302b (322-fold). Similar to SH-SY5Y, long-term exposure to ethanol induced miR-497 and miR-302b in IMR-32, another human neuroblastoma cell line. Using *in silico* approaches, BCL2 and cyclin D2 (CCND2) were identified as probable target genes of these miRNAs. Cotransfection studies with 3′-UTR of these genes and miRNA mimics have demonstrated that BCL2 is a direct target of miR-497 and that CCND2 is regulated negatively by either miR-302b or miR-497. Overexpression of either miR-497 or miR-302b reduced expression of their identified target genes and increased caspase 3-mediated apoptosis of SH-SY5Y cells. However, overexpression of only miR-497 increased reactive oxygen species formation, disrupted mitochondrial membrane potential, and induced cytochrome c release (mitochondria-related events of apoptosis). Moreover, ethanol induced

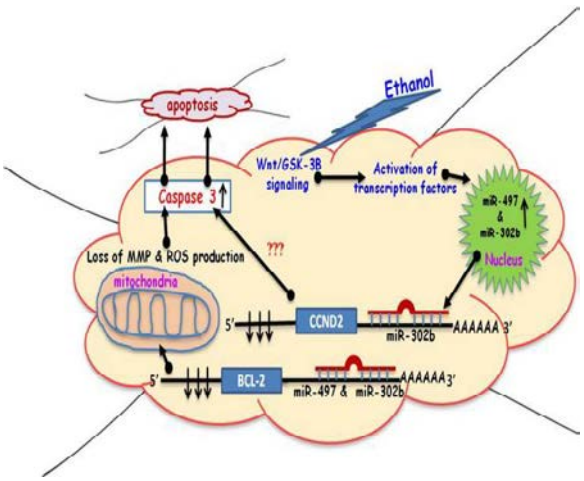

Fig:1.28 Pictorial summary of results. The arrows with ● at the bottom indicate a connection between two events. ↑ indicates an increase in activity or expression, and ↓ indicates decreased expression

changes in miRNAs, and their target genes were substantially prevented by pre-exposure to GSK-3B inhibitors. In conclusion, CSIR-IITR has have shown that ethanol-induced neuronal apoptosis follows both the mitochondria-mediated (miR-497- and BCL2-mediated) and non-mitochondria-mediated (miR-302b- and CCND2-mediated) pathway.

Linear PEI nanoparticles: efficient pDNA/siRNA carriers *in vitro* and *in vivo*

Linear polyethylenimine (lPEI, 25 kDa) nanoparticles' (LPN) series was synthesized by varying percentage of cross-linking with 1,4-butanediol diglycidyl ether (BDE) and their size, surface charge, morphology, pDNA protection/release, cytotoxicity and transfect ion efficiency were evaluated by CSIR-IITR. Synthesized nanoparticles (NPs) were spherical in shape (size: ~109 - 235 nm; zeta potential: +38 to +16 mV). These NPs showed increased buffering capacity with increasing percent cross-linking and also exhibited excellent transfection efficiency (i.e. ~1.3 - 14.7 folds in case of LPN-5) in comparison with lPEI and the commercial transfection agents used in this study. LPN-5 based GFP-specific siRNA delivery resulted in ~86% suppression of targeted gene expression. These particles were relatively nontoxic *in vitro* (in cell lines) and *in vivo* (in Drosophila). *In vivo* gene expression studies using LPN-5 in Balb/c mice through intravenous injection showed maximum expression of the reporter gene in the spleen. These results together demonstrate the potential of these particles as efficient transfection reagents.

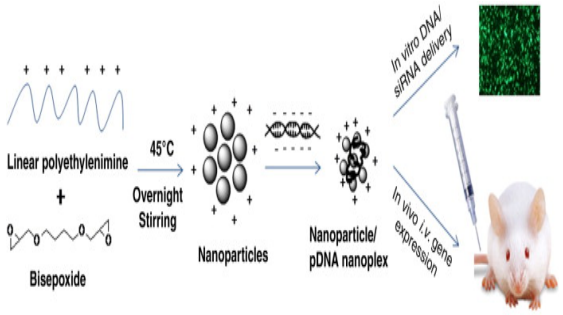

Fig.1.29 Linear PEI nanoparticles; efficient pDNA/siRNA carriers in vitro and in vivo

