Annual Report 2009-10

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भाकृअनुप
ICAR

भारतीय कृषि अनुसंधान परिषद
Indian Council of Agricultural Research

NBAIM
रा.कृ.उ.सू.ब्यू.

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो
NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT MICROORGANISMS

Understanding and conserving our national heritage of agriculturally important microorganisms

Published by

Dilip K. Arora
Director, NBAIM

Editorial Board

Rajeev Kaushik
Alok K. Srivastava
Sudheer Kumar
D. P. Singh

Secretarial Assistance

Manish Kumar Jain
Anchal Kumar Srivastava

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Executive Summary

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National Bureau of Agriculturally Important Microorganisms (NBAIM) is among one of the premium organizations of agricultural and microbial biotechnology holding its responsibilities in the area of collection, isolation, conservation, management and utilization of agriculturally important microorganisms (AIMs) in the country. The Bureau is engaged in the multifarious activities leading to the establishment of microbial diversity, biological control, genomics, culture collection, maintenance and microbial gene pool acquisition. Developing the technical and scientific skills among the researchers, scientists, students and industry people through education and training for the molecular identification and characterization is among the prime aims of the bureau and it is making significant progress in this area with commendable dedication. The mandate of the Bureau has been set to undertake basic, applied and adaptive research programs for the successful functioning of the microbial repository and to generation of highly skilled knowledge base and technical expertise among researchers, students and in-service scientists to combat the upcoming challenges of microbial diversity, quarantine and protection from invasion of species across the borders.

NBAIM has made it competent enough in the past years technically and scientifically to act as a vibrant, dynamic and responsive organization in the mainstreams of microbial culture collection, genomic resource repository and microbial biotechnology. With its sincerely dedicated scientific and technical staff, modern infrastructure, Research and Development activities and state-of-the art instrumentation facilities, we are devoted for the great national cause. The salient multi-dimensional activities observed in the year 2009-2010 were…

- This year, the Bureau has reached a target of acquiring 3500 microbial accessions cultures in its culture collection including bacteria, fungi, actinomycetes and cyanobacteria. The Culture collection unit is equipped with well established laboratory for characterization, preservation and maintenance of different types of microbial cultures, passport data information of microbial holdings in electronics format in a custom made data management software for easy access and fast monitoring for lyophilization schedule and revival alerts and the electronic surveillance system.
- Microbial Genome Resource Repository (MGRR) has made significant achievements in this year. A total of 801 genomic DNA from 350 Bacteria, 304 Fungi, 65 *Rhizobium*, 55 Actinomycetes, 27 Cyanobacteria have been conversed. Besides, 139 metagenomic clones of Lalkuan paper mill effluents from agriculturally irrigated lands are preserved. 203 gene sequences have also been preserved from in-house projects of NBAIM. Various T-vectors, markers assisted vectors like pGFT, pUC^{18}, pUC^{19}, THα-5 are preserved in glycerol stocks.
- With efficient scientific and technical team, the Bureau is strengthening its linkages with National organizations including Indian Council of Agricultural Research, Council of Scientific and Industrial Research, Department of Biotechnology and Department of Science and Technology, State and Central Universities, Agricultural Institutes and International Microbial Resource Centers.
- Scientific, academic and industrial communities including scientists, teachers and research students from all over the country and abroad have visited and revisited NBAIM for enhancing and up-scaling their knowledge-base on molecular identification and characterization of bacteria, fungi, actinomycetes, methylotrophs

and cyanobacteria. Three Two training programs and one Summer school have been organized during 2009-10 that has witnessed overwhelming response and great success.

- NBAIM is a consortium partner in the National Agriculture Innovation Project (NAIP) Mega Project "Bioprospecting of microbial genes and Allele mining for Abiotic Stress Tolerance". The Bureau is a consortium leader of another NAIP funded project "Diversity analysis of *Bacillus* and other predominant genera in extreme environment and its utilization in agriculture"
- NBAIM is successfully handling the nation-wide Network Project "Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)" as a nodal center and managing and coordinating the research activities being carried out at 61 centers working in all over India with 81 Principal Investigators, 139 Co-Investigators and 159 research students (Senior Research Fellows and Research Associates).
- Significant research and development under AMAAS has been documented with the whole genome sequencing of one of the important microbe, *Mesorhizobium ciceri* using next generation 454 Pyrosequencer. About 849 million base pairs sequence data generated in the form of 2684068 (2.68 million) sequence reads. Total 64 contigs formed having average length of 112361 (0.11Mb) base pairs. Largest contig having 577250 (0.57 Mb) base pairs. Complete assembled genome is about 6.7 MB of estimated genome size and 70X coverage depth.
- More than 20 explorations and extensive surveys in different parts of the country and national hot-spots have been undertaken during the year 2009-10. The states that were covered are Uttar Pradesh, Himachal Pradesh, Rajasthan, Bihar, Orissa, West Bengal, Arunachal Pradesh, Assam, Sikkim, Meghalaya, Kerala for the collection of samples from different habitats (soils, plants, fresh water, hot-water lakes, extreme cold conditions, marine ecosystems, mangroves, agro-waste soils etc.) in order to isolate, identify and characterize microbes and their products.
- Molecular fingerprinting for many useful microbes including *Bacillus, Pseudomonas, Fusarium, Macrophomina* and *Serratia* has been completed. Gene sequences from potential microbes have been submitted to the Genbank and NCBI and accession numbers have been obtained.
- Reproducible, rapid and sensitive protocols have been developed or modified for the isolation of genomic DNA from filamentous cyanobacteria.
- Bioprospecting and chemoprospecting of different groups of bacteria including methylotrophs, actinomycetes, cyanobacteria and plants inoculated with these organisms is baing undertaken using High Performance Liquid Chromatograph (HPLC) for phytohormones, phenolics, flavonoids, amino acids, carbohydrates and alkaloids.
- Currently, NBAIM is maintaining a vast diversity of important microbes with significant importance in crop productivity (i) Biocontrol Agents: *Trichoderma* spp., *Paecilomyces thermoascus, Paecilomyces lilacinus, Beauveria* spp., *Gliocladium verens, Verticillium* spp.; (ii) Biopesticides*: Beauveria* spp., *Metarhizium* spp., *Paecilomyces* spp., *Verticillium* spp., *Nomurea* spp.; (iii) Growth Promoters: *Pseudomonas fluorescens, P. syringae, Rhiobium* spp., *Bradyrhizobium* spp.; (iv) Enzymes/Antibiotics/Toxins producers: *Fusarium pallidoroseum, Fusarium oxysporum, Pencillium citronum, Penicillium frequentens,* isolates of *Aspergillus; (v)* Entomopathogenic: *Beauveria* spp., *Metarhizium* spp., *Verticillium* spp., *Nomuraea* spp.; (vi) Egg Parasitic Fungi: *Paecilomyces lilacinus, Verticillium chalamydosporium, Mycoparacitic* fungi, *Gliocladium* spp.; (vii) Bacteria possessing nematicidal and insecticidal properties: *Bacillus aerculans, Bacillus brevis, Paenibacillus alvei, Brevibacillus laterosporus;* (viii) Biofertilizers: Species of *Nostoc* and *Anabaena*, *Rhizobium, Azospirillum* and *Azotobacter*.

Prof. Dilip K. Arora
Director

Introduction

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National Bureau of Agriculturally Important Microorganisms (NBAIM) was set up in the IX Plan in the year 2000-01 with an outlay of Rs. 533 lakhs for the first two years. However, the Bureau actually started functioning since July 2001 with an Officer–on Special Duty (OSD) and one Assistant Finance and Account Officer (AF&AO) as regular staff. The Bureau has made a modest beginning with the appointment of one Assistant Administrative Officer (AAO) and a few contractual administrative staff. Dr. Dilip K. Arora joined NBAIM as founder Director on 29th April, 2002.The Bureau started functioning at Old NBPGR Building, New Delhi with the manpower of Director, AAO and AF&AO only and remained there until 31st May 2004. On June 01, 2004, the Bureau was shifted to the left-over building of National Institute of Sugarcane Technology (NIST) at Maunath Bhanjan, Uttar Pradesh. Now, NBAIM is working at the village Kusmaur, about 15 km away from Maunath Bhanjan city.

The Bureau draws the guidelines from the Crop Sciences Division of Indian Council of Agricultural Research (ICAR), Institute Management Committee (IMC), Research Advisory Committee (RAC) and Institute Technical Committee (ITC). The NBAIM has been sanctioned a total strength of 20 scientists, 30 technical staff, one AAO, one AF&AO and 24 administrative staff. At present it has 34 employees (Director, 9 Scientists, 3 technical staff, 6 administrative staff and 15 supporting staff).

Looking into the prospects of the most modern research trends including microbial ecology, genomics, bioprospecting, gene mining and bio-product development, the Bureau has focused on taking a lead in research and development in these areas and consequently has acquired state-of-art facilities like genome sequencing units, DNA fingerprinting unit, Shotgun Cloning Lab, Sequencing Laboratory and Genoinformatics centre, Confocal and SEM microscopy, HPLC and GC units and separate unit for computerized documentation. A Local Area Network and Website of NBAIM was created and all the units of the NBAIM are linked with various ICAR institutes and research organizations in the country.

Mandate

"To act as the nodal Institute at national level for acquisition and management of indigenous and exotic microbial genetic resources for food and agriculture, and to carry out related research and human resource development, for sustainable growth of agriculture.

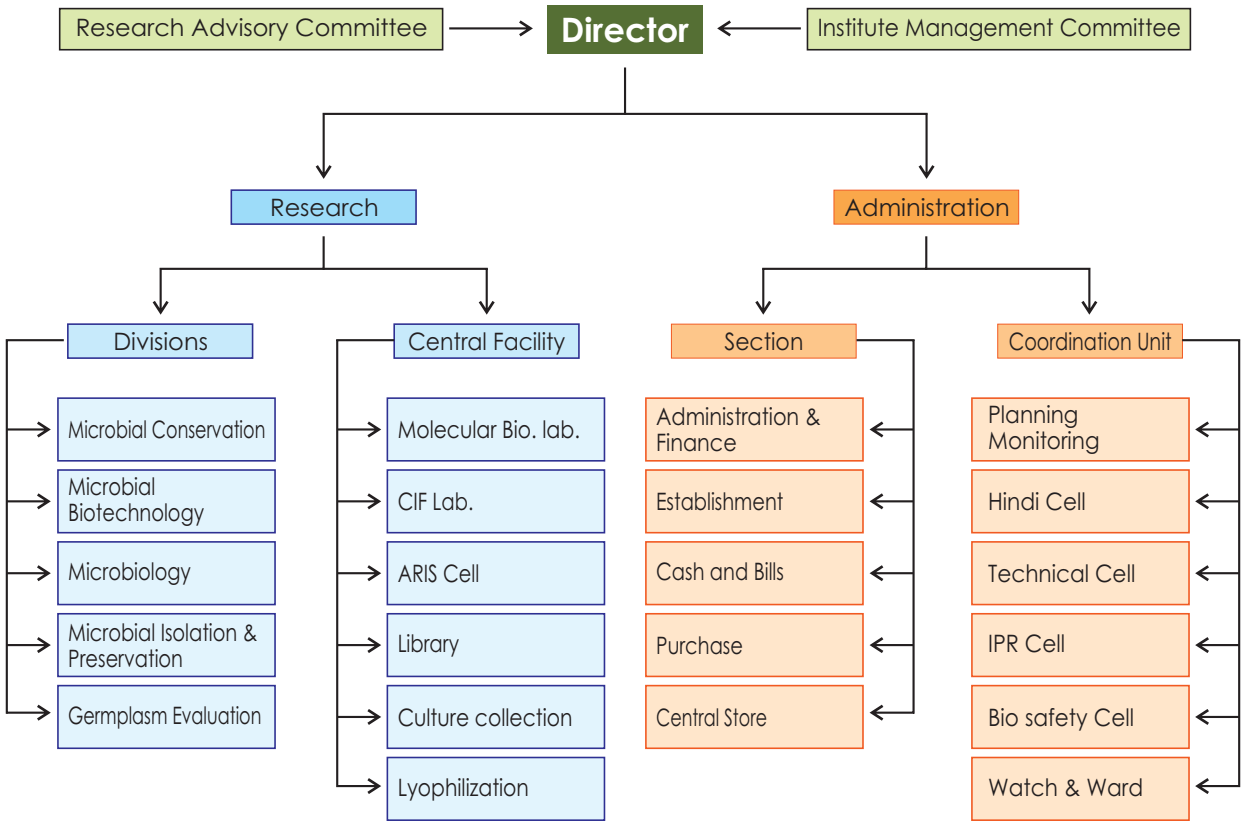
Objectives

- Exploration and collection of agriculturally important microorganisms (AIMs)
- Identification, characterization and documentation of AIMs
- Conservation, maintenance and utilization of AIMs
- Surveillance of indigenous/exotic AIMs
- Microbial biodiversity and systematics
- Human resources development (HRD)

Structural Organization of the Bureau

The major research activities of the Bureau are being carried out in different sections/units like Microbial conservation, Microbial biotechnology, Microbial isolation and preservation, Microbial Germplasm evaluation. The faculty comprises entry level as well as Senior and Principle Scientists who are supported by a number of technical and administrative personnel. Administration, auxiliary and supporting personnel help in attaining Bureau's mandated objectives of research, training, consultancy and related activities.

The Organogram



Research Perspectives

India is immensely rich in its diversity of biological forms, which include all living organisms from terrestrial, marine and other aquatic ecosystems and the ecological complexes of which, they are the part. The Convention on Biological Diversity is a common concern of all Nations. The preamble to the convention on Biological Diversity aptly emphasizes, that the countries have sovereign rights over their biological resources, and are responsible for conserving and using their biological resources in sustainable manner for the benefit of present and future generations. The convention has also established commitment from the signatory countries to develop national strategies for the conservation of biological diversity. The importance of biological diversity, especially microbial entities in India has also been realized and the Indian Council of Agricultural Research established National Bureau of Agriculturally Important Microorganisms for exploration, evaluation and conservation of agriculturally important microorganisms. Due to intensification of agricultural practices and population pressure, the microbial variability is eroding at a faster pace. It is well recognized that once a variant is lost, it is lost forever. Therefore, it is imperative to conserve and characterize the variability to the extent possible for its utilization by the coming generations..

Till now very limited and discrete efforts were made to tap microbial diversity for the purpose their identification, evaluation and preserving them for different applications. NBAIM aims at putting coordinated efforts leading to the exhaustive surveys, isolations, identification and molecular characterizations, biodiversity analysis, bioprospecting and conserving the microbial diversity from various niches for varied applications. The Bureau foresees the following essential activities to be carried out:

- Selection of sites for collection of rhizospheric and non-rhizospheric soil samples, plant parts, water and other entities for isolation of microorganism
- Isolation of extremophiles from exotic zones,

extreme environments, hot springs and dunes

- Exploration and collection of Agriculturally Important Microorganisms (AIMs) from soils, plants, fresh water etc. covering different agro climatic regions
- Augmentation of cultures through exchange from various national and international organizations and culture collections
- Inventorization, characterization and documentation of AIMs
- Integrated electronic database development that includes habitat, geographic, phenotypic, genotypic, morphological, and accession information
- Deciphering the functional and structural genomics of microorganisms for their better exploitation in agriculture and allied sectors
- Short term and long term preservation of microbes using various techniques
- Development of diagnostic kits for plant pathogens and soil microbes.
- Characterization of microbes for
- Biocontrol of plant pathogens, insect pests and weeds
- Bioremediation of heavy metals, toxic metabolites and xenobiotics
- Production of value added products like bio-fuels; enzymes and mushrooms
- Development of biofertlizers and biopesticides
- Novel bioactive metabolites
- Construction of microbial gene bank
- Isolation of genes and their alleles for abiotic and biotic stress tolerance
- Sequence determination of the isolated genes
- Functional validation of selected alleles in microbes and model plants
- Identification and characterization of unculturables through metagenomics and other molecular techniques.
- Strengthening of the Bioinformatics for genomics, proteomics and phylogenetic studies
- Strengthening linkages with various institutions throughout the world

Prioritized research activities being carried out by the Bureau is as under…

1. Exploration and collection of AIMs from soils, plants, freshwater etc. from different agro-climatic regions. Collection and repatriation of AIMs from existing culture collection centers, institutions and Universities in India and abroad. Information exchange.
2. Identification, characterization and documentation of AIMs: Morphological, physiological, bio-chemical and molecular characterization. Development of molecular markers and diagnostic tools. Database of the entire collection on electronic format for easy access to information. Preparation of monographs and synoptical keys for identification of AIMs.
3. Conservation, maintenance and utilization of AIMs: Short-term and long-term preservation. Conservation of obligate parasites on host plants under controlled conditions. Utilization of AIMs as biofertilisers, biopesticides, growth promoters, bio-indicators, bio-degraders, in bio-remediation and biocomposting, food processing etc. Use of molecular and immunological markers for diversity analysis.
4. Surveillance of indigenous/exotic AIMs : Isolation and collection of exotic AIMs from different agro-climatic zones of India. Characterization of exotic AIMs on the basis of morphological, biochemical and molecular characters. Isolation and identification of bioactive compounds. Exploitation of these AIMs for sustainable agriculture.
5. Microbial biodiversity and systematics : Analysis of microbial diversity using molecular methodologies. Inter- and intra-species variation among microbial populations, its identification and quantification. Digitization of the national microbial biodiversity data.
6. Human resources development (HRD): Training to researchers and students for molecular identification of AIM's, tools for biotechnological approaches and its implementation. Transfer of technology from the laboratory to land. Identification of hitherto microbial diversity of the country.

Infrastructure

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Research Facilities

NBAIM is having various units including offices, guest house, laboratories, hostels and residential quarters for the need of the scientists and staff posted. During 2008-09 the administrative and financial units have been expanded with more facilities. The hostel facility at the Bureau is upgraded and now it is having 110 rooms to house the Research Associates, Senior Research Fellows and the trainees coming from various Institutions to carry out research work. The hostel also has a mess run by the staff and students at no-profit-no-loss basis. To ensure regular water and electricity supply, tube-wells, irrigation facilities and power generators have been installed within the campus. Electricity supply is being substantially enhanced and provided with new high-power DG sets to run the controlled working environment in the laboratories. Whole campus is equipped with the internet facility through Wi-Fi.

