



Microbial Biotechnology Division

Overview

Our main focus is on exploration and exploitation of India's rich microbial diversity (both cultivable and uncultivable) for novel bioactive molecules and biocatalysts with application in health, agriculture and industry, and their overproduction using conventional and molecular biology tools. The group is also working on development of new fungal expression systems, Diagnostic Microbiology and expression of cryptic biosynthetic pathways of microorganisms.

- Development of metagenomic libraries from unexplored ecosystems for new/novel bioactives/biocatalysts
- Production of microbial enzyme and high value microbial products from wild type and genetically modified strains
- Metabolic pathway engineering for production of novel/ desired metabolites
- Hyperexpression and protein engineering of biocatalysts for their application in biotransformation of important drug auxiliaries/intermediates

Mission and Goals

- Development of microbial repository from extreme environments and endophytes of endemic plants
- To study the plant- microbe interactions for disease management, adaption and optimum production of key metabolites in selected medicinal plants
- Bioprospecting and characterization of novel endophytes and extremophiles for bioactives and biocatalysts

Competencies

- Repository of psychrotrophic, mesophilic and thermo-tolerant microorganism including bacteria, actinomycetes, fungi and endophytes
- Metagenomic libraries of environmental samples from unique niches
- High altitude medicinal plant gene bank
- Indigenous *in vitro* propagation methods for many high value medicinal plants

- Indigenous methods for rapid molecular detection of several microbial pathogens
- Indigenous novel expression system for heterologous protein production in fungi
- Hyperexpression and protein engineering
- Strain development for production of bioactive molecules and novel enzymes using traditional and molecular tools (genomics and proteomics)

- Experimental farms at various locations in Jammu and Srinagar with a large collection of medicinal and aromatic plants suitable for studying plant-microbe interactions, endophytic, ectophytic and other symbiotic associations
- Well equipped plant tissue culture Lab at Srinagar to carryout *in vitro* propagation studies for high value medicinal plants

Facilities

- Well equipped Microbiology laboratories at IIM, Jammu and branch laboratory Srinagar to carryout cutting edge research on isolation and characterization of microorganisms and their bioactive metabolites
- State-of-the-art facility to undertake nucleic acid amplification, biomarker development, gene cloning, heterologous gene expression, protein purification, DNA sequencing and differential expression studies
- Mycology unit at Branch Laboratory Srinagar undertaking collection, culture and identification of mushrooms for characterization of bioactive molecules

Areas of Research

- Plant- microbe interactions for disease management, adaption and optimum production of key metabolites in selected medicinal plants
- Molecular and biochemical characterization of bioactive molecules and enzymes from cultivable microorganisms with special properties from extremophiles (thermophiles, Psychrophiles, alkalophiles etc) and endophytes
- Characterization of cultivable and non-cultivable microbial populations from extreme environments
- Screening and characterization of bioactive molecules and new/novel

enzymes from metagenomic libraries constructed from environmental samples from unique niches

- Heterologous protein production in filamentous fungi and use of novel promoters in expression system
- Mechanism of drug resistance in *Salmonella*, particularly with reference to role of different efflux pumps
- Development of molecular methods for culture-independent and rapid detection of food-borne and clinical pathogens

(SSP0406 PNG-CSIR Project, 2007-2010)

- Plant and microbial molecular biology (Measure Lab Fund, 2007-2010)
- Development of biocatalytic tool box (2007-2010)
- Bioprospecting of *Epimedium elatum*, GAP-1108 (DBT, India, 2007-2010)
- Bioprospecting of *Melissa officinalis* (DST, 2008-2011)

Project

- Plant, Microbe and Soil interactions (CSIR-Network Project; 2012-2017)
- Exploration of micro-flora isolated from north western Himalayas for anticancer molecule(s) (DBT, India; 2012-2015)
- Bioprospecting microbial species from unexplored ecological niches for novel molecules and enzymes (MLP-1008)
- Bioprospecting of endophytic fungi from high value medicinal plants (MLP-1009)
- Bio prospecting microbial strains from North Western Himalayas (NWP006 CSIR Networking, 2007-2012)
- Fractionation characterization and molecular identification of SK-II Pitera