Identification of environmental reservoirs of nontyphoidal salmonellosis

CSIR-IITR carried out a study of identification of environmental reservoirs of *Salmonella enterica* subsp. enterica serovar Typhimurium (abbreviated as *Salmonella Typhimurium*) in sediments, water, and aquatic flora collected from the Ganges River (Ganges riverine material) was carried out by adopting a two-step strategy. Step 1 comprised a selective serovar-specific capture of *Salmonella typhimurium* from potential reservoirs. Step 2 involved culture-free detection of selectively captured *Salmonella Typhimurium* by ttr gene-specific molecular beacon (MB) based quantitative polymerase chain reaction (q-PCR). The ttr gene-specific MB designed in this study could detect 1 colony-forming unit (cfu)/PCR captured by serovar-specific DNA aptamer. Sediments, water, and aquatic flora collected from the Ganges River were highly contaminated with *Salmonella Typhimurium*. The preanalytical step in the form of serovar-specific DNA aptamer-based biocapture of bacterial cells was found to enhance the sensitivity of the fluorescent probe in the presence of nonspecific DNA. Information about the presence of environmental

reservoirs of *Salmonella Typhimurium* in the Ganges River region may pave the way for forecasting and management of nontyphoidal salmonellosis in south Asia.

Industrial hygiene and toxicity studies in unorganized bone-based industrial units

A large variety of ornamental and decorative items are manufactured from bone waste by various unorganized sectors in India. An initial survey by CSIR-IITR indicated that workers were exposed at various phases of final product. The subjects (12 industrial units) were tested for total suspended particulate matter (TSPM), particulate matter <10 micron PM_{10}, and particulate matter <2.5 microm $PM_{2.5}$. Prevalent levels of TSPM ranged between 2.90 and 5.89 mg/m^3. Respirable fractions of occupational dust as PM_{10} and $PM_{2.5}$ were found in the range of 0.30-2.08 and 0.26-0.50 mg/m^3, respectively. Cytotoxicity study was conducted using hemolysis as a sensitive marker. In an *in vitro* study, rat RBCs were exposed to the concentration of 25-1,000 microg/ml for 15-120 min. A considerable variation was observed in the hemolytic activity of samples collected from different areas. At 500 microg/ml concentration, the hemolytic activity (12 h) was found to be in the range of 18-25%. Due to limitation in sample mass of respirable fractions, only one concentration (100 microg/ml/2 h) was used for comparative study on hemolysis of RBCs caused by PM_{10} and $PM_{2.5}$. Interestingly, the hemolytic activity was more at $PM_{2.5}$ than PM_{10} and TSPM. These results suggest that the respirable particles are capable of reaching deep into the respiratory system. The finding is significant notably when there are no standards available in occupationally exposed populations. This is the first such study. Data could be of importance to policy makers and regulators.

Optimization and validation of an extraction method for the analysis of polycyclic aromatic hydrocarbons in chocolate candies

Chocolate is a key ingredient in many foods such as milk shakes, candies, bars, cookies, and cereals. Chocolate candies are often consumed by mankind of all age groups. The presence of polycyclic aromatic hydrocarbons (PAHs) in chocolate candies may result in health risk to people. A rapid, precise, and economic extraction method has been optimized and validated by CSIR-IITR for the simultaneous determination of polycyclic aromatic hydrocarbons in chocolate candy by high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GS-MS) as a confirmatory technique. The method was optimized by using different solvents for liquid-liquid extraction, varying volume of de-emulsifying agent, and quantity of silica gel used for purification. The HPLC separation of 16 PAHs was carried out by C-18 column with mobile phase composed of acetonitrile : water (70 : 30) in isocratic mode with runtime of 20 min. Limit of detection, limit of quantification (LOQ), and correlation coefficients were found in the range of 0.3 to 4 ng g^{-1}, 0.9 to 12 ng g^{-1}, and 0.9109 to 0.9952, respectively. The exploration of 25 local chocolate candy samples for the presence of PAHs showed the mean content of benzo[a]pyrene as 1.62 ng g^{-1}, which representing the need to evaluate effective measures to prevent more severe PAHs contamination in chocolate candies in future.