Instrumentation

Most modern equipments required to carry-out research work in the molecular biology and microbial biotechnology are not only introduced but fully operational in the NBAIM. To name a few are

- Pyrosequencer
- 16 Capillary DNA Sequencer
- Confocal laser microscope
- Microbial growth kinetic analyzer
- Robotic DNA extraction system
- Pulse field gel electrophoresis system
- Electroporator
- Scanning Electron Microscope
- Media Preparator
- Automatic DNA extraction System
- Atomic Absorption Spectrophotometer
- High Thoroughput electrophoresis
- Nitrogen Analyzer
- UV-Vis Spectrophotometer
- Centrifuges
- Fermentor
- Bead Beater
- ELISA Reader
- Gene Analyzer
- Water Bath Circulator
- Ultra-Sonicator
- PCR machines
- RT-PCR Facility
- GLC and HPLC Facility
- Gel Documentation Facility
- BIOLOG Microbial identification System
- Lyophilizers
- Ultra Centrifuge
- High Speed Centrifuge
- Freeze Drier
- Water Purification System
- BOD Incubators
- Ovens
- Dish Washers
- Ice Flaking Machine
- Plate Centrifuge
- Greenhose facility
- Liquid Nitrogen Production plant

Agricultural Research Information System (ARIS) cell

The ARIS cell is fully functional in the Bureau with the latest facilities and user friendly softwares. The cell is also providing internet facilities to scientists and other staff.

IPR and Bio-safety Cell at NBAIM

The Indian Council of Agricultural Research (ICAR) envisions harnessing science through generation, refinement and assessment of appropriate technologies that will ensure comprehensively sustained physical, economic and ecological access to food, nutrition and livelihood security for all. The issues of Intellectual Property Rights (IPR) provided agricultural scientists with a rich pool of technological gain for the development of commercial products while protecting our own natural resources by law. In response to the changing

scenario of technology generation and dissemination, ICAR has developed a policy framework that will guide the management of Intellectual Property (IP) created by its scientists/innovators at its institutions or elsewhere, and that developed with its support.

ICAR Guide lines for Intellectual Property Management, Technology Transfer and Commercialization states the fact that ICAR will seek patents on microorganisms as per the Patents Act. In particular, it will not seek patent on a microorganism in the same form in which it is retrieved from its natural habitat but, in the form of its usage in agriculture, food and allied industries.

NBAIM has established its own IPR cell which is equipped with wealth of information on IPR. The Bureau is making efforts to identify, register and document the novel microorganisms, genes, and microbiological processes for patents as per the ICAR and other GOI guidelines. The Manual of Patent Practice and Procedure of Indian Patent Office as described by them, is also being applied as guidelines for the Bureau.

- The living entities of natural origin such as animals, plants, in whole or any parts thereof, plant varieties, seeds, species, genes and micro-organism are not patentable. Any process of manufacture or production relating to such living entities is also not patentable.
- The living entity of artificial origin such as microorganism, vaccines are considered patentable.
- The biological materials such as organs, tissues, cells, viruses etc. and process of preparing thereof are not patentable under Section 3 (c).
- The biological material such as recombinant DNA, Plasmids and processes of manufacturing thereof are patentable provided they are produced by substantive human intervention.
- Gene sequences, DNA sequences without having disclosed their functions are not patentable for lack of inventive step and industrial application.
- The processes relating to microorganisms or producing chemical substances using such microorganisms are patentable.

NBAIM follows strict quality control and biosafety standards in culture collection as well as in the laboratories. Various types of microorganism including filamentous fungi, bacteria, actinomycetes and yeasts are maintained under long-term preservation. Each culture is preserved by at least two methods according to the type of microorganism. Fungi are preserved either under mineral oil or by freeze-drying/ lyophilization. The bacteria, actinomycetes and yeast are preserved either by freeze-drying/ lyophilization or in glycerol at – 80° C. For short term storage the cultures are maintained on slants in appropriate medium at 4^0C. Biosafety cell at NBAIM collects information regarding safe usage and handling of agriculturally important microorganisms in the laboratories and the fields.

Organisms for deposit meet the acquisition criteria of the Collection; fall into the groups agreed in the Biosafety and have documentation providing isolation data, in particular the country of origin and identity or characterization information. The culture collection checks dangerous pathogens listed in Indian biosafety rules and regulations **(**Rules for the manufacture, use, import, export & storage of hazardous micro organisms, genetically engineered organisms or cells, 1989) before accepting a strain. The depositor must provide proof that prior informed consent to collect and deposit the strain in a collection had been obtained or reasonable efforts had been taken to do so. Conditions of deposit must be determined and agreed e.g. laid down in a materials transfer agreement to meet the Convention on Biological Diversity. Organisms are received directly into a laboratory where a laid down procedure is followed to ascertain whether the organisms can be handled safely i.e. the appropriate containment level is in place (see Laboratory Procedures Manual). A unique accession number is allocated to the strain, which is never reassigned if the organism is later discarded. The viability, purity, identity, growth requirements, and methods of maintenance and/or preservation of the strain are determined and the information recorded (as laid down in Laboratory Procedures Manual). These records are retained along with information on preservation and growth.

NBAIM Website

NBAIM website (http://www.nbaim.org) reflects different activities of the Bureau in different profiles viz., mandate, about the Bureau, culture collection, scientific plan, gene bank, library, album (photo gallery), future activities etc. A list is also displayed about available agriculturally important fungi, bacteria and actinomycetes at culture collection with information regarding utility, preservation and conservation. Very soon, it will be designed as per the latest format given by the ICAR.

Genome Sequencing Lab

The National Bureau of Agriculturally Important Microorganisms has well equipped Genome Sequencing Lab. Lab contains 4 separate dedicated rooms assigned for different applications to avoid the cross contamination of genomic materials. These rooms used for Shotgun Genomic Library preparation (Hydro shear and Bio Analyzer), PCR (Amplicon room), Electrophoresis & Gel Documentation and DNA Sequencing section respectively.

Genome Sequencing Lab has Next generation sequencing facility with next generation Pyro Sequencer (454 Life Sciences). These next generation sequencers generate very huge amount of sequence data with massive parallel sequencing. Next generation sequencers can be used for the whole genome sequencing, Re-sequencing, comparative sequencing, and metagenomic sequencing and for other applications also. The NBAIM is involved in the first genome sequencing project of India. The NBAIM is sequencing whole genome of an agriculturally important bacterium. After the sequencing and annotation of bacterial genome, there will be large number of new genes and gene promoter and transcriptional factor can be predicted. This information would be highly useful for the agriculture and can be utilized for the preparation of transgenic crops and genetically modified microbes.

Microbial Genomic Resource Repository

MGRR is a well equipped repository that deposits the Genomic materials of Microorganisms. Genomic material is of different types these are genomic DNA, Plasmids, Cloning Vectors, Cloning hosts, Expression vectors, Genomic DNA libraries, Diagnostic primers and probes and many other agriculturally important microbial genes. MGRR has a state-of-the-art facility which include Robotic DNA extractor, Automated DNA electrophoresis, Pulse field gel electrophoresis, electro-porator, automated media preparator and microbial growth kinetic Analyzer. MGRR have 1000 accessions of different types of genomic material of different use.

National Agriculturally Important Microbial Culture Collection

The National Agriculturally Important Microbial Culture Collection (NAIMCC) at NBAIM preserves and conserves the microbial diversity of the country. The collection is enriched every year through isolation efforts made at the Bureau, deposits from organizations or research workers throughout the country and repatriation of cultures of Indian origin. The collection has wide diversity of fungi and includes more than 750 species belonging to 250 genera. Likewise the bacterial collection has more than 125 species belonging to 39 genera.

NAIMCC has developed state-of-the art facilities for short conservation of AIMs for 5-10 years in mineral oil and glycerol and for long term (20-25 years) preservation of AIMs under lyophilization . NAIMCC exchange the cultures under MOU basis with different National Institutes/Organizations. NAIMCC has a good collection of very useful microbes which could be used as PGPR Biofertilizers, Biopesticides, Biodegrader and in Biocomposting, Bioremediation industries etc. such as (i) Biocontrol agents: *Trichoderma*spp., *Paecilomycesthermoascus, Paecilomyceslilacinus, Beaveria*spp., *Gliocladium*, *Verticillium* spp., (ii) Biopesticides: *Beaveria*spp., *Metarhizium* spp., *Paecilomyces*spp., *Verticillium* spp., (iii) Growth promoters: *Pseudomonas florecens, P. syringae, Rhizobium* spp., *Bradirhizobium* spp., (iv) Potential Enzyme/ Antibiotic / Toxins producers: *Fusariumpallidoroseum, Fusariumoxysporum, Peniciliumcitronum, Peniciliumfrequentence*, (v) Entomopathogenic: *Beaveria*spp., *Metarhizium* spp.,*Verticillium* spp., *Nomuraea* spp., (vi) Biofertilizers: Species of *Rhizobium, Azospirillum* and *Azotobacter,* (vii) Bacteria possessing insecticidal properties: *Bacillus circulans, Bacillus brevis, Paenibacillusalvei, Brevibacilluslaterosposus*.

NAIMCC has published its first edition of "Catalogue of Strains - 2009" which has more than 3000 holding fully characterized and documented. The relevant information regarding cultures like source of isolation, place of isolation, growth conditions, depositor and year of deposit were also given with each accession. Catalogue also has composition of different culture media, deposition forms, long term storage protocols and information for registration of microbial cultures

NAIMCC also offers the facility for registration of elite microbial germplasm to facilitate the flow of such trait specifies germplasm among scientist under MoU for further research. The "Guidelines for the Registration of Microbial Germplasms" has been developed.

Major thrusts at NBAIM

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Characterization of microbes is of paramount importance, not only from the point of view of conserving them and protecting their gene-pool resources but also, for enhancing crop productivity by the ways of integrated nutrient management, soil improvement and pest and disease control. The identification of indigenous species, strains, races and types of microorganisms would also help in identifying and developing suitable bioinoculants, which in the present century will be the main armory for the eco-friendly management of biotic and abiotic stresses. The Bureau is aimed to excel in the isolation and utilization of genes for conventional and unforeseen products of high economics and values in environment and agriculture. Such efforts will greatly strengthen National capabilities in quarantine and other regulatory matters. Bureau will also perform an important function of depositing AIMs, which will facilitate the process of registration and patenting. *Above all, the Bureau will also help in understating and conserving our national heritage of microorganisms not well understood and conserved so far.*

Diversity of exotic AIMs in different ecological niche

- Modern tool and technology, especially in the field of diagnostic of AIMs will prove valuable tools in the process of planning and management of microbial diversity, allowing the incorporation of multiple criteria for a better use of AIMs.This is particularly important in developing countries like India, where ecological imbalance has reached to alarming proportion
- To conserve and characterize the variable AIMs for its optimum utilization by the coming generations. A better understanding of microbial diversity promises to provide array of new products and processes as well as a better awareness of the microbial biosphere the earth's life support system. The understanding of microbial diversity will be a critical aspect of future agricultural since this is the basis for emergence of plant diseases and so, the control of their productivity, as well as providing new ways to identify products of microbial origin.
- The microorganisms present in the diversified agro-ecosystems of India will also provide a valuable source of novel bioactive compounds.
- NBAIM will pool all the available resources and upgrade the facilities to meet the current and the future requirement for the conservation and characterization of AIMs in the country.

Biosystematics of AIMs

- The discipline of "Microbial Taxonomy and Biosystematics" has been losing ground in universities/research institutes and agricultural universities, though its importance has increased with the changing IPR scenario. There is an urgent need to revive it and it is in this context, work on "Microbial Taxonomy", "Biosystematics" and "Evolution" has become more relevant.
- Due to enormity of biodiversity of AIMs in the country, the existing facilities are far from adequate, as a result of which many scientists send their collections outside the country for identification, and in the process, the country looses valuable gene pool resource and also foreign exchange for the services which can be easily provided in India with required infrastructure.
- Biosystematics of microbial isolates of Indian origin is urgently needed. NBAIM is the only National body which can take lead in this matter and scientists and researchers from all over the country could get "identification and

diagnostics" of AIMs.

- Development of National Culture Collection Centre as per Budapest Treaty with the state-of-the-art facilities for identification and taxonomic studies of Agriculturally Important Microorganisms. This is of utmost importance, as at present no Centre/Institutes/University in India is providing the services of "Identification of AIMs" to the scientists engaged in the agricultural and scientific research and industry. NBAIM may act as a nodal centre for developing a "National Facility for the Identification of AIMs".

Exploration and survey

NBAIM acts as a nodal agency for taking appropriate measures for system-wide management of AIMs e.g. (i) constituting microbial genetic resource advisory committees, (ii) preparing national exploration maps, (iii) developing and widely disseminating guidelines for handling and storage of microbial isolates, (iv) registration and notification of microbial deposits, (v) developing/implementing coordination, linkage and cooperation mechanisms, (vi) technical backstopping for the development of national policy and its implementation, and (vii) handling matters/ concerns related to biosafety, biopiracy and IPR issues etc.

Characterization

- Morphological, physiological, and biochemical.
- Molecular characterization based on prioritization with emphasis on IPR regimes.
- Development of molecular diagnostic tools.

Documentation and inventorization

- Database of the entire collection on electronic format for essay access of information.
- Short and long term conservation of AIMs

Utilization

- Build up and exchange of exsiccate sets.
- Identification of AIMs for utilization as bio-fertilizer, bio-pesticides, growth promoting microorganism, bio-indicators and for biodegradation, bio-remediation, bio-composting.

Isolation and identification of AIMs

- Isolation, identification and utilization of AIMs in the processes of biofertilization, bioprocessing and bioremediation or addressing the pathogens causing either diseases or spoilage in agri-products constitute important national priority.
- To quite an extent, this has been attended in the ongoing agricultural research and education programs in the NARS.
- Mechanism to be developed for sending and receiving referral samples of AIMs for maintenance, cataloguing and facilitated access for use in public interest.
- Exploration, characterization, evaluation, maintenance, conservation, and documentation of various categories of microbes important to agriculture/animal science /fisheries have to be facilitated in the national system.

Externally funded research projects

- **Bioprospecting of microbial genes and allele mining for abiotic stress tolerance. NAIP**
- Diversity analysis of *Bacillus* and other predominant genera in extreme environment and its utilization in agriculture. NAIP
- Diversity and conservation of agriculturally important microorganisms and their potential as biocontrol agents". APCESS Project, ICAR
- Digitization of taxonomic data of fungi from Indian origin earlier deposited in UK-CABI. NATP
- Wilt of crops with special reference to cultural, morphological, molecular characterization and pathogenic variability. ICAR Network Project
- Collection and digitization of agriculturally important microorganisms and their DNA fingerprinting. APCESS, ICAR
- Development of sustainable management strategies for the control of *Parthenium* weed in U.P. using biotechnological approaches. DBT
- Development of molecular markers for the identification and characterization of *Fusarium* groups of plant pathogenic fungi. ICAR Network Project

Institute Projects

- Molecular and functional diversity of microorganisms isolated from extreme environments
- Assessment of genotypic diversity of *Bacillus, Bacillus*-derived genera and fluorescent Pseudomonads in Indo-Gangetic plains
- Exploration, collection, biochemical, molecular and genetic characterization of actinomycetes in Indo-Gangetic Plains of India

- Microbial management of soil borne plant pathogens in salt affected soils
- Diversity analysis and utilization of some motile and non-motile actinomycetes from mangrove ecosystem of India

Exploration, collection and identification of agriculturally important microorganisms (AIMs) collected from diseased plant materials and soils from different agro-climatic regions of Indo-gangetic plains (IGP)

- **Microbial diversity analysis of soils contaminated with industrial effluent in Northern plains of Indo-gangetic regions**
- **Evaluation of rhizosphere fungi for plant growth promotion and induced resistance in some vegetable crops**