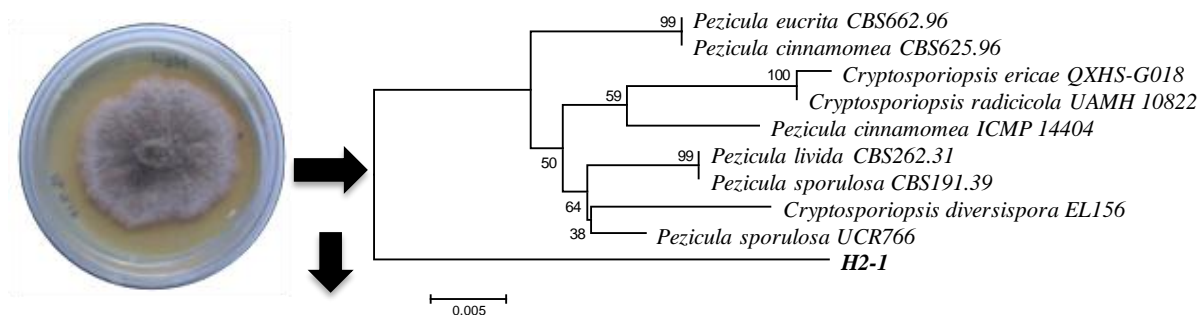
People

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Current Research

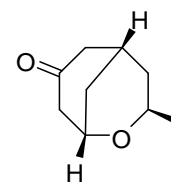
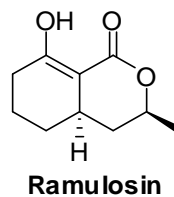
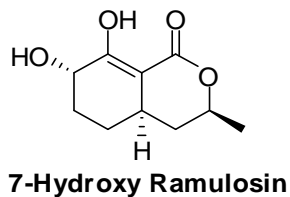
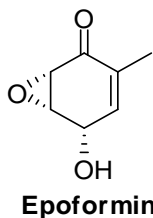
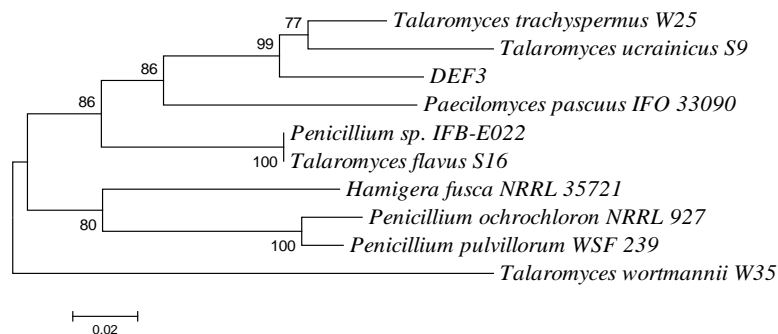
Bioprospecting for endophytes and their bioactive molecules

The project seeks to establish a repository of endophytes and isolation and characterization of novel bioactive agents. These organisms are obtained from specific areas of the state in which there are areas of endemism, ethnobotanical applications of plants and strong botanical biodiversity. Our rationale is based on the fact that certain microbes, selected from their natural plant ecological settings, may provide new and useful leads in industrial, medicinal and agricultural product discovery. The microbes are screened for antimicrobial, anti-cancer and immune suppressant activities. The endophytes are taxonomically characterized with conventional and molecular phylogeny. Preference is given to microbes representing unique taxa. The bioactive compounds are purified and characterized. Potent bioactive microorganisms will be selected for metabolic engineering of the polyketide pathway to produce new and desired molecules. The project will lead to establishment of a repository of endophytic microorganisms, isolation of new bioactive molecules against infectious agents, cancer and inflammation. It will also generate intellectual property and publications.



Phytochemistry (2013), doi:pii: S0031-9422(13)00249-5.

Cryptosporiopsis was isolated as an endophyte of *Clidemia hirta* as evidenced by ITS sequencing and microscopic examination of its fruiting structures. We report two new and one known bioactive metabolites (1-3), in which the compound 1 exhibited moderate cytotoxic activity in the human leukemia cell line, HL-60 ($IC_{50} = 4 \mu\text{g/ml}$).