Usage pattern of synthetic food colours and exposure assessment through commodities preferentially consumed by children

Exposure studies in children are emphasized nowadays given children's higher consumption vulnerability. CSIR-IITR has generated national-level data covering 16 major states of India on the usage pattern of colours and it identified food commodities through which a particular colour has the scope to exceed ADI limits. Out of the total analysed samples, 87.8% contained permitted colours, of which only 48% adhered to the prescribed limit of 100 $mg.kg^{-1}$. The majority of candyfloss, sugar toys, beverages, mouth fresheners, ice candy and bakery product samples exceeded the prescribed limit. Non-permitted colours were mostly prevalent in candyfloss and sugar toy samples. Though sunset yellow FCF (SSYFCF) and tartrazine were the two most popular colours, many samples used a blend of two or more colours. The blend of SSYFCF and tartrazine exceeded the prescribed limit by a factor of 37 in one sample, and the median and 95th percentile levels of this blend were 4.5- and 25.7-fold, respectively. The exposure assessment showed that the intake of erythrosine exceeded the ADI limits by two to six times at average levels of detected colours, whereas at the 95th percentile level both SSYFCF and erythrosine exceeded the respective ADI limits by three- to 12-fold in all five age groups. Thus, the uniform prescribed limit of synthetic colours at 100 $mg\,kg^{-1}$ under Indian rules needs to be reviewed and should be governed by consumption profiles of the food commodities to check the unnecessary exposure of excessive colors to those vulnerable in the population that may pose a health risk.

Cholinesterase levels and morbidity in pesticide sprayers in North India

Pesticide sprayers in North India use different application methods for different crops. CSIR-IITR has compared cholinesterase activity and symptoms in knapsack and tractor-mounted pesticide sprayers. Blood cholinesterase activity and symptoms were recorded for 42 knapsack and 66 tractor-mounted sprayers attending a health camp in North India and for 30 controls. 108 out of 197 (55%) eligible sprayers consented to participate. Mean acetylcholin-esterase (AChE) and butyrylcholinesterase activity was 33 and 60% lower, respectively, in knapsack sprayers than in controls (P,0.001) and 56 and 62% lower, respectively, in tractor-mounted sprayers than in controls (P,0.001). AChE depletion was greater in tractor-mounted sprayers than in knap-sack sprayers (P,0.001). In knapsack sprayers compared to controls, odds ratios (OR) were significantly raised for musculoskeletal symptoms (OR 3.9, 95% CI 1.03–18) but not for other symptoms. In tractor-mounted sprayers compared to controls, ORs were significantly raised for neurological (OR 7, 95% CI 2–23), ocular (OR 8.7, 95% CI 2.7–32), respiratory (OR 5.14, 95% CI 1–29), cardio-vascular (OR 7.5, 95% CI 2–42), gastrointestinal (OR 5.43, 95% CI 2–18) and musculoskeletal (OR 6.12, 95% CI 2–26) symptoms but not for dermal symptoms (OR 1.93, 95% CI 0.3–20). The risk of cholinesterase inhibition and symptoms is greater in tractor-mounted than in knapsack pesticide sprayers and in both groups compared to controls. Occupational exposure in pesticide sprayers in North India needs better control, perhaps through redesign of spraying equipment.

Studies on urban drinking water quality in a tropical zone

Anthropogenic activities associated with industrialization, agriculture and urbanization have led to the deterioration in water quality due to various contaminants. To assess the status of urban drinking water quality, samples were collected from the piped supplies as well as groundwater sources from different localities of residential, commercial and industrial areas of Lucknow City in a tropical zone of India by CSIR-IITR during pre-monsoon for estimation of coliform and faecal coliform bacteria, organochlorine pesticides (OCPs) and heavy metals. Bacterial contamination was found to be more in the samples from commercial areas than residential and industrial areas. OCPs like α,γ-hexachlorocyclohexane and 1,1 p,p-DDE {dichloro-2, 2-bis(p-chlorophenyl) ethene)} were found to be present in most of the samples from study area. The total organochlorine pesticide levels were found to be within the European Union limit (0.5 µg/L) in most of the samples. Most of the heavy metals estimated in the samples were also found to be within the permissible limits as prescribed by World Health Organization for drinking water. Thus, these observations show that contamination of drinking water in urban areas may be mainly due to municipal, industrial and agricultural activities along with improper disposal of solid waste. This is an alarm to safety of public health and aquatic environment in tropics.