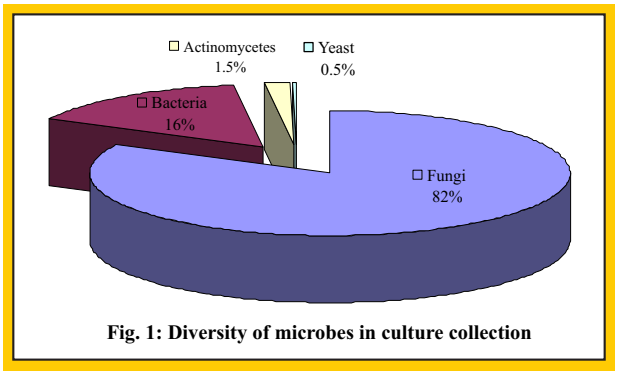
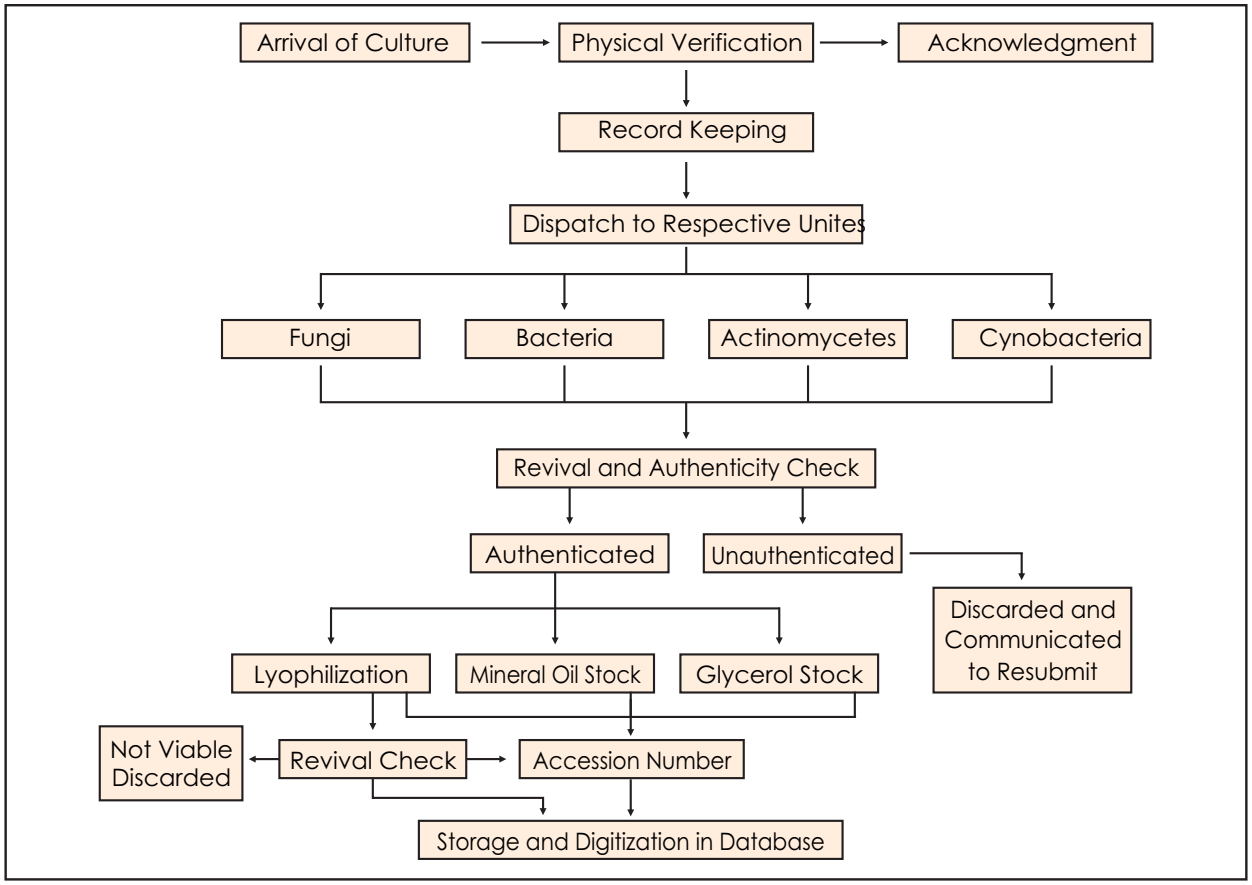


Fig. 1: Diversity of microbes in culture collection

Management chart of culture collection at NAIMCC



- Exploring cyanobacterial biodiversity in extreme habitats for potential applicability in agriculture
- Diversity analysis of plant growth promoting epiphytic and endophytic methylotrophic bacteria from different agro-ecological zones of India

Culture Collection

NBAIM has developed state-of-the-art culture storage facility in the form of National Agricultural Important Microbial Culture Collection (NAIMCC) unit. The unit has well established laboratory for characterization, preservation and maintenance of different types of microorganisms. The passport data information of microbial holdings has also been put in electronic format in a custom made data management software for easy access and fast monitoring of maintenance plan, lyophilization schedule and revival alerts. Both the culture collection laboratory and storage facility has been made under the electronic surveillance system in 2008.

NBAIM culture collection is recognized as National Repository by Biodiversity Authority of India. NBAIM follows strict quality control and biosafety standards in culture collection as well as laboratories. Various types of microorganism including filamentous fungi, bacteria, actinomycetes and yeasts are maintained under long tern preservation. The culture collection holds the credit of having wide diversity in fungal and bacterial organisms. Now the cyanobacterial cultures have been included in NAIMCC. Many important and useful cyanobacterial strains are being conserved in culture collection.

Research Achievements

5

Project 1. Isolation and Functional Diversity of Microorganisms Isolated from Extreme Environments

PI : D. K. Arora
Co-PIs : Rajeev Kaushik, Alok Srivastava, Sudheer Kumar and Mahesh Yandigeri

Rationale

Microbes from extreme environments are attracting major attention of the scientific communities in recent years primarily due to their survival strategies and unique functionalities. The study provides important clues about the origin and evolution of life, since many environmental conditions on earth closely resemble to those existing on other planets and secondly, these conditions also significantly influence the world of microbial biodiversity. At present, only a fraction of microbial life (less than 1 %) is known and explored. The microbes from extreme habitats constitute important component of the vast unexplored biological potential. The limited studies on the diversity of the extreme organisms from varied habitats have indicated highly diversified population dynamics. Therefore, exploration of many more extreme habitats would be of great significance. Thirdly and last application indeed is their utilization in biotechnological applications. The robust biocatalysts and unique metabolic capabilities of extremophiles are the major points of attraction in developing processes towards production of biofuels and bioremediation. The value based molecules from microbes dwelling in unexplored and extreme habitats have recently prompted interest among the scientific communities and industries.

Objectives

- Microbial diversity analysis in different climates
- Characterization of novel microorganisms for their utilization in biodegradation of agricultural residues, bioremediation and mining of genes for abiotic stress tolerance

Significant achievements

Diversity analysis of bacteria in North Sikkim

- Aerobic, thrermophilic bacteria were isolated and characterized from water and sediment samples collected from different hot springs of Yumtang and Yumesamdong in North Sikkim having pH 6.5.
- The total number of microorganisms in the sediment and water samples was $2x10^3$ cfu ml^{-1}.
- 53 morphotypes were selected out of which 19 were able to grow at temperature 45C and 11 at 60C.
- In soil samples of Gurudongar Lake, North Sikkim the bacterial count ranged from 1.2 to 3.7 x $10^3 ml^{-1}$
- A total of 50 bacteria were picked that were able to grow at 4^0C
- All bacterial isolates showed production of copious amount of exopolysaccharide
- Among the isolates, 12 bacteria were found to be psychrotolerant and could not grow beyond 15^0C.
- 16S rDNA-RFLP analysis with *Hae* III and *Alu* I revealed greater diversity among the isolates.

Conclusion:

Isolation and identification of microbes from extreme habitats has biotechnological implications. Microbes that are able to grow at high temperature were identified and explored for the possibilities of having valuable mechanisms and genes for temperature tolerance.

Project 2. Microbial Diversity Analysis of Soils Contaminated With Industrial Effluents in Northern Plains of Indo-gangetic Region

PI : Dr. Rajeev Kaushik

Rationale

Despite great progress in overall agricultural productivity in recent decades, land degradation has reduced the productive capacity of soils up to nearly 40% of the world's agricultural land. These soils suffer biological degradation by organic matter depletion and loss of biodiversity; physical degradation, such as erosion and compaction; and chemical degradation due to acidification, nutrient depletion, pollution from industrial wastes, and over use of pesticides and fertilizers. In light of these threats, there is growing interest in the factors governing soil health, biodiversity and resilience as well as in the fundamental relationship between them.

The boon in industrial development and continuous increase in population since last several decades has led to decline in availability of groundwater for irrigation, especially in the regions where the practice of intensive irrigated agriculture is followed such as Indo-gangetic Plains of South Asia. Due to depletion of ground water and non availability of water at right stages of irrigating a crop, the practice of using organic and inorganic matter rich industrial effluents of agro based industries such as paper mill, molasses based distillery in India is on increase. Organic wastes, e.g. pulp and paper mill effluent, molasses based distillery effluent, tannery effluent etc, can benefit plant growth by providing essential plant nutrients, especially N which appears predominantly in organic forms. However, long term application and indiscriminate use of such "mixed bag" of compounds may cause significant shifts in soil microbial community structure, which in turn may influence the viability of the soil for agriculture. Thus, there is a need to study the shift in microbial diversity of such soils in relation to changes in soil physicochemical properties, which are governed by agricultural management practices.

Objectives

- Isolation, characterization and identification of bacteria from agricultural sectors contaminated with paper and alcohol industry effluent in Northern plains of Indo-gangetic region
- Deciphering shifts in bacterial structural and functional diversity in soils contaminated with such effluents
- Development of microbial informatics relating soil microbial diversity and soil physicochemical properties

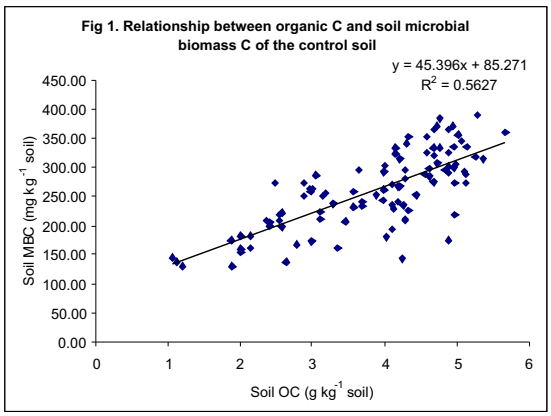
Significant Achievements:

Changes on soil biological properties

The short-term changes in soil microbial biomass are useful indicator for understanding the long-term productivity of soil. It is also frequently used as an early indicator of changes in soil physico-chemical properties resulting from soil management and environmental stresses in agricultural ecosystem. The long term irrigation of agricultural fields with anaerobically digested molasses based distillery effluent in Gajraula, Western Uttar Pradesh caused significant increase in microbial biomass carbon to microbial biomass nitrogen ratio (Cmic: Nmic ratio) from 3.72 to 6.48 in Kharif season and from 4.34 to 7.62 in Rabi season. It has been reported that Cmic:Nmic is affected by soil properties such as application of organic effluents, pH, N-fertilization etc. The Cmic:Nmic ratio is often used to describe the structure and the state of the microbial community. A high Cmic:Nmic ratio of 7 - 12 indicates that the microbial biomass contains a higher proportion of fungi, whereas a low value of 3 – 6 suggests that bacteria predominate in the microbial population. In the present study the Cmic:Nmic ratio varied between 3.72 to 7.62 in effluent irrigated soils. The increase in Cmic:Nmic ratio suggests shift in bacterial community structure.

Long term application of distillery effluent also showed increase in soil organic carbon (SOC) over the period of 20 years. The percentage of Cmic in total soil OC showed significant variation in effluent irrigated fields of rice and wheat (1.46 – 2.16 and 2.18 – 3.43) respectively, whereas, in control soil it was 1.18 and 1.51, respectively. Although, the Cmic constitutes only 1–3% of SOC but it gives an estimation of the quantity of carbon in microbial biomass, quality of organic matter in soil, its availability and soil health. Increase in soil Cmic to SOC ratio up to 2 to more than 3 due to application of effluent in rice and wheat indicates that soil health may deteriorate if the practice of effluent application in regular manner is

not stopped. The Cmic values obtained in effluent irrigated and control soil were significantly correlated with Corg (r2= 0.54 and 0.56 respectively) (Fig 1 and 2).



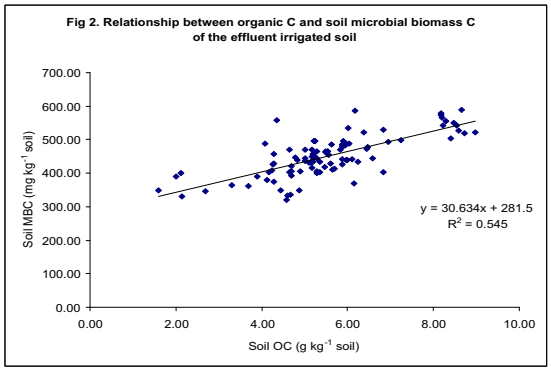


Fig. 1 & 2 Cmic values obtained in effluent irrigated and control soil

Community Level Physiological Profiling

Carbon is a key factor governing microbial growth in soil, and functional aspects related to substrate utilization could provide important information beyond that afforded by taxonomic level investigations or structural investigations based on rRNA analysis. The functional diversity of microorganisms, particularly as defined by the substrates used for energy metabolism, is integral to our understanding of biogeochemistry. Indeed, it has been argued that it is diversity at the functional level rather than at the taxonomic level that is crucial for the long-term stability of an ecosystem. The method involves direct inoculation of environmental samples into BIOLOG ECO microtiter plates (containing 31 different C sources plus a well having water as blank in three replications), incubation, and spectrometric detection of heterotrophic microbial activity. The EcoPlates contain substrates that are known to be plant root exudates or that have previously been found to have a high discriminatory power among soil communities.

Three kinds of soil samples from pulp and paper mill effluent irrigated soils (control soil, diluted effluent irrigated field soil and concentrated effluent irrigated field soil samples) were used for community level physiological profiling in three replications. The first principal components accounted for about 95% of variance. An ordination diagram from the CLPP results produced clear separation of the control as well as treated soil samples. Significant effect of pulp and paper mill effluent application on soil metabolic profiling was observed in 32 hrs of incubation (Table 1).

Table 1. Variation in C utilization as depicted by the normalized values of average well colour development in Eco plates

Carbon Source	Control Soil	DEIF Soil	CEIF Soil
Glycogen	0.31	2.52	2.04
α- D – Lactose	0.29	3.07	0.20
β- Methyl-D-Glucoside	1.60	3.21	1.84
D- Mannitol	3.63	2.09	1.55
D- Galactonic Acid Lactone	2.84	0.00	0.82
l- Arginine	0.62	1.74	1.41

DEIF: Diluted Effluent Irrigated Field; CEIF: Concentrated Effluent Irrigated Field

Changes on soil microbial diversity of unculturable microflora

Soil DNA was extracted from distillery effluent contaminated and control soils of Gajraula, UP. 16S rDNA from the soil metagenome was amplified using universal primers. The 16S region of approximately 1500 bp was cloned using TOPO TA cloning kit. Clones were randomly picked from each of the 16S product and ARDRA was performed for similarity analysis between clones. Based on ARDRA pattern it

was observed that control field showed more variability in the diversity of unculturable microflora as compared to effluent contaminated field.

From distillery effluent contaminated field the dominant microflora mainly belonged to *Bacillus, Lysinibacillus fusiformis, Flavobacterium columnare, Kochuria* sp. and *Streptomyces*. Phosphate solubilizing bacteria, Nitrogen fixers and Pseudomonad group of bacteria were found absent in distillery effluent contaminated field.

Development of mathematical correlation for analyzing microbial shift

Mathematical correlations were developed for assessing the changes in soil fertility and shift in microbial diversity based on data of microbial diversity, C-utilization pattern, soil microbial biomass carbon, soil microbial biomass nitrogen, total soil organic carbon and microbial quotient. The long-term irrigation of agricultural fields with anaerobically digested molasses based distillery effluent in Gajraula, Western Uttar Pradesh caused significant increase in microbial biomass carbon to microbial biomass nitrogen ratio (Cmic : Nmic). The increase in Cmic : Nmic suggests shift in bacterial community structure. The increase in MBC:MBN ratio shows domination of actinomyctes and fungi in the soil irrigated with distillery effluent. The ratio of % soil microbial biomass carbon to total Soil OC in effluent irrigated soil was 1.12 as compared to 2.36 in control soil (Average of 100 samples). The ratio of MBC:MBN in control soil was between 4-7, whereas in effluent irrigated soil it varied from 4-12. It shows effluent irrigation his causing significant change in total soil microbial dynamics

Conclusion:

Irrigation of farmer's field with distillery effluent released from Jubilant Organosys, Gajraula, UP, India for over 25 years in succession caused increase in soil organic carbon (SOC). The ratio of Cmic in total soil OC showed indicated deterioration in soil health. The Cmic values obtained in effluent irrigated and control soil were significantly correlated with Corg. Soil DNA was extracted from distillery effluent contaminated and control soils of Gajraula, UP. The amplification of 16S rDNA from the soil metagenome of this region and its subsequent analysis revealed that control field showed more variability in the diversity of unculturable microflora as compared to effluent contaminated field. From distillery effluent contaminated field the dominant microflora mainly belonged to *Bacillus, Lysinibacillus fusiformis, Flavobacterium columnare, Kochuria sp* and *Streptomyces*. P solubilizing bacteria, Nitrogen fixers and Pseudomonad group of bacteria were found absent in distillery effluent contaminated field. Mathematical correlations were developed for assessing the changes in soil fertility and shift in microbial diversity based on data of microbial diversity, C-utilization pattern, Soil microbial biomass carbon, soil microbial biomass nitrogen, total soil OC and microbial quotient.

Project 3. Evaluation of Rhizosphere Fungi for Plant Growth Promotion and Induced Resistance in some Vegetable Crops

PI : Alok K. Srivastava
Co-PI : Sudheer Kumar

Rationale

Cultivation of Cucurbitaceous and Solanaceous vegetables in Indo-gangetic plain (IGP) ecosystem constitutes a distinct type of farming in India. The cropping pattern in these areas is based on rainfed subsistence farming and wholesome indigenous vegetables grown in the lower IGP region of India. The direct effects of the rhizosphere fungi on plant growth and development are important for agricultural uses and for understanding the roles of these fungi in natural and managed ecosystems. Root colonization by *Trichoderma* and other fungal strains frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients. Recently, various strains of *Trichoderma* that produce cytokinin-like molecules, viz. zeatin and gibberellins GA3 or GA3-related compounds have been detected and characterized. The controlled production of these compounds could improve biofertilization. Together with the synthesis or stimulation of phytohormone production, most of the rhizosphere fungi acidify their surrounding environment by secreting organic acids and able to solubilize phosphates, micronutrients and mineral cations like iron, manganese and magnesium etc.

Plant diseases play a direct role in the destruction of natural resources in agriculture. In particular, soil-borne pathogens cause important losses, fungi being the most aggressive. The distribution of several phytopathogenic fungi, such as *Phythium*, *Phytophthora*, *Botrytis*, *Rhizoctonia* and *Fusarium*, has spread during the last few years due to changes introduced in farming, with detrimental effects on crops of economic importance. Chemical compounds have been used to control plant diseases (chemical control), but abuse in their employment has favored the development of pathogens resistant to fungicides. By contrast, the use of microorganisms that antagonize plant pathogens (biological control) is risk-free when it results in enhancement of resident antagonists.

The ability of antagonistic fungal strains to protect plants against root pathogens has long been attributed to an antagonistic effect against the invasive pathogen. However, these root-fungus associations also stimulate plant defensive mechanisms. Strains of *Trichoderma* added to the rhizosphere protect plants against numerous classes of pathogens, e.g. those that produce aerial infections, including viral, bacterial and fungal pathogens, which points to the induction of resistance mechanisms similar to the hypersensitive response (HR), systemic acquired resistance (SAR), and induced systemic resistance (ISR) in plants. The fungi themselves (both *Trichoderma* spp. and other beneficial fungi) have many proven abilities to affect plant productivity and health positively. These fungi can be exploited in efficient manner by understanding the insight mechanisms and systems operating during the interactions of beneficial fungi with plant pathogens.