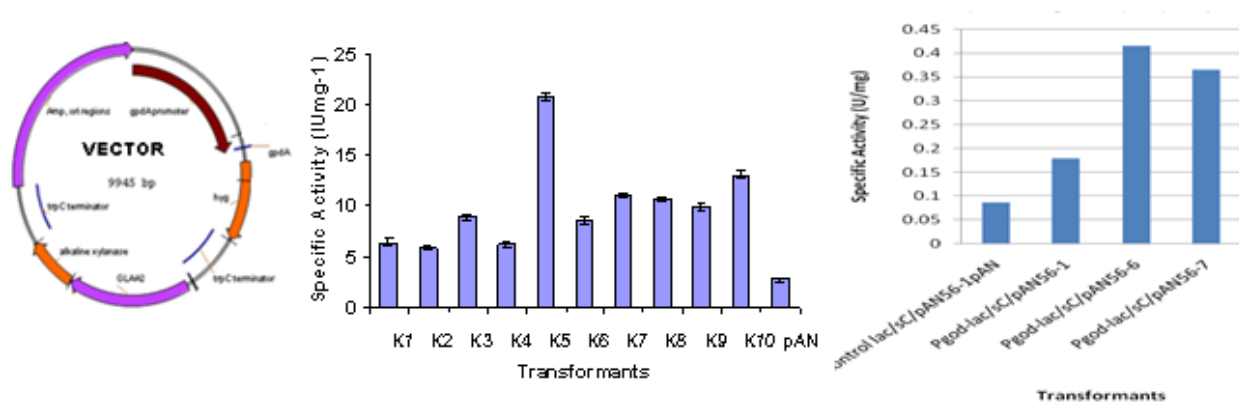


Characterization of Lovastatin Gene Cluster of Aspergillus niger

Filamentous fungi produce many secondary metabolites with complex chemical structure via the polyketide pathway. Lovastatin and its derivatives are the most commercially important polyketide drugs, with a market value of over US\$12 billion in 2007 (Broad Institute). Lovastatin can be produced by *Aspergillus terreus*, and *A. flavipes*. The biosynthetic pathway of lovastatin in *A. terreus* has been investigated. *A. niger* is an important industrial strain and given 'Generally Regarded As Safe' status. Recently its genome has been sequenced. Annotation of genomic sequences suggested that it also has lovastatin biosynthetic gene cluster. Because of the importance of this molecule, gene cluster of lovastatin biosynthesis will be characterized from *A. niger* and recombinant clones expressing these proteins will be utilized for generating the new analogues of lovastatin which can be exploited for various bioactivity other than cholesterol lowering.

Novel Expression System for filamentous fungi

Filamentous fungi have ability to produce proteins in large quantity (30g/l). To exploit this quality of filamentous fungi and non-availability of commercial expression system, an expression system was developed. Two promoters (of glucose oxidase/catalase gene) from *Aspergillus niger* strain were isolated and used in the development of expression vector for expressing the heterologous proteins. Under these promoters, expression of reporter proteins was upregulated suggesting the efficient working of system.



FEMS Microbiology Letters (2012) 327, 33-40.

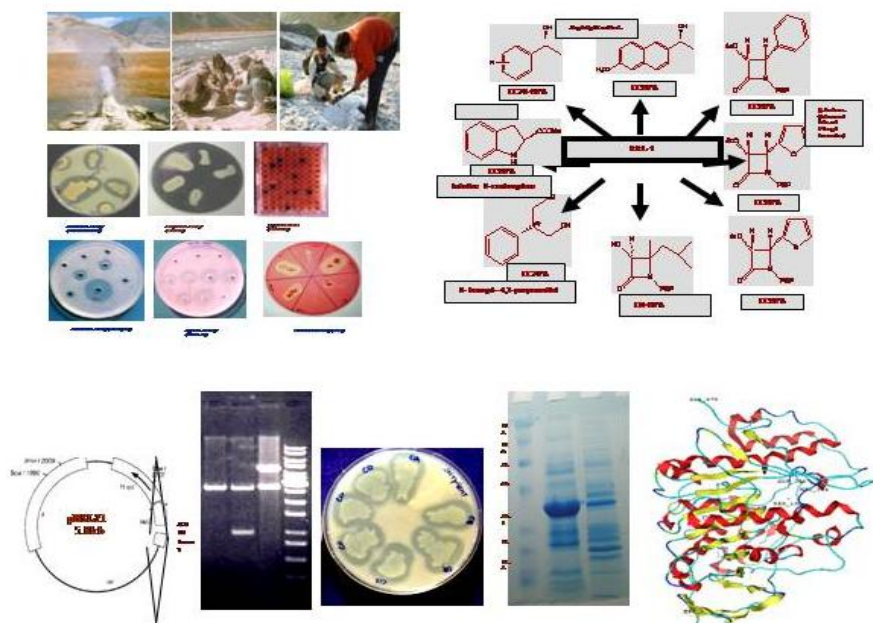
Characterization of bioactive molecules and microbial biocatalysts by metagenomic approach

Metagenomic libraries were constructed from soil sample collected from Apharwat glacier (13,000 ft above sea level) from North-west Himalayas and forest soil (Kupwara) of Kashmir for characterization of new antifungal and antibacterial compounds and novel enzymes. Identification of multifunctional protease, phospholipase C and amylase from unculturable was done by function driven approaches. Analysis of Microbial (bacterial and archaeal) diversity in the soil samples collected from Kupwara and Apharwat from Kashmir and saltpan sediment of Mumbai was done using 16s ribosomal DNA sequencing.