1.1.2 Technology Developed

Baseline Information on select protected areas of Himachal Pradesh

The physical, soil and geological maps of the Dhauladhar Wildlife Sanctuary (DWLS), Great Himalayan National Park (GHNP), Rupi-Bhabha Wildlife Sanctuary (RWS) and Pin Valley National Park (PNP) have been prepared in GIS environment by CSIR-IHBT. The base information was obtained from Survey of India (SOI), Dehradun; National Bureau of Soil Survey & Landuse Planning (NBSS & LUP), Nagpur and Wadia Institute of Himalayan Geology, Dehradun as hard copy maps which were scanned, geo-reverenced and digitized. The physical maps depict information on major locations, transport network, contours, glaciers and rivers in the area of study. The soil and geological maps provide information on soil taxonomy and lithological units of the region.

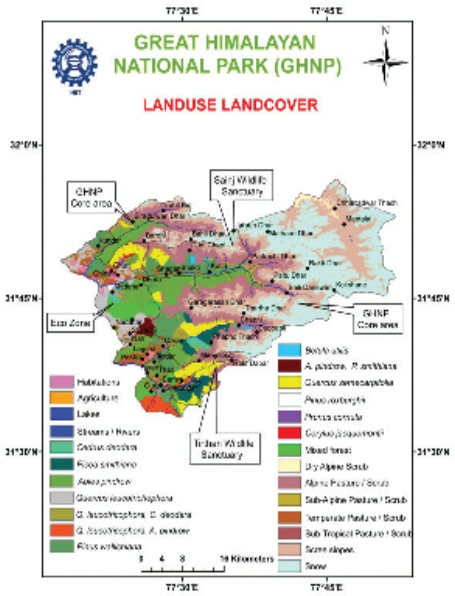

Fig. 1.30 Landuse/landcover map of GHNP

Institute has also carried out the landuse/ landcover classification of GHNP using ETM satellite images. It reveals that 44.37% of its area is occupied by scree/ stony and snow classes. The areas under forest cover and pastures are 31.94 and 20.54% respectively. The water bodies and habitations collectively constitute 1.92% area of GHNP. The forest cover densities of these protected areas were derived from forest

cover map generated using Landsat TM satellite data. The Eco Zone (72.18%) maximum density had followed by Tirthan Wildlife Sanctuary (56.13%) and Sainj Wildlife Sanctuary (40.5%). The core area had lowest forest cover density (15.73%).

Apple pomace

Apple pomace is a solid biomass residue generated at fruit juice extraction industries which goes waste. CSIR-IHBT has explored the possibility of developing value added products from Apple Pomace. They have high moisture content and undergoes rapid oxidation. Further, presence of seeds create hindrance in its application for development of value added food products. Manual separation of seeds is very difficult from huge piles of apple pomace. Before venturing into product development, efforts were made to develop a process/ prototype for seed separation. A lab scale prototype of seed separation unit of 50 liters capacity was designed and manufactured (patent filed in India). Process was standardized to develop dietary fibers. The safety evaluation of the developed dietary fibre fractions was cross validated by an independent agency. A number of value added products were also developed using the extracted dietary fibre.