Objectives

- Isolation, identification and characterization of fungi from North-Eastern Indo-gangetic plains of Uttar Pradesh
- Screening of the fungal isolates for their ability to enhance plant growth and induced resistance in some vegetables

Significant Achievements

Greenhouse assay was performed to evaluate the efficacy of *Trichoderma* isolates as resistance inducer and biocontrol agent. All *Trichoderma* strains significantly reduced the severity of the disease. Out of six selected *Trichoderma* strains, four including TM-43, TM-38, TM-22 and TM-25 gave significant enhancement in plant growth in absence of pathogen inoculation. The maximum enhancement in root length (9.05±0.85cm), shoot length (17.78±0.78 cm), root fresh weight (1.14±0.05 g) and root dry weight (0.52±0.11 g) was given by TM-43 as compared to control.

The biocontrol potential of these six isolates against *Rizoctonia solani* with or without supplementation of chitin was also evaluated. TM-38 showed minimum disease incidence (19.44±3.89) in tomato plants. Enhanced level of protection was achieved when TM-38 strain was supplemented with chitin (13.89±2.22). It was also observed that the chitin amendment in soil exhibited superior

stimulation of *Trichoderma* isolates in comparison to infested control. In chitin amended treatments, superior growth of tomato plants were recorded as 35-51% increase in root length and 35-40% increase in shoot length.

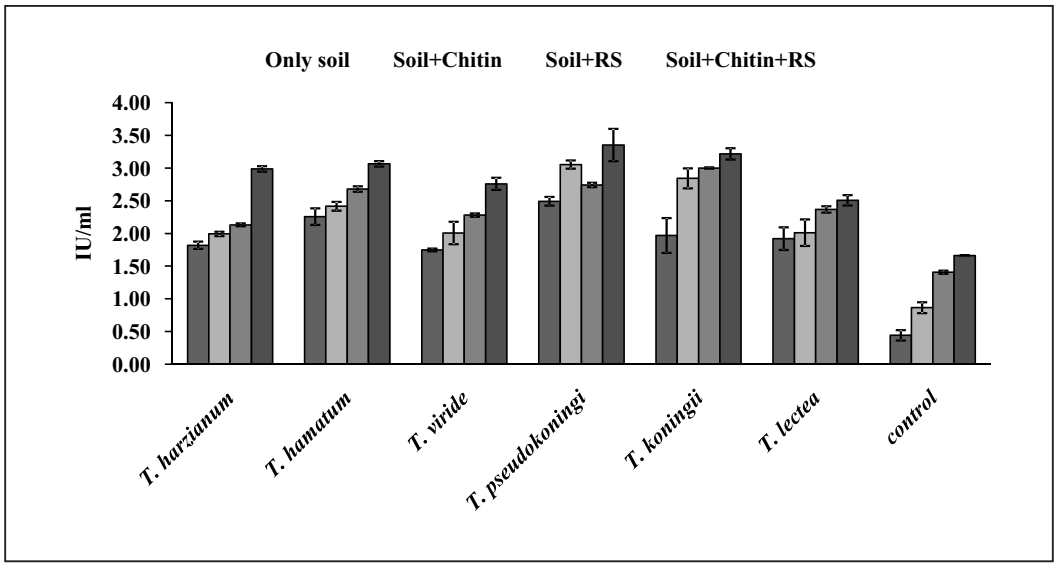


Fig 1: Level of chitinase in the plants inoculated with *Trichoderma* isolates under different soil amendments

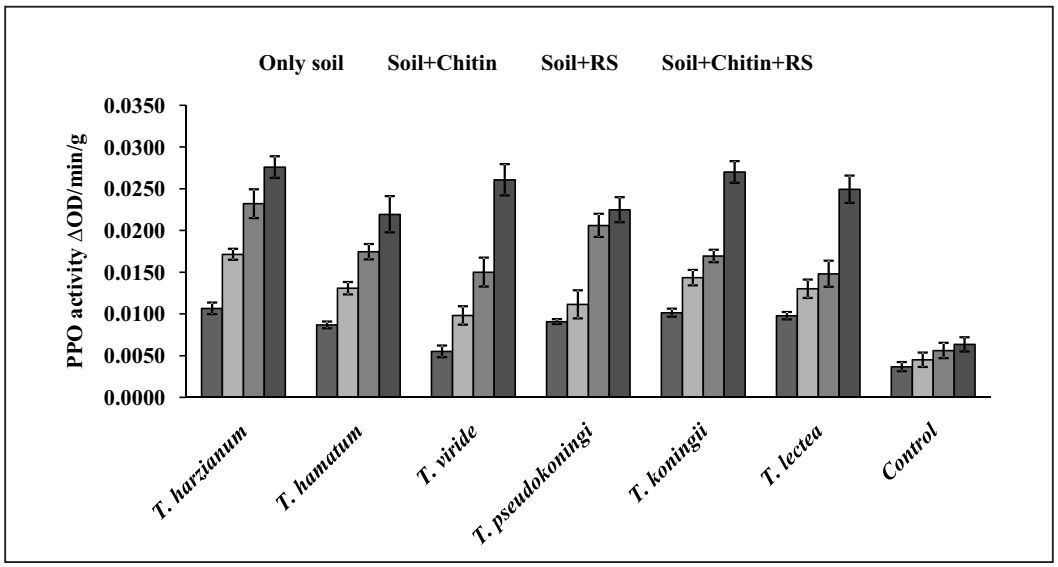


Fig 2: Level of polyphenoloxidase in the plants inoculated with *Trichoderma* isolates under different soil amendments

The levels of defensive enzymes *viz*. chitinase and PPO in tomato plants were evaluated. Higher level of chitinase activities was observed in tomato plants inoculated with pathogen and chitin with TM-29 and TM-38 isolates. All plants grown in chitin amended pots showed higher level of PO and PPO activity as compared to control.

Higher peroxidase activity was resulted in *T. koningi* with CC, it significantly ($P<0.05$) different in all treatments. The level of PO ranged from 195-330%

Fig 3: Effect of Chitin amendment on Growth of Tomato

with CC over control and 136-206% higher in the plants treated with *Trichoderma* alone. The polyphenoloxidase activity varied up to 146-243 % without CC and with CC 265-350% increase over control seedlings.

Conclusion

The present study was focused on the effect of chitinolytic substrate amendment on the improvement of mycoparasitic invasion shown by various *Trichoderma* strains and improvement of biocontrol efficacy against *R. solani* causing root rot of tomato. The results indicated that chitin directly or indirectly stimulates the growth of antagonists and plants which help plant to activate their self protection mechanism in better way. Amendment with chitin alone increased the population density and biocontrol efficiency of *Trichoderma*.

Project 4: Microbial Management of Soil Borne Plant Pathogens in Salt Affected Soils

PI : Sudheer Kumar
Co-PI : Mahesh Yandigeri

Rationale

Among biotic stress, fungi are considered important plant pathogens, particularly members of the genera *Rhizoctonia, Sclerotinia* and *Fusarium* which are able to infect a wide range of plants including several crop, vegetable, ornamental, and fruit species. The control of these diseases becomes difficult because of the formation of resistance structures like sclerotia or clamydospores.

In recent years, there is an increasing interest in the exploitation of fungi as biocontrol agents (BCAs) for the management of diseases of agricultural crops worldwide to replace/minimize the usage of harmful chemical pesticides. The excessive dependence on chemical pesticides leads to the development of resistance in plant pathogens and occurrence of residues in food chain. And also because of the increased demand for organically grown agricultural products, BCAs have become an important alternative disease control agents. Biological control offers safer, cheaper and environmental friendly management of plant pathogens.

Many soil borne plant pathogens like *Fusarium* spp., *Verticillium* spp. etc cause the disease under salt stress conditions. It is reported that mycelial growth and sporulation of different *Fusarium* spp. were motivated under salt stress conditions. Soil is an unpredictable environment and an intended result is sometimes difficult to obtain. The immediate response to soil inoculation biocontrol agent with PGPR varies considerably depending on the bacteria, plant species, soil type, inoculum density, and environment conditions. The inoculated bacteria sometimes do not survive in the soil when competing with the better adapted indigenous microflora. An effective biological control strain isolated from one region may not perform in the same way in other soil and climatic conditions. For this reason, salinity is an important factor to be considered when screening new isolates in particular different soil salinity levels.

The most commonly used antagonistic fungi are *Trichoderma* species. The pH and salt concentration effects mycilial growth, spore germination, spore production and enzyme involved in mycoparasitism. The maximum biomass production by same isolate of *Trichoderma* reported at pH range from 4.6–6.8. One of the most crucial boundary to use *Trichoderma* strains as biocontrol agent is their low salt tolerance. Thus the research programme is formulated to select the salt tolerant stain of different bacterial biocontrol agents including *Trichoderma* spp.

Objectives

1. Collection, isolation and screening of antagonists against locally important soil borne fungal pathogens
2. Screening of promising biocontrol agents for salinity tolerance
3. Mechanism of antagonism

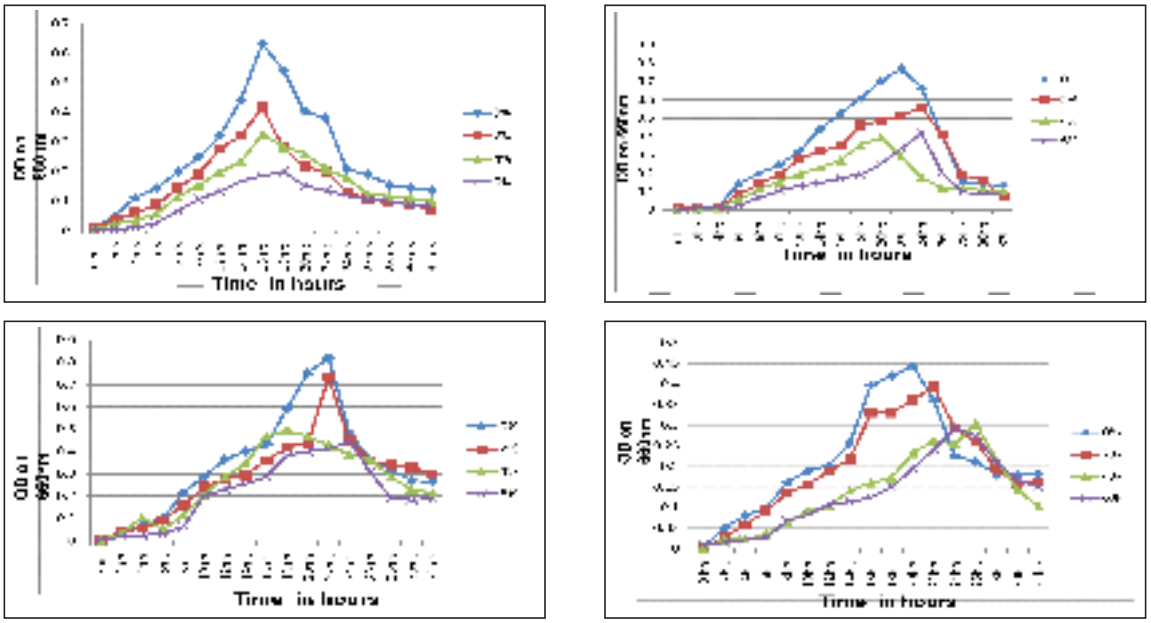
Significant Achievements

Soil samples of rhizospheric and non-rhizospheric soil were collected from salt affected areas of eastern IGP region *viz*. Kanpur, Unnao, Lucknow, Allahabad, Mau, Varanasi, Gauzipur Etawah, Agra, Pratapgarh and Raibareilly. The different selective and semi-selective media were used for isolation and a total 130 bacterial and 68 fungal were isolates and evaluated against *Rhizoctonia solani*, *Fusarium udum* and *Sclerotium rolfsii*.

The fungal and bacterial isolates were also characterized for biochemical biocontrol traits like siderophore, Ammonia, HCN and Chitinase production. The isolates showed multiple traits for growth promotion and biocontrol were analyzed for production of non-volatile inhibitory compound through well diffusion test against *Rhizoctonia solani*, *Fusarium udum* and *Sclerotium rolfsii*.

On the basis of biochemical characterization and screening for biocontrol potential finally 45 isolates have been selected for their evaluation for salt tolerance. The screening was done on the basis of growth, sporulation and dry weight of the mycelium grown in broth culture of fungal isolates. In general the growth reduces as increasing of salt concentration but some fungal isolates given stable growth up to 6% of NaCl. Whereas bacterial biocontrol agents growing well in salt on semisolid media. The population dynamics of bacterial antagonist under different salt concentration was carried out upto 6% of NaCl.

Fig. Growth kinetics of promising bacterial strains under different salt (NaCl) concentration



The isolates showing salt tolerance with biocontrol and plant growth promoting traits were further evaluated under pot conditions against root rot of tomato for their disease inhibition and plant growth promotion. The seedlings were bacterized by dipping in cell suspension (10^6 ml^{-1}) for 2 hrs and pathogen was inoculated in the soil (2%). The experiment was laid out in complete randomized block design and all the treatments were maintained in three replications. The disease severity was recorded in 5th week after transplanting. The significant reduction in disease severity was recorded in all the treatments over control. The minimum diseases intensity was recorded on seedling inoculation with strain B-123 under pot condition followed by B-101 and B-99.

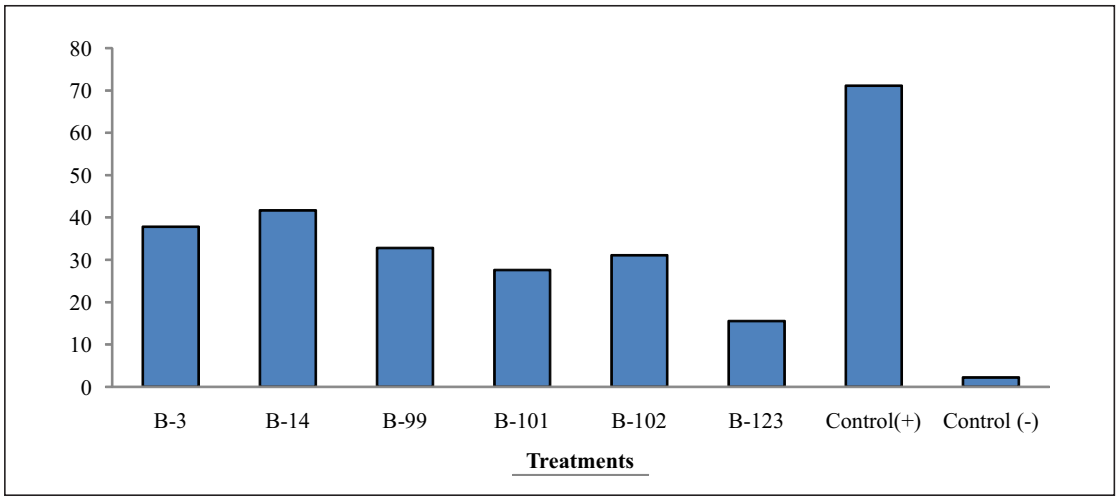


Fig. Effect of different treatments on root rots intensity (%).

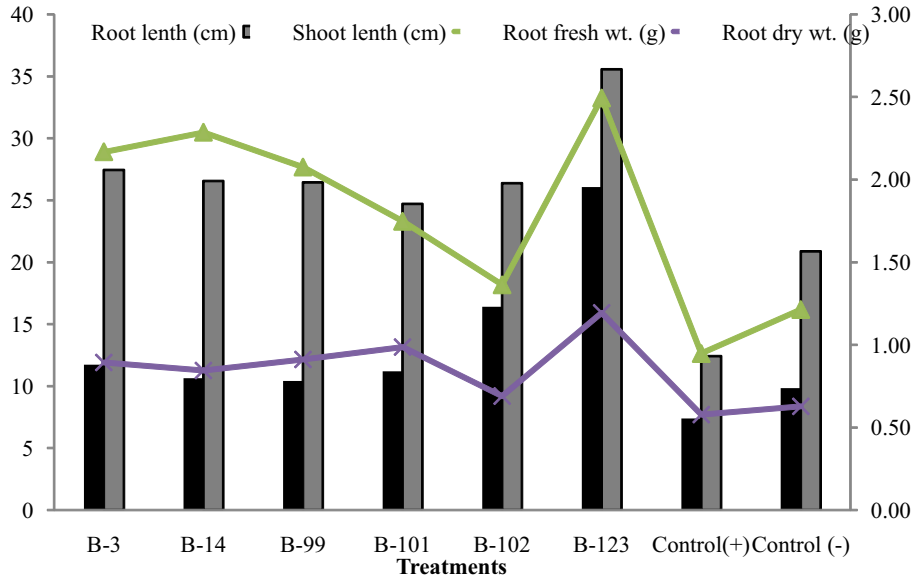


Fig. Effect of different treatments on growth promotion traits.

Significant effect of all the treatments was observed in growth promotion. The strain B-123 were also gave the maximum enhancement in root length, shoot length root fresh and dry weight.

Conclusion

The strain B-123, B-101 and B-99 showed good biocontrol potential as well as growth promotion in tomato that may be formulated as consortia to manage root rot under marginal saline conditions.