Hyperexpression of genes encoding novel enzymes like carboxylesterase/lipases (with broad substrate specificity and high enantioselectivity) from culturable microbes was carried out. Protein engineering (directed evolution/site directed mutagenesis) of these enzymes for thermal stabilization and better catalytic activity has been undertaken. Work is also being done on cloning and hyperexpression of genes encoding highly thermostable endocellulase from a salt

tolerant mesophilic bacterium and genes encoding cellobiohydrolase1 and glucose tolerant β -glucosidase from a biomass degrading fungal endophyte.

Bioprospecting microbial biodiversity of north-west Himalayas for biocatalysts for industrial application



Extremophiles. (2013) 17(2): 229-239.

Exploration of extremophiles/high altitude medicinal plants from Kashmir valley for novel secondary metabolites and biocatalysts

Samples were collected from different geographical high/low altitude locations of Kashmir valley and subjected to isolation of extremophiles (psychrophiles, psychrotrophs, thermophiles, alkalophiles and acidophiles). Characterization of one cold active and one thermostable stable lipase/cutinase from two psychrotrophic bacterial strains IIM3 and IIM5 has been completed. These two enzymes are optimally active both above and below the optimal growth temperature of the microorganisms from which they were isolated. The cloning & hyperexpression of genes encoding these enzymes is underway.

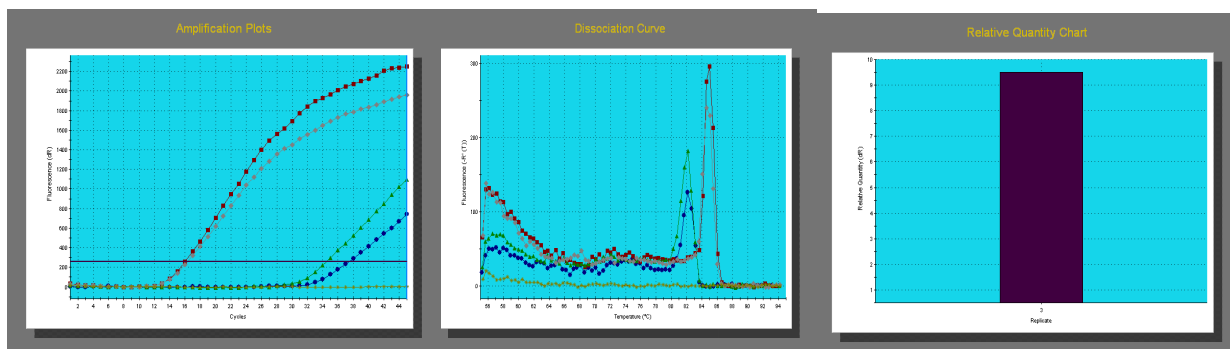
We are also exploring high altitude medicinal plants and their endophytes for bioactive molecules. Icariin and Epimedin B two principal bioactive molecules from *Epimedium elatum* have been isolated and characterized so far. Our focus is study the pathways of these

pharmaceutically active secondary metabolites in extremophilic bacteria / fungi and high altitude medicinal plants and use of genetic tools for their applications in human health, agriculture etc.

Mechanism of Drug resistance in Salmonella

Salmonella spp. are facultative, intracellular parasites that invade the mucous membrane, and are transmitted to humans mainly through water and food. *Salmonella* infection is the most frequent food-borne gastrointestinal disease transmitted from animals to humans. It is estimated to be responsible for the death of more than 500 people each year, with costs of \$1.1 billion to \$1.5 billion annually in the United States alone. The occurrence of resistance to multiple antibiotics presents a serious problem in the treatment of bacterial infections. Resistance can be caused by a variety of mechanisms, active efflux of the antimicrobial agents being one of the most important.

We are studying the role of known and putative efflux pumps in conferring drug resistance to *Salmonella* using molecular biology tools.