Apple rootstock - Virus free

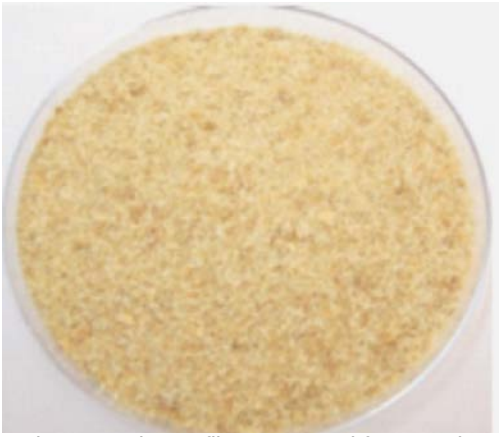

Fig 1.31. Dietary fibre extracted from apple

Apple is an important crop for economy of Himachal Pradesh. The crop get infected with several diseases due to grafting and budding practices adversely affecting productivity and quality of fruits. CSIR-IHBT has developed a technology for raising virus tested root-stock and scion materials having potential to generate saplings producing healthy fruits for better returns. The startup culture and training have been provided to M/s Rajat Biotech, Ghumarwin, Distt. -Bilaspur (H.P.), M/s Kunal Bio-Tech, Nagwaine, Distt. Mandi (H.P.) and M/s Neva Plantation, Palampur, Distt. Kangra (H.P.). Virus tested materials are being mass multiplied by these tissue culture (TC) industries for farmers so that new orchards with increased productivity may be raised. Several thousand plants have been taken up by farmers and there is a huge demand for certified quality planting material with the TC industries.

Identifying microbes useful for alleviating stress induced damages

An efficient Cr(VI) reducing bacterial strain *Microbacterium* sp. (JN674183) has been isolated by CSIR-CIMAP from rhizospheric soil irrigated with tannery effluent completely (100%) reduced the Cr(VI) at 0.2 mM concentration within 24 hours and could reduce the damage of plants caused by chromium toxicity. An efficient ACC-deaminase producing bacteria *Achromobacter* provided significant protection to *Ocimum sanctum* plants under flood stress; an improvement of over 40% in herb yield when exposed to 15 days of water logging. Application of these microbes is

expected to reduce stress induced damage in the plants and will help in effective utilization of waste lands.

High speed and low cost non-invasive method for determination of drug concentration in animal plasma for pharmacokinetic analysis

The method developed by CSIR-CIMAP for non-invasive concentration determination of drug in animal plasma is faster than Schebb's procedure and does not require any solid phase extraction of the drug. FT-NIR spectroscopy analysis combined with partial least squares (PLS) regression model was undertaken with validation through HPLC analysis. Pharmacokinetic parameters obtained through FT-NIR and HPLC were found to be statistically similar with errors below the acceptable limits. The study demonstrates the use of FT-NIR for pharmacokinetics and bio-availability studies. This high throughput method analyses more than 50 samples in an hour without solvents usage and provide ample scope for automation and commercial utilization.

Design of Ankle foot orthosis for patients with Diabetic foot ulcer

The accepted etiology of diabetic plantar wounds is excessive pressure on the insensitive foot that leads to callus formation, skin breakdown and infection. Thus off-loading of peak plantar pressures (PPPs) reduces the risk for skin breakdown and allows healing of open wounds. One of the effective offloading devices is Ankle foot orthosis (AFO). Among the pressure offloading techniques, AFO offers lot of advantages for physicians and patients. But the current design and materials used for fabrication of AFO make the treatment expensive which is not affordable by the patients in India. Therefore a new design of AFO for patients with diabetes having diabetic foot ulcer has been developed by CSIR-CLRI.

Fig.1.32 Ankle foot orthosis

AFO was designed in such a way that it can be worn either in left or in right foot. The foot part and the ankle part are molded as a single piece so that the excess of plantar flexion movement can be arrested. The Velcro fastener from the foot part wrap over the dorsal part of the foot so that slipping of foot can be controlled. The insole plays the major role in offloading the pressure at the ulcer site. The insole was designed exclusively with three layers of foam in which the middle layer is formed with many holes and the upper layer has projections in its inner side which lock the holes in middle layer. The upper layer is segmented so that each hole of the middle layer is locked by each segment of the upper layer. The foot care specialist can remove the segments at the ulcer site so that offloading will take place effectively.

1.1.3 Unique Major Facilities

OSDD Chemistry Outreach Program (CSIR-CDRI Initiative)

Fig. 1.33 OSDD Chemical Outreach portal window

On the occasion of the International Year of Chemistry, CSIR has launched a Chemistry Outreach Program to create an Open Chemical Library mainly by the M.Sc. as well as Ph.D. students at universities/institutes across the length and breadth of the country to synthesize a large number of organic compounds which will be screened against various infectious diseases under the Open Source Drug Discovery (OSDD) program. The endeavour envisages to:

- Impart practical training to large number of M.Sc. students specializing in Organic Chemistry;
- Involve various universities, IITs, IISERs and other academic institutes;
- Besides, University departments designated for carrying out these works to train other students from nearby colleges and universities who do not have any facility; and
- The compounds generated from the exercise to be submitted to the repository for screening and archiving.