Project 5: Exploring Cyanobacterial Biodiversity in Extreme Habitats for Potential Applicability in Agriculture

PI : Dhananjaya P. Singh
Co-PI : Anurag Chaurasia

Rationale

Cyanobacteria (blue-green algae) are a diverse group of gram-negative, photoautotrophic prokaryotes that inhabit almost all kinds of natural habitats right from soil, water, sediments, agricultural fields, freshwater ecosystem and eutrophicated ponds, lakes, rocks, sea and even the walls of old buildings. Many diazotrophic heterocyst-forming cyanobacteria possess the ability to form associations with vascular/non-vascular plants and produce growth-promoting substances. Some cyanobacterial species fix atmospheric nitrogen while others form symbiotic associations with plants and fungi. Looking into their primitive existence, cosmopolitan occurrence and great potentialities in terms of bioactive metabolite production, plant growth promotion and soil health improvement, this project was undertaken to explore the diversity of these fascinating blue-green microbes in different habitats including the saline soil systems and in the eutrophicated ponds, ditches and rivers. The community structure of these organisms in the paddy fields, and bioactive metabolite production by these organisms in agricultural systems is being worked out under this project. Also, right from their origin during Mesozoic period, approximately, $3x10^9$ years ago, many species are surviving in a variety of extreme conditions such as high temperature, high salinity and eutrophicated conditions. The ability to resist high concentrations of sodium chloride (NaCl) makes these microorganisms more vulnerable to adaptations and therefore, many salt tolerant,

halophilic or marine organisms exist. Many organisms inhabit paddy fields and in view of their importance as biofertilizer, nitrogen-fixing salt tolerant cyanobacteria are of considerable application and of fundamental interest.

Objectives

- Collection, isolation, purification and identification of cyanobacteria from different habitats including alkaliphilic and eutrophicated conditions
- Morphological, chemotaxonomic and molecular biodiversity of cyanobacteria in selected habitats
- Development of molecular tools for the identification of cyanobacteria and probe designing
- Functional biodiversity entwined with metabolic diversity in the organisms for potential implications in agriculture

Significant Achievements

Morphological and molecular characterization of the isolates led to the characterization of isolates up to genera level as *Anabaena, Nostoc, Aulosira, Cylindrospermum, Hapalosiphon, Tolypothrix, Oscillatoria* and *Scytonema* (Fig 1 and 2).

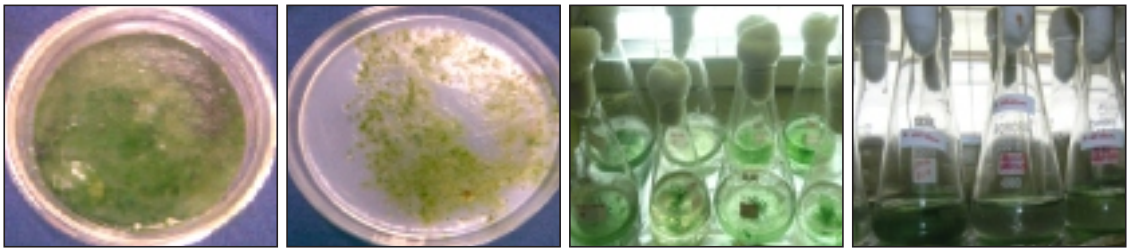
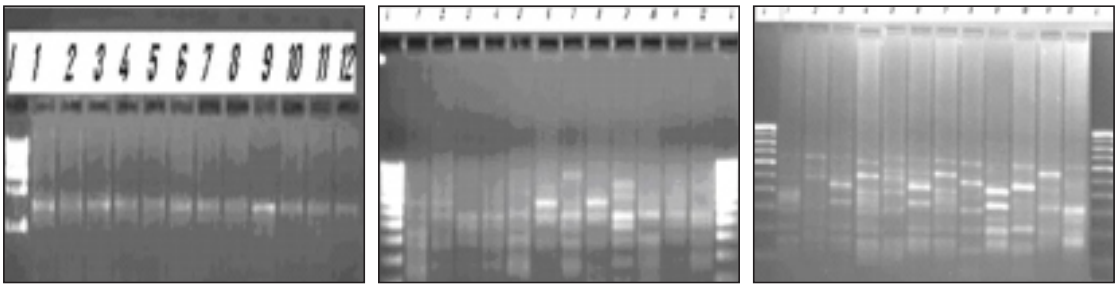


Fig 1. Isolation of cyanobacterial strains from Kanpur region



A B C

Fig 2: A. Specific amplification of 16S rDNA fragment (1.5kb) of extracted genomic DNA electrophoresed in 1.2% (w/v) agarose gel from cyanobacterial species. *l*- ladder, Lanes 1 to 12 are PCR products from different isolates of cyanobacteria from Mau region; **B&C.** Specific amplification fragment length polymorphism using *Hae* III (A) and *Alu* I (B) enzymes of the 16S rDNA fragment of cyanobacterial species under study. l-Ladder, lanes 1 to12 are from different isolated of cyanobacterial from Mau region DNA thus digested was resolved on 2.2% (w/v) agarose gel.

Fifteen cyanobacterial strains were evaluated for their potential activity against bacterial strains (antibacterial activity). Cell free extract in methanol:water (1:1) was prepared and antibacterial activity at different concentrations was tested against bacteria (*Pseudomonas* sp., *Enterobacter* sp. and *Exguobacterium* sp. Prominent activity was observed with the extracts of *T. tenuis* followed by *O. acuta* and *H. intricatus* against *Pseudomonas* sp. Against *Enterobacter* sp., extract of *P. boryanum* was found to be more effective while against *Exguobacterium* sp., *O. acuta* was more prominent (Fig 3). The activity was observed to be concentration dependent and maximum effect was found at high concentration.

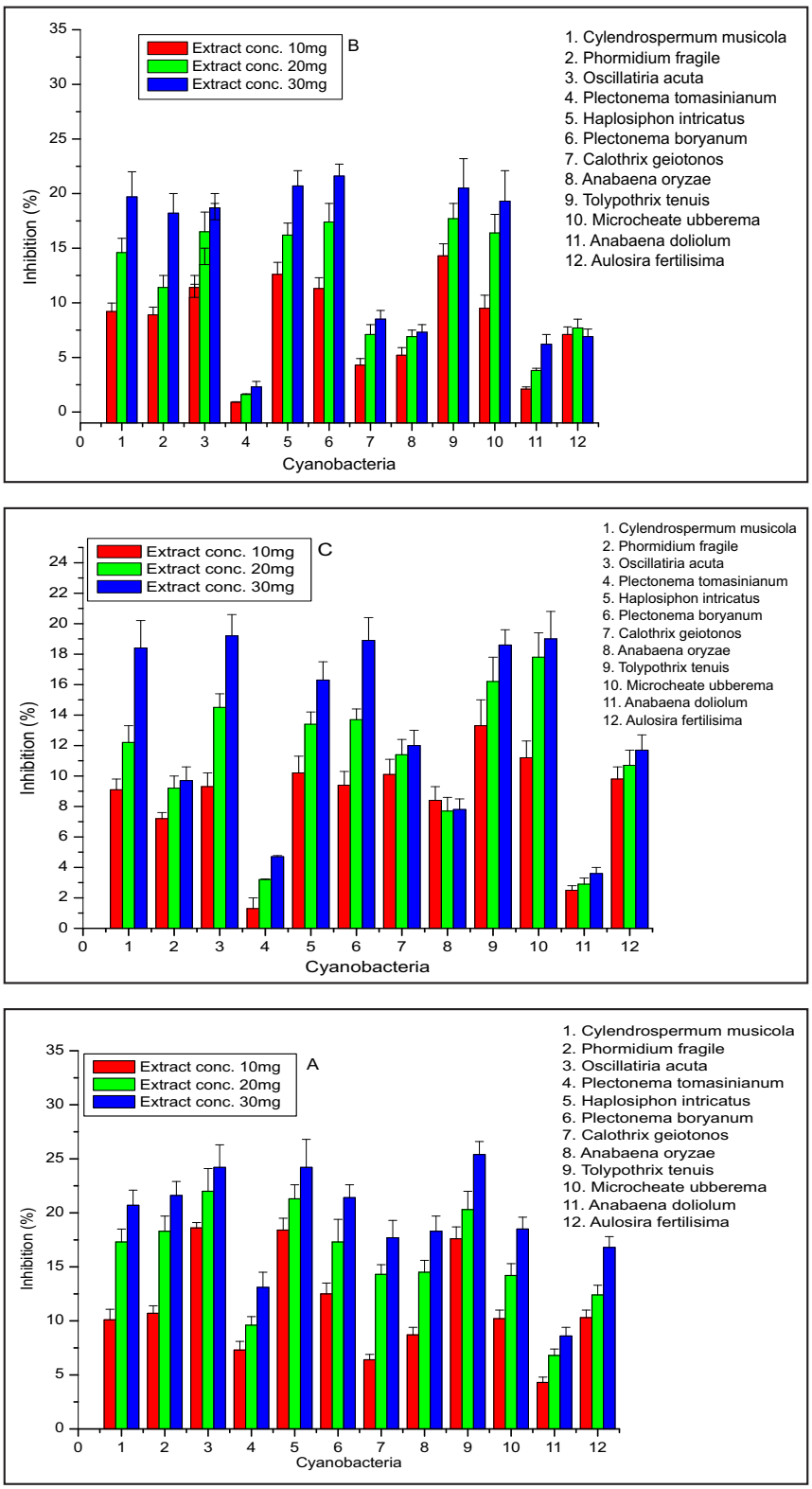


Fig 3: Effect of cyanobacterial extracts on A) *Pseudomonas* sp., B) *Enterobacter* sp. and C) *Exguobacterium* sp.

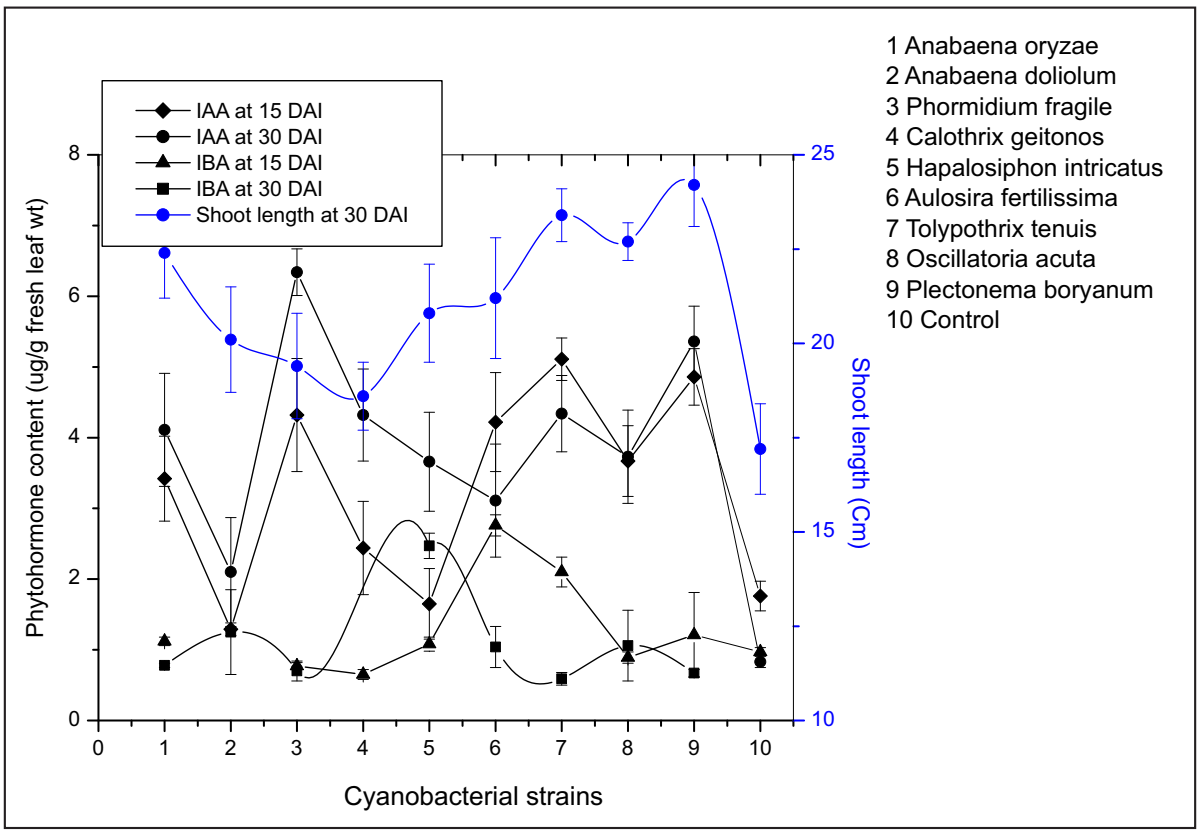


Fig 4. Accumulation of phytohormones in the leaves of rice plants inoculated with cyanobacterial strains and its correlation with shoot length. Two population t- test for Indole acetic acid (IAA) t = 0.43944, p = 0.66557, at 0.05 level, the two means are NOT significantly different, Indole butyric acid: t = -0.66988, p = 0.51312, at 0.05 level, the two means are NOT significantly different, Between IAA content at 15 DAI and shoot length : t = 6.30287, p = 6.09732E-6, at 0.05 level, the two means are significantly different.

HPLC profile of both the extracellular and cell-free extracts revealed that the extracts contain fairly good quantities of phenolic acids namely gallic, genteisic, ferulic and cinnamic acids. High concentration of ferulic acid was recorded in the extracts of *P. boryanum* (94.52 g/g fresh cell wt) followed by *P. fragile* (57.22g/g fresh cell wt). Presence of high quantities of phenolics in *P. boryanum* can be directly correlated its high antibacterial activity against the test bacteria.

The profile of individual phenolics (gallic, genteisic, chlorogenic, ferulic and cinnamic acids), flavonoids (rutin and quercetin) and plant growth regulators (indole acetic acid, IAA and indole butyric acid, IBA) in a rice variety (*Oryzae sativa* var. UPR 1823) inoculated with different cyanobacterial strains namely *Anabaena oryzae, A. doliolum, Phormidium fragile, Calothrix geitonos, Hapalosiphon intricatus, Aulosira fertilissima, Tolypothrix tenuis, Oscillatoria acuta and Plectonema boryanum* and the associated rhizospheric soil was evaluated using HPLC in pot conditions. Consistent accumulation of phenolic acids, flavonoids and phytohormones in rice and plant growth promotion (shoot, root length and fresh wt) positively correlated with total protein and chlorophyll content were recorded periodically. Phenylalanine ammonia lyase (PAL) activity and total phenolic content was fairly high in rice leaves inoculated with *O. acuta* and *P. boryanum* after 30 days (Fig 4). Rice leaves when challenged with the phytopathogen, *A. triticina* showed less lesion development due to infection in comparison to control.

Conclusion

Cyanobacterial isolates were identified form different location and bioprospecting of many of the strains has led to the evaluation of several properties and identification of certain molecules.

Project 6: Diversity analysis and utilization of some motile and non-motile actinomycetes from mangrove ecosystem of India

PI : Mahesh Yandigeri
Co-PI : Dilip K. Arora

Rationale

Actinomycetes are phenotypically diverse group of gram positive bacteria. Many species of actinomycetes produce a wide range of secondary metabolites, including antihelminthic compounds, antitumour agents, and many of known antibiotics produced by these organisms, have been exploited for their use in medicine and agriculture. They are tolerant to alkaline conditions and in general approximately, 95% of the microbial population dominated by actinomycetes in alkaline soils. With in soil, these actinomycetes act as a regulator switch for the decomposition and efficient operation of mineralization cycles which ultimately resulted in the production of diverse types of extracellular enzymes viz. cellulases, chitinases and lignin peroxidases etc. Ninety percent of the actinomycetes from soil belong to *Streptomyces* group and this genus alone represents 5-20% of total microbial population residing in the soil. Moreover, majority of the antibiotics used for curing plant and animal ailments are produced by *Streptomyces.*

Mangrove ecosystem is a bridge between terrestrial and marine ecosystem and harbours unique microbial diversity. In India, the area under mangroves is distributed over 4,900 sq. km, which accounts for about 8% of India's coastline. Mangrove forests are among the world's most productive ecosystems. They enrich coastal waters, yield commercial forest products, protect coastlines, and support coastal fisheries. However, mangroves exist under conditions of high salinity, extreme tides, strong winds, high temperatures and muddy, anaerobic soils. Although mangrove ecosystem is rich in organic matter, by and large they are nutrient-deficient especially in nitrogen and phosphorus. Diversity of microbial communities inhabiting this unique swampy, saline, partially anaerobic environment is useful, as it provides clue of the microorganism and their adaptability in such habitats. Mangroves and mangrove ecosystems have been studied extensively but remain poorly understood. Very little information is available regarding the microbial diversity, mechanisms and their interactions in the mangrove ecosystem. Mangroves provide a unique ecological environment for diverse bacterial communities like sulfate-reducing bacteria, methanogenic bacteria, N_2-fixing bacteria, halotolerant bacteria etc. Also they are niche for group of fungi called manglicolous fungi, microalgae, algal population and actinomycetes. Mangroves harbor both motile and non motile actinomycetes population. Many of these communities are involved in various mineralization cycles, biodegradation, nutrient cycling and decomposition. Apart from this mangrove microbial flora are known to possess halotolerance and survive under stresses. Thus such microflora can be utilized under stressed soils to improve the crop ecosystems. Keeping these views in mind, survey and collection of motile and non motile actinomycetes from mangrove ecosystems will be useful in characterizing the potent isolates. Hence, the project was formulated to isolate, analyze the diversity and utilize the potent isolates for their use in agriculture.

Objectives

- To isolate and purify motile and non-motile actinomycete isolates from mangrove ecosystem of India
- Morphological, biochemical and molecular characterization of the isolates
- To evaluate the role of some isolates for their plant growth promotion activities and nutrient management

Significant Achievements:

Extensive exploration, survey and collection of soil samples were carried out from Mangroves of Orissa (Bitarkanika National Park) and Mangroves of Goa during 2009-10 (Fig 1). Survey and collection of rhizospheric and non rhizospheric soil samples were carried out from Mangroves of Orissa (Bitarkanika, National Park) (Fig.1, Plate 1, Table 1). Soil samples were enriched with 1% $CaCO_3$, phenol, sodium dodecyl sulphate (SDS) and incubated for 7 days. Out of eleven soil samples collected from Bitarkanika mangroves, 23 morphologically distinct actinomycetes were isolated. These isolates were studied by using the morphological parameters like colour of aerial mycelium, colour of substrate

mycelium, colour of soluble pigments produced (if any) and growth pattern. Cultures were purified by using antibiotics and repeated streaking. Most of the actinomycetes were forming wet spreading type of colonies, representing genera other than non streptomyces actinomycetes (NSA) genera. Six actinomycetes belonging to *Streptomyces* genus were identified on the basis of morphological characteristics.These actinomycetes were further screened with various biochemical and actinomycetes specific tests. Among all the collected isolates, 12 were positive for casein hydrolysis, 6 for gelatin hydrolysis, 9 for urea hydrolysis and 13 positive for resistance to lysozyme. Intrinsic salt tolerance ability of the actinomycetes was tested by growing these actinomycetes on varying concentrations of NaCl. Among these actinomycetes, 12 isolates were able to tolerate up to 8% (1.03M) NaCl, where as none of the isolates could tolerate 10% (1.71 M) NaCl concentration. It was observed that all the isolates were invariably positive for ammonia production. Characterization of isolates for carbohydrate utilization pattern and siderophore production is under progress.