Relative expression (9.5 fold) of *AcrD* in drug resistant mutant of *Salmonella typhimurium*

Molecular Diagnostics

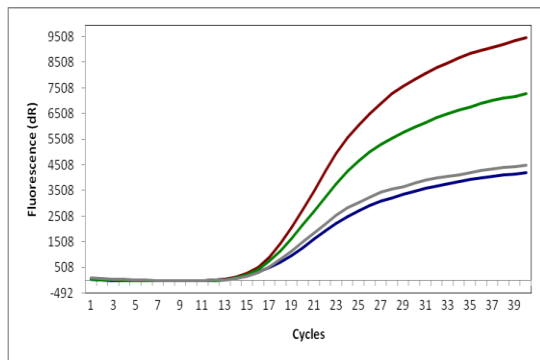
Culture-independent Real-Time PCR assays were developed for the detection of important pathogens in clinical and food samples. The protocols were validated on the micro-PCR system devised by the industrial partner, M/S Bigtec Pvt. Ltd., Bangalore. The reaction mix was immobilized on the PCR chip and real-time PCR tubes to simplify the whole process.

Real-time PCR assays based on SYBR Green or Taqman chemistry were developed for the detection of

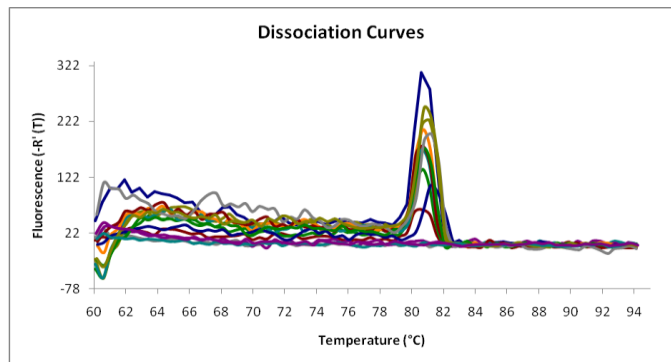
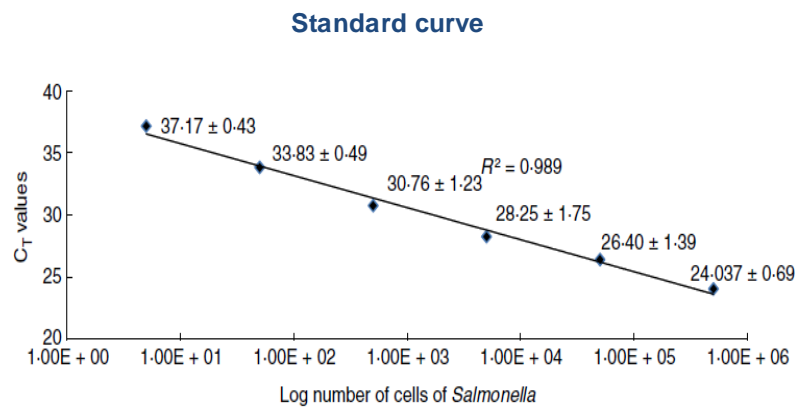
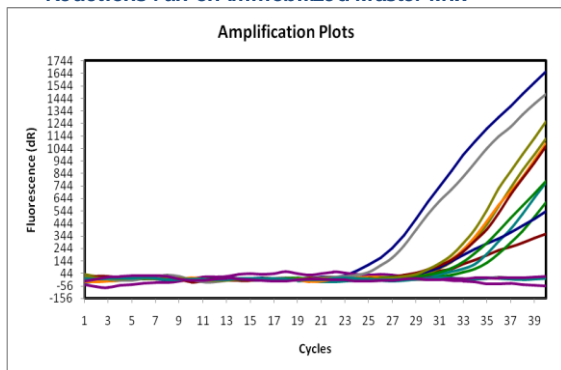
- *Salmonella* spp.
- *Shigella* spp.
- *E. coli*

- *Shiga-toxic E. coli*
- *Aspergillus flavus/A. parasiticus*
- Hepatitis B

A protocol was developed for the extraction of metagenomic DNA from whole milk and subsequent detection of pathogens by real-time PCR. 150 samples of raw milk were collected from the local market and analyzed. All samples tested positive for *E. coli*, nine samples for shiga toxin producing *E. coli* (STEC) and 30 for *Salmonella*. The validation and field application of these assays are being carried out.



Reactions run on immobilized Master Mix



Detection of *Salmonella* by Real-time PCR: addressing some core issues

Letters in Applied Microbiology (2013), 56: 275–282 (Editor’s Choice paper of the month)

PCR based Diagnosis of *Cryptosporidium parvum*

Cryptosporidium is a protozoan parasite responsible for diarrhea to humans and animals. Cryptosporidiosis is a zoonotic disease, transmits from animals to human through food and water. In AIDS patients, diarrhea is mainly because of this disease. It even becomes a cause of

death due to excessive dehydration. Diagnostics of this parasite is a paramount issue. Different human and animal samples were diagnosed for this disease using ELISA and PCR. Identification of the species which would be the main causative agent of human infection will lead us to control the disease accordingly.