The details are available on http://crdd.osdd.net/syncdb/index.html

G.N. Rahmachandran Protein Centre

Fig:1.34 Glimpses of GNR protein Centre

G.N. Ramachandran Protein Centre was inaugurated in the CSIR-Institute of Microbial Technology, Chandigarh campus on 24, January, 2012 by Prof. Balaram, Director, Indian Institute of Science, Bengaluru. This Protein Centre is one-of-its-kind facility in India, solely focused on diverse forms of integrated activities on proteins. It houses groups involved in Research & Development using basic building blocks of proteins i.e. amino acids to supramolecular assemblies. In-silico as well as experimental science research is also being carried out for better understanding of functioning of proteins. This unique Protein Centre has all the requisites to translate findings from basic research into applications for societal good.

Incubator Facility for Biopharmaceuticals

Hon'ble Shri Pawan Kumar Bansal, the then Minister for Parliamentary Affairs, Science and Technology & Earth Sciences and Vice President CSIR inaugurated the Incubator facility for Biopharmaceuticals in the campus of CSIR-IMTECH. This state-of-the-art cGMP facility is approved by the Drug Controller General of India, for production of biopharmaceuticals, such as therapeutic proteins through microbial fermentation for toxicology and clinical trials. The facility is equipped with cGMP and validation compatible fermenters (5 L, 15 L, 40 L, 150 L), downstream processing equipments such as centrifuges (batch and continuous), lab and pilot scale micro-ultrafiltration units, cell disintegration systems, pilot and process scale chromatography systems, analytical equipments etc.

Fig:1.35 Glimpses of Incubator Facility

Tissue and cell culture Facility

A Central Tissue Culture Facility has been established at CSIR-CDRI with an objective of development, maintenance, propagation, cryopreservation, revival of cell

lines and extending the necessary support for research. Various cell lines were provided to user scientists for research and development. Some new cell types are also added on a need-based basis. Help has also been provided to students and investigators in trouble-shooting common problems encountered with the *in vitro* work. During the year, 129 flasks were provided to the users upon request. Besides, facility continued to provide short term training to personnel from academic institutions and industries in techniques of tissue and cell culture.

Biological screening of outside samples

CSIR-CDRI has excellent facilities for carrying out *in vitro* and *in vivo* biological screenings against various disease models and the facility is being provided to R&D institutions, Universities, academic organizations and industrial houses within the country on payment basis. During the year, more than 35 external samples were screened against various models and report sent to the respective researchers.

New Models/Facilities in Animal House

CSIR-CDRI has sufficient number of healthy animals available for research. Some new disease specific animals such as specific hypertensive rat has been included in the facility.

Two new labs viz. Animal Genetic Monitoring Lab and Animal Health Monitoring Lab has been set up at CSIR-CDRI which would be very helpful in experimentation with laboratory animals.

Fig1.36 Facilities in Animal House

Bamboo Museum

To highlight the importance of bamboo, a unique museum with a total plinth area of 400 sqm has been constructed at CSIR-IHBT using bamboo as the building material. The walls, roof and the floors of the museum are made of treated bamboos and bamboo panels. The opening in the ceiling is aesthetically fitted with fiberglass to allow sunlight for natural lighting. The articles and products made of bamboos are displayed in the museum.

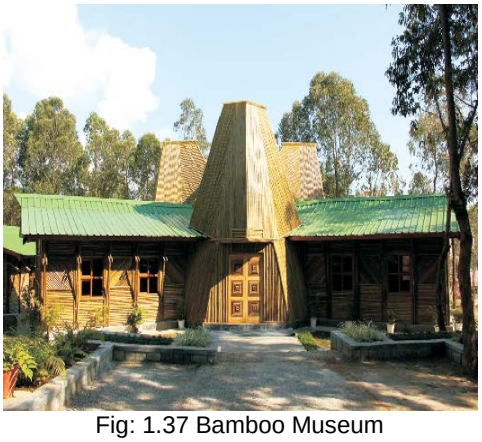
Fig: 1.37 Bamboo Museum