Fig. 1. Map depicting the mangrove regions chosen for the survey and diversity analysis of actinomycetes

Table 1. Location of sampling sites and enumeration of actinomycetes from Mangroves of Orissa

Sample	Sample type	Actinomycetes (cfu x 10^3 g^{-1} soil)	Morphotypes picked
Initial forest nearby Gupti guest house	Rhizospheric soil of mangrove plant	5	1
From 1 km away from Gupti guest house	Rhizospheric soil of mangrove plant	18	4
From 2 km away from Gupti guest house	Rhizospheric soil of mangrove plant	7	2
From 3 km away from Gupti guest house	Rhizospheric soil of mangrove plant	34	5
From 4 km away from Gupti guest house	Rhizospheric soil of mangrove plant	25	1
From 5 km away from Gupti guest house	Rhizospheric soil of mangrove plant	10	2
From 6 km away from Gupti guest house	Rhizospheric soil of mangrove plant	17	2
From 7 km away from Gupti guest house	Rhizospheric soil of mangrove plant	12	2
From 8 km away from Gupti guest house	Rhizospheric soil of mangrove plant	1	1
From 9 km away from Gupti guest house	Rhizospheric soil of mangrove plant	5	1
From 10 km away from Gupti guest house	Rhizospheric soil of mangrove plant	8	2
		Total	**23**

Plate 1. Photographs of survey and soil sampling from Mangroves of Orissa

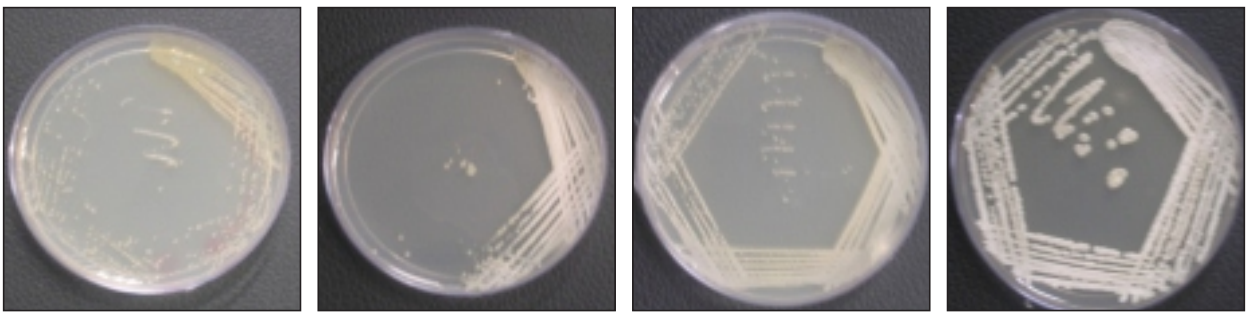


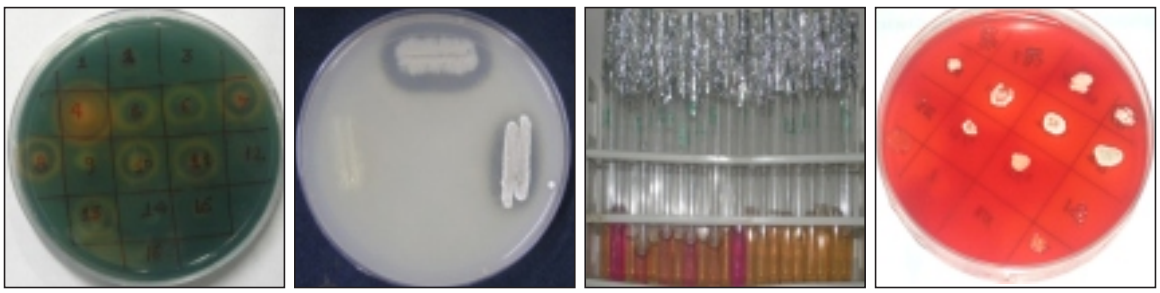
Plate 2. Plates showing the representative morphotypes of actinomycetes isolated from mangroves of Orissa.

Plate 3. Photographs of survey and soil sampling from Mangroves of Goa

Soil and water samples from Goa (old Goa, Dhauji, Diwar Naroa, Ribander, Sapedro from Mandovi river basin, Chorao mangroves, Salim Ali bird sanctuary, Colvale, Chapora, Mapusa, Panjim, Kabharjua canal, Zuari river basin, Zuari) were collected using systematic sampling procedure (Fig. 1, Plate 3, Table 2) from the rhizospheres of pneumatophores (breathing root hairs) of mangrove plant species namely *Rhizophora mucornata, Rhizophora avicennia, Sonneratia alba, Excoecaria agallocha, Avicennia alba*, *A. officinalis* etc. From 26 soil and water samples, 25 distinct morphotypes of actinomycetes were isolated using starch casein agar, actinomycetes isolation agar and Streptomyces agar. The soil enrichment using $CaCO_3$ and SDS yielded better results and morphotypes in comparison to the phenol and heat treatment of soil samples. Morphological characterization of actinomycetes was carried out for colour of aerial mycelium, substrate mycelium and soluble pigments produced and recorded.

Table 2: Location of sampling sites and enumeration of actinomycetes from Mangroves of Goa

Location	Sample type	pH	Colony forming units	Morphotypes picked
Old Goa and Dhauji (Mandovi river, Old Goa)	Rhizosphere soil of mangrove plant	7.9	$10X10^3$	2
Old Goa and Dhauji (Mandovi river, Old Goa)	Rhizosphere soil of mangrove plant	7.5	$18X10^3$	5
Old Goa and Dhauji (Mandovi river, Old Goa)	Soil from the mangroves swamp	7	$3X10^3$	1
Diwar Naroa side (Mandovi river, Old Goa)	Rhizosphere soil of mangrove plant	7.5	$12X10^3$	2
Diwar Naroa side (Mandovi river, Old Goa)	Rhizosphere soil of mangrove plant	7	$25X13^3$	6
Sapedro, Ribander (Mandovi river, Old Goa)	Rhizosphere soil of mangrove plant	7.6	$15X10^3$	4
Salim Ali Bird Sanctuary, (Mangrove plants) Chorao	Rhizosphere soil of mangrove plant	7.5	$5X10^3$	1
Salim Ali Bird Sanctuary, (Mangrove plants) Chorao	Rhizosphere soil of *Excoecaria agallocha*	7	$4X10^3$	1
Salim Ali Bird Sanctuary, (Mangrove plants) Chorao	Rhizosphere soil of *Avicennia officinalis*	7	$8X10^3$	2
Salim Ali Bird Sanctuary, (Mangrove plants) Chorao	Water sample from mangrove basin	7.5	$5X10^3$	1
Salim Ali Bird Sanctuary, (Mangrove plants) Chorao	Rhizosphere soil of *Sonneratia alba*	7	$4X10^3$	1
Salim Ali Bird Sanctuary, (Mangrove plants) Chorao	Water sample from beds of *Sonneratia alba*	7	$15X10^3$	3
Salim Ali Bird Sanctuary, (Mangrove plants) Chorao	Rhizosphere soil of *Rhizophora mucornata*	7.5	$9X10^3$	2
Colvale, Chapora	Rhizosphere soil of *Rhizophora avicennia*	7.5	$10X10^3$	2
Camurlim, Chapora, Bardez	Rhizosphere soil of *Avicennia officinalis*	7	$6X10^3$	1
Camurlim, Chapora, Bardez	Rhizosphere soil of *Avicennia alba*	7	$5X10^3$	1
Peddem, Mapusa, Goa	Rhizosphere of *Kandelia rheedii*	7.6	$10X10^3$	2
Peddem, Mapusa, Goa	Rhizosphere of *Avicennia alba*	8.2	$5X10^3$	1
Peddem, Mapusa, Goa	Water sample from mangroves	7.8	$6X10^3$	1
Panjim (Mandovi river creek), Goa	Rhizosphere soil of *Avicennia officinalis*	7	$12X10^3$	3
Gondalim, Goa	Rhizosphere soil of *Avicennia officinalis*	7	$7X10^3$	1
Kumbarjua canal, old Goa	Rhizosphere soil of *Rhizophora apiculata*	7.4	39×10^3	1
Zuari river back waters, Karmali, Velha, old Goa	Mangrove rhizosphere	7.2	7×10^3	2
Coralim Dhado, Zuari river back waters, Goa	Mangrove rhizosphere	7.3	5×10^3	4
Coralim Dhado, Zuari river back waters, Goa	Mangrove rhizosphere	7.6	3×10^3	3
Colva Beach	Salt water from the beach	7.2	8×10^3	1
			Total	25



Siderophore Production | Caseinase activity | Urease activity | Xylanase activity

Plate 4. Biochemical characterization of the actinomycetes isolated from mangroves of Goa

Purified actinomycetes from mangroves of Goa were subjected to characterization with respect to plant growth promotion traits. Out of 25 actinomycetes, 14 were found positive for siderophore production, 6 for phosphate solubilizers, 22 for ammonia producers and one for HCN producer. Among the 25 isolates, 12 isolates showed caseinase activity, 4 for gelatinase, 3 for xylanase and 6 for urease (Plate 4). One isolate was positive for melanin pigment production on tyrosine agar and three isolates showed resistance to lysozyme. The actinomycetes were tested for intrinsic resistance salinity using varying concentrations of NaCl. Five isolates could grow up to 10% NaCl concentration.

PCR amplification of genomic DNA isolated from different isolates resulted in ~1400bp fragment of 16S rDNA in all the isolates using universal 16S rDNA primers. Restriction analysis of amplified ribosomal product was carried out using *Hae*III, *Hha*I and *Taq*I restriction enzymes (Plate 4). *Hha*I enzyme did not reveal much polymorphism between the isolates in comparison to *Taq*I and *Hae*III enzymes.

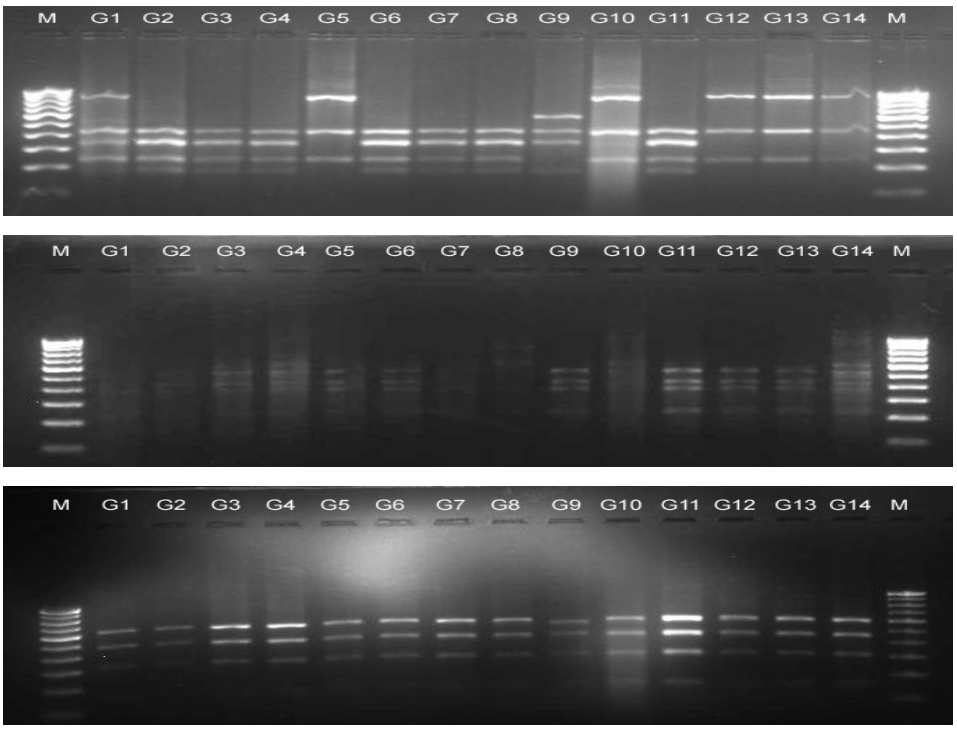


Plate 4. Restriction analysis of ribosomal DNA of the actinomycetes obtained from mangroves of Goa

Conclusions

Actinomycetes isolated from all the mangroves ecosystems were found to be structurally and functionally diverse. Among the actinomycetes isolated from mangroves of Gujarat, West Bengal, Tamil Nadu, Andhra Pradesh, Goa and Orissa, it was found that highest population of actinomycetes was obtained from mangroves of Andhra Pradesh followed by mangroves of West Bengal, where as structural diversity of actinomycetes based on the morphotypes was observed to be more in West Bengal and Andhra Pradesh mangrove ecosystems in comparison to mangroves of Tamil Nadu, Gujarat, Goa and Orissa. The actinomycetes isolated possessed various attributes including biocontrol and plant growth promoting traits. These traits will find utilization in increasing the crop growth and yield. Many actinomycetes had ability for production of various enzymes like xylanase, cellulase, caseinase etc. which will be helpful in assessing the isolates for their industrial and agricultural uses. The diversity of actinomycetes will be documented for further utilization in the future.

Project 7: Culturable and unculturable diversity of Methylotrophic bacteria in brackish water of Chilka Lake

PI : Kamlesh Kumar Meena
Co-PI : Mahesh Yandigeri

Rationale: Methylotrophs are ubiquitous bacteria inhabiting water, soil and plants and playing an important role for cycling of hazardous compound in the environment. Apart from this they are agriculturally important bacteria. They are having very strong associations with crops plants and living as epiphytes as well as endophytes on the plants.

Aerobic methylotrophs contain specialized pathways for dissimilatory metabolism during Methylotrophic growth. Rather than biochemical characterization, molecular techniques are more authenticate tools providing significant information about diversity indices and community analysis of microorganisms. The main purpose of this research was to isolate aerobic methanol utilizing methylotrophic strains from water and sediment of the lake and to characterize them with respect to some biochemical, physiological and molecular approach.

Lake Chilka is one of the important hot spots for microbial diversity in India. No reports are available on the methylotrophic diversity in the Lake Chilka. Therefore, the present study was aimed to explore the genotypic diversity of methylotrophic bacteria isolated from the Chilka Lake. In the present study, the isolates obtained from the Chilka Lake were grouped based on ARDRA analysis of PCR amplified 16Sr DNA and the representative isolates were sequenced to reveal their identity.

Significant Achievements

- For the diversity analysis a total of the 10 sediment and 5 water samples were collected from the Lake. The water and sediment sample were slightly alkaline with pH ranging from 7.0 to 8.5. The total average viable count of aerobic methylotrophic bacteria in the samples was $180X10^{2}$ cfu g^{-1} or ml^{-1}. After enrichment of methanol the samples were diluted and plated on different selective media with methanol as a sole carbon source of energy. The pH of the media was adjusted according to the pH of samples. A total of 80 isolates were selected on the basis of different pigmentation and colony morphology.
- Most of the isolates were found to be gram-ve rod shaped. Almost all isolates were Oxidase, Catalase and Urease +ve. All these biochemical and microscopic result gave an indication that these cultures isolated belong to the genus methylotroph.
- To see the variation at species level for the different selected morphotypes, PCR amplification of 16SrDNA followed by ARDRA analysis using three different restriction endonucleases was carried out. When 16SrDNA amplicon were digested with restriction enzyme, the fragments ranging in size up to 750 base pairs were seen for the different isolates.
- All the 24 repersentative isolates were sequenced and the sequence data was analysed by BLAST and the nearest match from the GenBank data was reported. DNA sequencing and phylogenetic analysis revealed that all the isolates btained from the lake Chilka showed 98 to 100% similarity with the sequences with in the GenBank.
- The partial 16S r DNA sequences of the isolated strains were compared with those available in the databases. Identification to the species level was determined as a 16S rDNA sequence similarity of $\geqslant$ 97% with that of a type strain sequence in the GenBank. Sequence alignment and comparison was performed using the multiple sequence alignment program CLUSTALW with default parameters. Among the isolates, majority showed sequence similarity to the genus *Methylobacterium* (53.50%) followed by *Hypomicrobium* (15%), *Methylophillus* (7.6%), *Mehthyloversatiles* (7.6%), *Acenetobactor* (3.84%), *Azospirillum* (3.84%), *Mycobacterium* (3.84%) and *Pseudomonas* (3.84%).

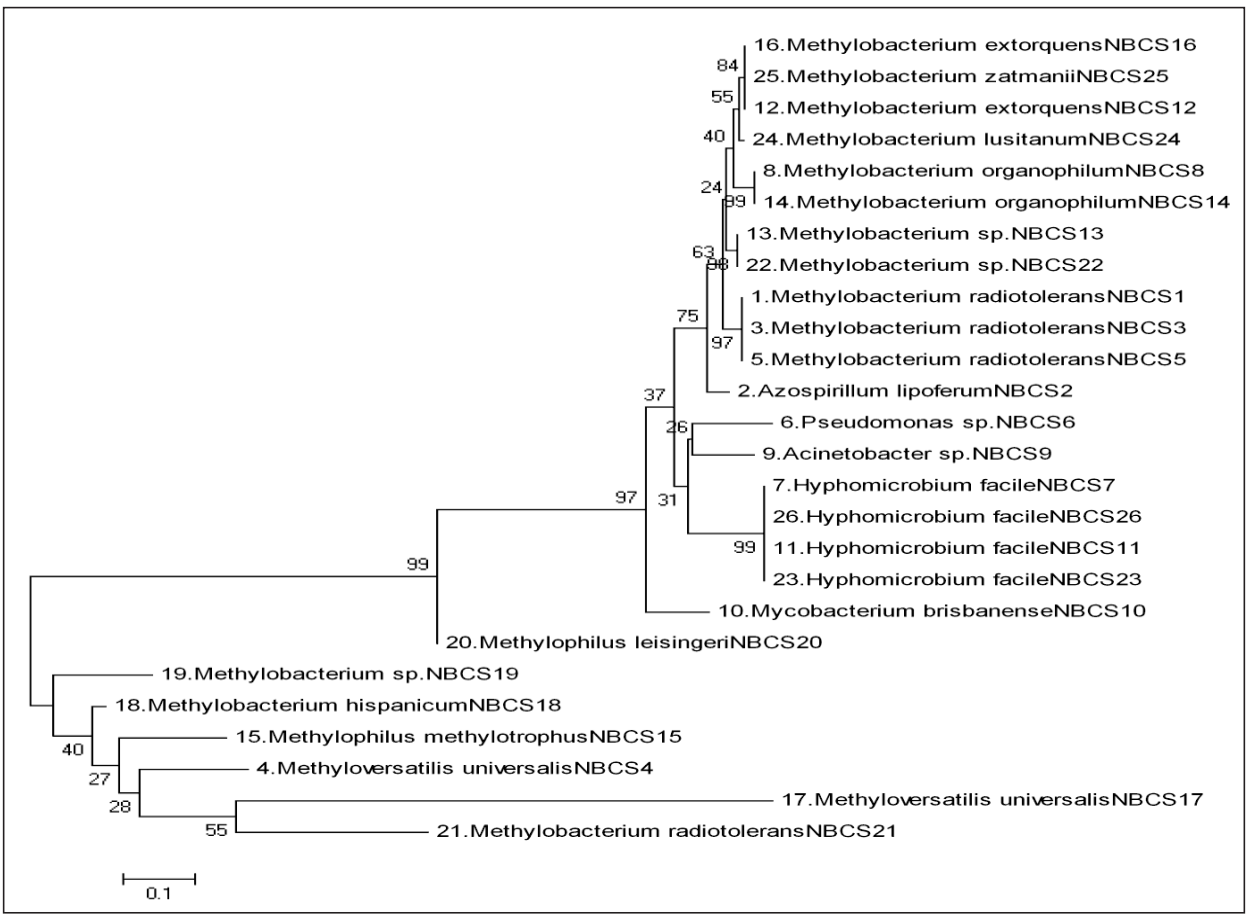


Fig 4. Bootstrap neighbor-joining tree of Methylotrophs generated from the alignment of 16S rDNA sequences. The horizontal bar represents 10% difference in DNA sequence

Carbon Utilization Test

- All the selected isolates were analyzed for different carbon substrate utilization, *viz.* acetate, fructose, D-glucose, arabinose, galactulose, maltose, sucrose, lactic acid, d-mannitol, D-galactose, erythritol, dextrin, glycogen, tween-80, NAG (N-acetyl glucosamine), D-lactose, d-raffinose, D-sorbitol, aminoethanol, 2,3-butane-diol, glycerol, D-glucose-6-phosphate. These carbon substrates were substituted for methanol in NMS liquid medium at 0.5% (w/v) level. Presence of growth was observed after 15 days of incubation at 30^{0}C in an orbital shaking incubator and growth was compared to a negative control containing no carbon substrate.
- Physiological similarities of 24 representative strains were assessed by cluster analysis based on the substrate utilization pattern. Three major clusters were obtained from the dendrogram based on carbon utilization pattern. Cluster I and II comprises α, β- and γ-Proteobacteria, while Cluster III comprises only α and β-Proteobacteria. From the carbon utilization pattern it is clear that Methylotrophic strain isolated either from sediments or from water, utilizing the similar carbon substrate. Like, *Methylobacterium* genus, either isolated from sediments or from lake water, lies in the same cluster.

Functional approach: mxaF gene sequencing

- Since mxaF gene is universal in all methanol oxidizers (except very few), the gene coding for the large subunit of methanol dehydrogenase was sequenced. *Methylobacterium* genera was found to be dominant followed by *Methylophilus* and the *Hyphomicrobium* compared with the 16S rRNA gene sequences. Two distinct major clusters I and II were found.

Table 1. Characteristics of representative strains of Chilka Lake and their phylogenetic rsemblance (+Ve and - Ve).

Character -istics / Isolate No.	Sample type	colony color	Cell morphology	Gram's staining	Catalase	Urease	Oxidase	Salt tolerance (M)	Division	Phylogenetic similarities (%)
NBCS1	soil	Pink	Rods	–	+	+	–	0-1.5	α-Proteobacteria	*M.radiotolerans (99%)*
NBCS2	soil	Pink	Rods	–	+	+	–	0-1.5	α-Proteobacteria	*M.radiotolerans (99%)*
NBCS3	soil	Cream	Rods	–	+	+	–	0-2.5	β-Proteobacteria	*Methyloversatilis universalis(85%)*
NBCS4	soil	Pink	Rods	–	+	+	+	0-0.89	α-Proteobacteria	*M.radiotolerans (99%)*
NBCS5	soil	Pink	Rods	–	+	+	+	0-1.95	γ-Proteobacteria	*Pseudomonas sp.(99%)*
NBCS6	soil	White	Rods	–	+	+	+	0-1.95	α-Proteobacteria	*H.facile (99%)*
NBCS7	soil	Pink	Rods	–	+	+	–	0-1.5	α-Proteobacteria	*M.organophilum (98%)*
NBCS8	soil	Cream	Rods	+	+	+	+	0-1.5	γ-Proteobacteria	*Mycobacterium brisbanense(99%)*
NBCS9	soil	Cream	Rods	–	+	+	–	0-0.80	α-Proteobacteria	*H.facile (99%)*
NBCS10	soil	Pink	Rods	–	+	+	–	0-0.90	α-Proteobacteria	*M.extorquens (99%)*
NBCS11	soil	Pink	Rods	–	+	+	_	0-2.5	α-Proteobacteria	*Methylobacterium spp.(100%)*
NBCS12	water	Pink	Rods	–	+	+	–	0-15	α-Proteobacteria	*M.organophilum (98%)*
NBCS13	water	White	Rods	–	+	+	+	0-1.5	β-Proteobacteria	*Methylophilus methylotrophus (99%)*
NBCS14	water	Pink	Rods	–	+	+	+	0-1.25	α-Proteobacteria	*M.extorquens (99%)*
NBCS15	water	White	Rods	–	+	+	–	0-1.68	β-Proteobacteria	*Methyloversatilis universalis (98%)*
NBCS16	water	Pink	Rods	–	+	+	–	0-0.90	α-Proteobacteria	*Methylobacterium hispanicum (98%)*
NBCS17	water	Pink	Rods	–	+	+	–	0-1.5	α-Proteobacteria	*Methylobacterium spp. (97%)*
NBCS18	water	White	Rods	–	+	+	–	0-0.89	α-Proteobacteria	*Methylobacterium spp. (93%)*
NBCS19	water	Pink	Rods	–	+	+	–	0-1.0	α-Proteobacteria	*M.radiotolerans (99%)*
NBCS20	water	Pink	Rods	–	+	+	+	0-2.5	α-Proteobacteria	*Methylobacterium spp..(99%)*
NBCS21	soil	Cream	Rods	–	+	+	–	0-1.45	α-Proteobacteria	*H.facile (99%)*
NBCS22	soil	Pink	Rods		+	+		0-1.8	α-Proteobacteria	*M.lusitanum (99%)*
NBCS23	soil	Pink	Rods	–	+	+	–	0-1.95	α-Proteobacteria	*M.zatmanii (99%)*
NBCS24	soil	Cream	Rods	–	+	+	–	0-1.25	α-Proteobacteria	*H.facile (99%)*

- The functional gene employed in this study *mxaF* was used to identify or to authenticate populations capable of methanol oxidation downstream of formaldehyde. The presence of mxaF gene in the isolates detected by the partial amplification of the gene using specific primers. The forward primer *mxaF*-1003 (5'GCGGCACCAACTGGGGCTGGT3') and reverse primer *mxaR*-1561 (5'GGGCAGCATGAAGGGCTCCC3') were used for amplification of *mxaF* gene, 550bp.

DGGE Profiling of mxaF gene amplified from metagenomic DNA (Culture independent approach)

- Five composite samples of sediments from different locations of the lake were subjected to the metagenomic DNA extraction. The *mxaF* gene coding the alpha subunit of methanol dehydrogenase were amplified with the primer sets *mxaF* f100/r 1557 having GC clamp (CGCCCGCCGCGCGCGGCGGGCGGGGC CCGCCCGC) at 5' end in one of the primer.

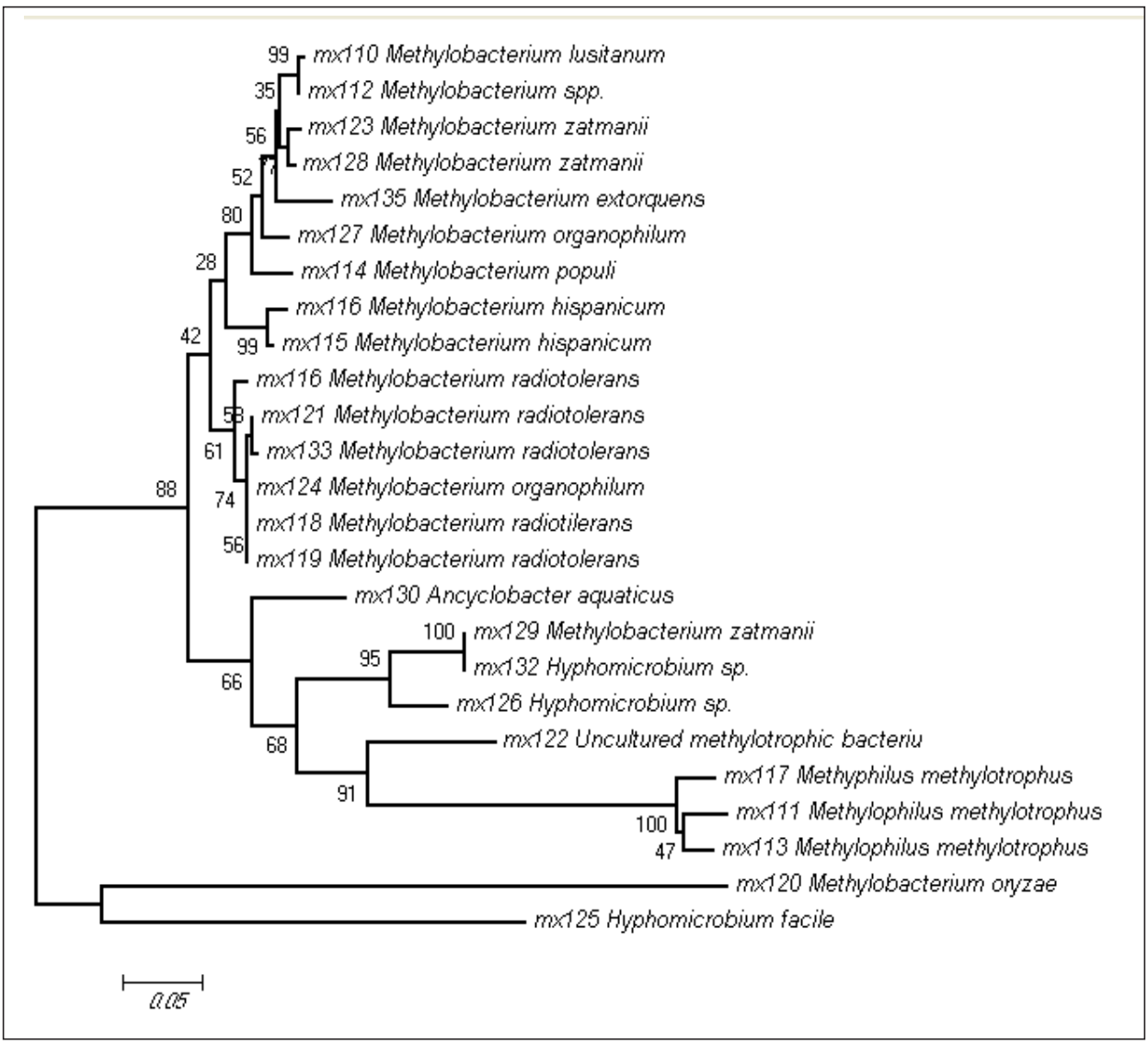


Fig 4. Bootstrap neighbor-joining tree of Methylotrophs generated from the alignment of mxaF gene sequences. The horizontal bar represents 10% difference in DNA sequence. The scale bar represents the 5% difference in the nucleotide sequences.

- The conserved region of the *mxaF* gene has been developed as a functional gene marker for the methylotrophic diversity. The large subunit of methanol dehydrogenase giving the distinct variation after DGGE profiling.
- A denaturing gradient of 30% to 70% was used (100% denaturant is 40% deionized formamide and 7M urea). A large 7.5% acrylamide (37.5:1) gel was poured using a homemade gradient delivery system. 40ul of the final amplification reaction was run in each well in 1X TAE buffer. Electrophoresis was performed at constant voltage (200V) at 60^{0}C for 5 hours. Gel was stained with EtBr and photographed.
- The eluted DNA was given to sequence to identify to know the antiquity of the uncultured methanol oxidizers. The maximum variation of the isolates obtained from Satpada (lane 5, Fig 1) nearby sea mouth.

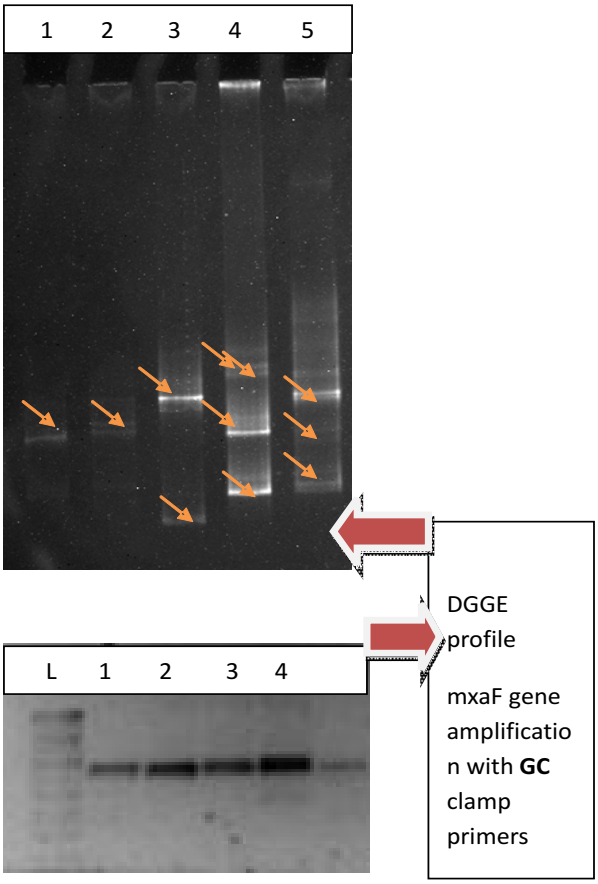


Fig 1. DGGE banding profile obtained from five different metagenomic mxaf gene amplified products

DGGE DNA band Sequencing and phylogenetic affiliation

- *Methylobacterium organophilum, Ancyclobacter aquaticus, Berkholdariales* bacterium and *Hyphomicrobium* spp. were detected in the environment.**Screening of the PHB producing methylotrophic bacteria**
- Isolation of the poly-b-hydroxybutyrate (PHB) producing bacteria, recognition of the brightly refractile cytoplasmic inclusions, lipophilic stains with the Sudan Black was used as the detection of the PHB producing bacteria.
- Colonies with different morphologies grown on the NMS media were transferred to the
- PYM media, which allows PHB accumulation. Sudan black (0.3g in 60% ethanol) was performed on the heat fixed samples for 10 min and counterstained with 0.5% aqueous safranine for 5 Sec. Accumulated granules were clearly visualized under compound microscope at 1000X.
- PHB granules at both ends of the rod shaped bacteria
- Out of 80 isolates from Chilka lake water and sediments, 69 were detected as PHB producers.

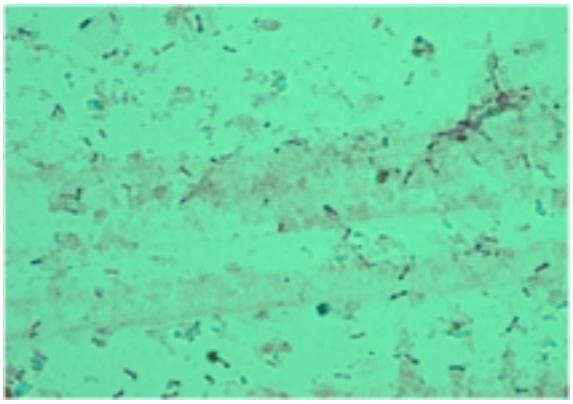


Fig: Microscopic observation of the Polyhydroxy butyrate granules stained by Sudan Black.

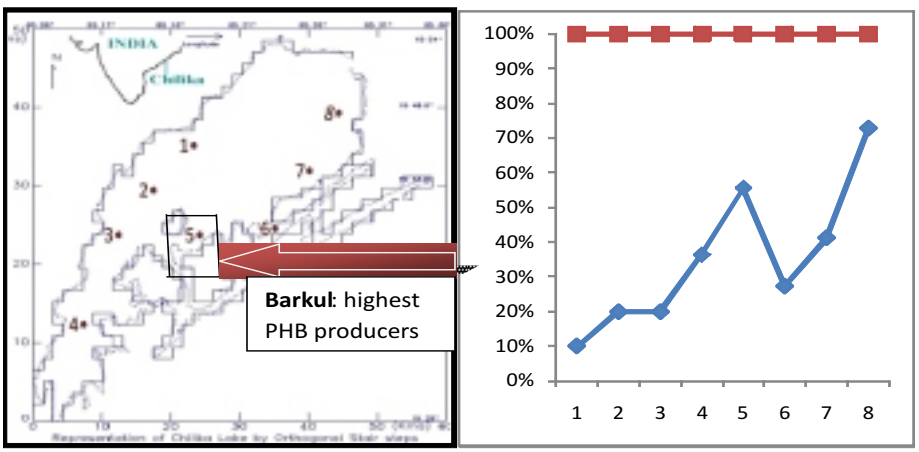


Fig: The variation in the PHB producing methylotrophs at different sites

Conclusions

The functional gene, coding methanol dehydrogenase coded by *mxaF* locus has been used as genus specific locus for restricting the subpopulation of Methylotrophs. The diversity indices calculated was more in the soil samples as compared to the water samples of the Chilka Lake.

A diverse group of Methylotrophic community was explored. In a total of 80 isolates from water and sediment, 53.5% were closely related to *Methylobacterium* genus with different species, 15.38% were *Hyphomicrobium facile*, 7.6% *Methylophilus sp.* 7.6% *Methyloversatilis universalis*, 3.84% *Acinetobacter* sp. 3.84% *Azospirillum lipoferum*, 3.84% *Mycobacterium brisbanense* and 3.84% *Pseudomonas sp.* Among these, *Methylobacterium* is the dominant genera, which are pink pigmented facultative methylotrophs.

Interestingly, in this study, more diverse group of methylotrophs like *Methylobacterium, Methyloversatilis, Pseudomonas, Hyphomicrobium* and *Mycobacterium* were explored from sediment. *Methylophilus methylotrophus* produces significant amount of a low viscosity extracellular polysaccharides from methanol under conditions of nitrogen limitation in chemostat culture. *Hyphomicrobium facile* is an aerobic chemoorganotroph plays an important role in denitrification to remove nitrate at drinking water treatment plants and sewage treatment plants. The high concentration of organic matter,including lipids, produce toxic effects on bacteria communities.

The variation in the *mxaF* gene sequences were clearly visible after looking at the DGGE pattern obtained from the amplified metagenomic DNA. The GC clamp primers for the methanol dehydrogenase gene was able to separate out the mixed amplified *mxaF* gene products. The phylogenetic affiliation reveals the uncultured Methylotrophic bacteria 77% while *Methylobacterium, Hyphomicrobium, Ancylobacter* 6.6%.So from the result obtained we can justify our culturable mxaF gene sequences with the metagenomic gene sequences obtained. It is clearly visible the alpha-proteobacterial group is dominant in the Lake which is obtained from both culture dependent and culture independent approach.

Synthesis of PHB has been proposed as a detoxifying mechanism of bacteria in water with high concentrations of fatty acids. Thus, PHB production in this brackish environment does not function only as a storage material, but also as a mechanism to cope with imbalanced environments. The present study not only describes the Methylotrophic diversity of Chilka Lake, but also indicates many biotechnologically potential cultures. The variation in the PHB producing methylotrophs were obtained too. Polyhydroxy butyric acid and similar bacterial polyesters are promising candidates for development of environment-friendly, totally biodegradable plastics. The use of methanol, one of the cheapest noble substrates available may help to reduce the cost of producing such bioplastics.

Project 8: Metagenomic approaches for exploring the biodiversity of antibiotic producing agriculturally important microorganisms (AIMs)

PI : Udai Bhan Singh

Rationale

Microbial secondary metabolites are good source for the discovery of novel antimicrobial compounds. Microbial metabolite exhibit versatile chemical structure with diverse biological activities that exceed the scope of synthetic organic chemicals. As a result of increasing environmental concern and the development of resistance in pathogens to synthetic chemicals, exploitation of antibiotics from microbial metabolites is being considered as an approach to the identification of novel antibiotics which meets environmental requirements also. Current research indicates that more than 99 per cent of microbes in the environment can not be cultivated. Regarding this, the present research project was proposed to exploit the metagenomic approaches to dig out the culturable as well as non-culturable microbial genomic diversity. To meet out these aspect formulate this project with the following objectives

Objectives

- Evaluation of genetic diversity of antibiotics producing agriculturally important microorganisms (AIMs) in rice- wheat cropping system.

- Screening and expression of antibiotic producing gene(s) and its possible application in agriculture.
- Detection, prediction and diversity of antimicrobial gene(s) by using metagenomic approaches.

Significant Achievements

- Survey and collection of soil and plant Samples from IGP region of Kanpur (Urban) and Kanpur (Rural) has been done. More than 125 villages of 15 blocks has been surveyed and 125 sample of rhizospheric soils with plant roots have been collected for the isolation of antibiotic producing agriculturally important microorganism (AIMs).
- Testing of physico- chemical properties of soil samples is going on.
- Isolation of microorganisms such as fungi, bacteria and acinomycetes are in the process.
- For the characterization of antibiotic producing microorganisms, primers were designed. Some of them are:
- 20-mer primers for antibiotics phenazine-1-carboxylic acid (PCA) and 2,4-diacetylphloroglucinol (Phl)

Phl2a	GAGGACGTCGAAGACCACCA
Phl2b	ACCGCAGCATCGTGTATGAG
PCA2a	TTGCCAAGCCTCGCTCCAAC
PCA3b	CCGCGTTGTTCCTCGTTCAT

- Oligonucleotides primers for type I polyketide synthase

PltBf	GG AGC ATG GAC CCC CAG C
PltBr	GTG CCC GAT ATT GGT CTT GAC CGA G
plt1	ACT AAA CAC CCA GTC GAA GG
plt2	AGG TAA TCC ATG CCC AGC
PrnCf	CCA CAA GCC CGG CCA GGA GC
PrnCr	GAG AAG AGC GGG TCG ATG AAG CC

- Oligonucleotides used to study Novel Nisin Variants

UbnPinvL	GGTAATATTGGGCTTTACAGTCATA
UbnPsauinvR	CAAGTAAATGGAACTGTACAAGATA
UbnPRinvL	GTTGCATAAATATCAATACTGTCTT
UbnPRinvR	TGCAATAATAGTGTAAAATAAAAG
UbnRSpeinvR	ACGGTGTTTGATTATGTACTGTTTGA
UbnRinvL	TTCCTATGGCGCTCTTCTCGTTTC
UbnRFwd	AATCGGATGCATTATTTCGTTCAA
FXbainvL2	TCAGTCCCCTCAGGCGTTTTG

- PCR amplification of aminoglycoside resistance genes (Streptomycin)

aadE (1268)	ACT GGC TTA ATC AAT TTG GG
(1845)	GCC TTT CCG CCA CCT CAC CG
aadA (564)	TGA TTT GCT GGT TAC GGT GAC
(829)	CGC TAT GTT CTC TTG CTT TTG
aac(6')- *aph*(2″)	
(348)	TGA TGA TTT TCC TTT GAT GT
(1723)	CAA TCT TTA TAA GTC CTT TT

Theme : Microbial Diversity and Identification

6

Project: Diversity analysis of *Bacillus* and *Bacillus*-derived genera in the Indo-gangetic Plains of India

PI : D. K. Arora
Co-PIs : Rajeev Kaushik
SRF : Shachi Vardhan

Rationale:

Indo-gangetic plain (IGP) also known as northern plain or the north Indian river plain is the major crop production belt of the country and is known to have rich biodiversity of flora and fauna. This region covers one-fourth of total agriculturally productive land available in India and produces three-forth food grains, majorly dominated by rice and wheat crops. These regions include Punjab, Haryana, Uttar Pradesh and West Bengal (Fig.1). However, majority of the rice-wheat cropping system are concentrated in Punjab, Haryana and Uttar Pradesh. During the last four decades, the wheat production has undoubtedly increased six times and that of rice two times, but today's picture reflects a stagnancy in wheat production and a decline in rice productivity. Factors responsible for declining productivity are: deteriorating agro-ecological conditions, lack of FYM application, nutrient mining, over exploitation of underground water and purturbance of soil microflora. Drastic shift in the dominant microbial community having Plant growth promoting traits plays an important role in declination of the crop productivity. To compensate, this declination trend of crop productivity, farmers have started applying higher doses of fertilizers than the recommended ones. Hence, indiscriminate use of fertilizers had further worsen soil productivity and nutrient imbalance in soil-plant systems resulting into various environmental problems and immensely impacted the dominant microbial community inhabiting in IGP region.

On the basis of microorganism cultivation studies, *Bacillus* spp. is recognized as one of the dominant genus in soils. They are able to exert a beneficial effect on plant growth. It is a Gram-positive, phenotypically heterogeneous, spore forming rod-shaped aerobic bacteria, with members exhibiting an extremely wide range of nutritional requirements, growth conditions, metabolic diversity and DNA base compositions. Members of this genus are used for the synthesis of a wide range of medical, agricultural, pharmaceutical and other industrial important products. Variability in their biochemical and physiological action is used to study the diversity. Successful plant-microbes relationships of *Bacillus* genera had been reported. PGP traits of microbes like indole 3-acetic acid (IAA) production, phosphate solubilization and siderophore production helps in the increment of crops productivity. The use of ribosomal RNA gene restriction pattern has proved very useful for classification purpose and is regarded as a key taxonomic tool for bacterial species and adapted for the identification of *Bacillus* species. Members of *Bacillus* genus has value for its novel bioactive compound for industrial and agricultural field, hence knowledge of their diversity, distribution and ecology will aid bioprospecting strategies. Moreover, the estimation of bacterial diversity is required for understanding bacterial biogeography, community assembly and ecological processes.

In the present work, a light has been thrown on the purturbance of microbial community as a reason for the declination in the crop productivity as well as

to show the diversity analysis of dominant *Bacillus* genus in the IGP region.

Objective

- To isolate and characterize the soil microbes (*Bacillus*).
- Biochemical characterization of the isolates with respect to the PGP traits.
- Molecular characterization including ARDRA with three restriction enzymes.
- Sequencing of the isolates.

Methodology:

Sampling sites and sample preparations

Based on random stratified sampling method, seventy three rhizospheric and non rhizospheric soil samples from wheat- rice cropping system prevalent in different IGP regions *viz*., Trans and Central IGP regions including Punjab, U.P and Bihar were collected. The roots along with the adhered soil on the root surface were allowed to dry overnight at room temperature and were collected in a fresh sterile container. Samples were brought to laboratory and preserved at 4°C for further studies.

Isolation and PGP characterization

Isolation of *Bacillus* was done by heat shock treatment of soils at 80°C for 10mins, to eliminate non-endospore forming microorganisms, followed by incubation at three different temperatures 30°, 37° and 45°C. Media used for isolation were Nutrient Agar with methyl red (10mg/ml). Isolates were assayed for various PGP traits such as phosphate solubilization (Pikovskaya, 1948), IAA production (Vikram *et al.*, 2007) and siderophore production (Schwyn and Neilands, 1987) etc.

Genomic DNA isolation and 16S rDNA amplification

Genomic DNA was extracted by following the Prep method devised by Pospiech and Newmann (1995). DNA purity is determined by measuring the OD_{260}/OD_{280} ratio. PCR amplification of 16S rDNA for the isolates was done by using two universal primer pA 5'-AGAGTTTGATCCTGGCTAG-3' and pH 5'-AGGAGGTGATCCAGCCGCA-3' (Edward *et al.*, 1989). PCR master mix contained 1.5 mM $MgCl_2$, 200mM dNTP, 1.5mM each primer, 3U of *Taq* polymerase (Genei, India) and 50 ng DNA template. The reaction conditions were: initial denaturation at 94°C for 5 min; denaturation at 94°C for 30 s for 40 cycles; annealing at 50°C for 40 s; extension at 72°C for 1 min; end filling at 72°C for 10 min and holding at 4°C. The amplified product was resolved on 1.2% agarose gel.

Amplified rDNA Restriction Polymorphism in *Bacillus*:

16S rDNA PCR product was digested with restriction endonucleases *Alu*I, *Msp*I, *Hae*III according to the manufacturer's instructions and restricted DNA was analyzed by horizontal electrophoresis in 2.5% agarose gels. Electrophoresis was carried out at 70V for 2.5 hr with standard gels (11 x 14 cm), and documented on a gel documentation system (Alpha Imager, USA).

Phylogenetic analysis

Restriction profiles obtained were scored in a binary matrix and the data was used for calculating Jaccard's similarity coefficient (Jaccard, 1908) for each pairwise comparison. Dendrogram was constructed from the similarity matrix by the unweighted pair group method with arithmetic mean (UPGMA) (Sneath and Sokal, 1928). In order to estimate the goodness-of-fit of cluster analysis, co-phenetic value matrices were calculated and compared with the original similarity matrices that were UPGMA clustered and tree was constructed to classify unknown isolates into closely related reference isolates by using the NTSYSpc analysis package (Version 2.02e; *Applied Biostatistics* Inc., Setauket, New York, USA).

Significant Achievements

- A total of 248 colonies were isolated from seventy three soil samples collected from different regions of Indogangetic plains, showing different morphological and cultural characteristics. Maximum numbers of *Bacillus* isolates were obtained from Kapurthala district of Punjab followed by Muzaffarnagar, Lucknow in Uttar Pradesh.
- *Bacillus* isolates from Kanpur, Dehradun and Saharanpur belt showed 74%, 68% and 52% isolates produced indole 3-acetic acid (IAA) respectively, whereas in Punjab region only 16% isolates were IAA producers (Fig 2). Higher quantity of IAA ranging from 1200µg/ mg of protein to 600 µg/mg of protein was recorded from Kanpur belt, followed by 18% and 20% of the isolates from Saharanpur and Kanpur belt were phosphate solubilizers, while Punjab region had only 6% of the isolates were solubilizing

phosphate. 20.5% of the isolates from Saharanpur and Kanpur each and 2% from Dehradun were found to produce siderophores, whereas from Punjab, 22.5% of the isolates produced siderophores, respectively (Fig. 2).

- Molecular characterization of all the 245 isolates from Central and Trans IGP region isolates by using PRA analysis of 16S rRNA with three restriction enzymes (*Msp*I, *Hae*III and *Alu*I) showed good diversity among the isolates in IGP regions (Fig. 3). On the basis of 16S rDNA sequencing results, we concluded that the large number of the isolates were from *Bacillus*-derived genera (Fig.4.).
- Isolates from the major cluster have been sequenced and identified as (with accession numbers) *Lysinibacills fusiformis* (EU430993.1), *Paucisalibacillus globulus* (EU430986.1), *Brevibacillus parabrevis*, *Bacillus humi*, *Bacillus clausii*, *Bacillus farraginis*, *Bacillus arbutinivorans*, *Pontibacillus* sp., *Bacillus casamancensis*, *Bacillus oleronius* (EU430987), *Bacillus circulans* (EU430989), respectively.

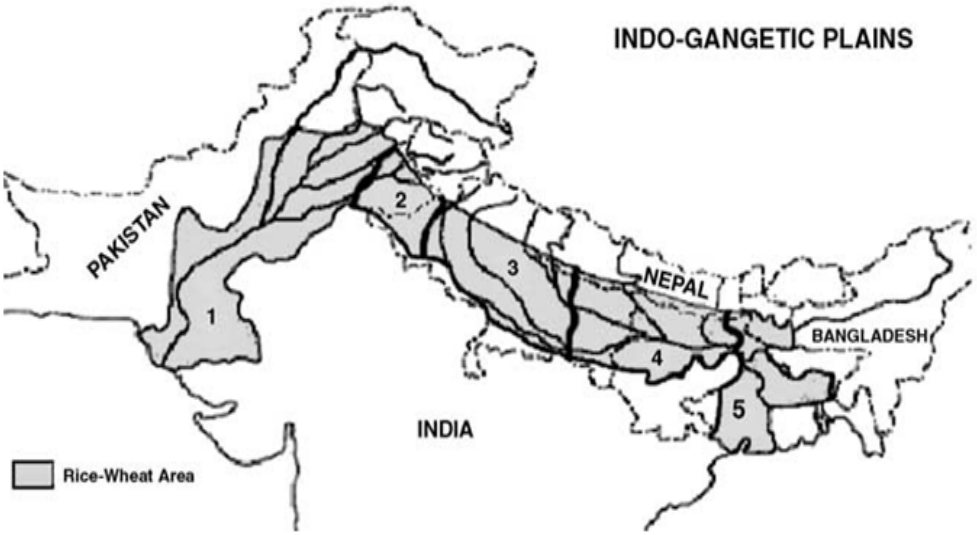


Figure 1: Generalized map of the Indo-Gangetic plains indicating homogenous regions along the transect. (1) Trans-Gangetic plain (in Pakistan); (2) Trans-Gangetic plain (in India); (3) Upper Gangetic plain; (4) Middle Gangetic plain; and (5) Lower Gangetic plain (Gupta *et al.*, 2001).

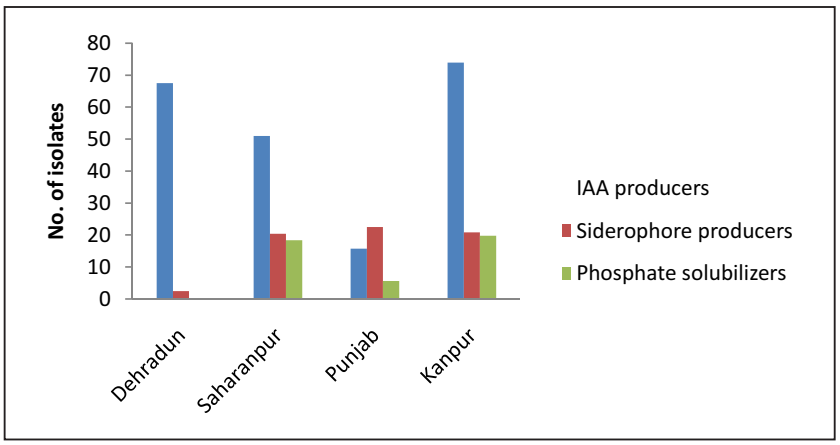


Figure.2: PGP attributes of isolates from different parts of IGP regions in terms of IAA, siderophores producers and phosphate solubilizers respectively.

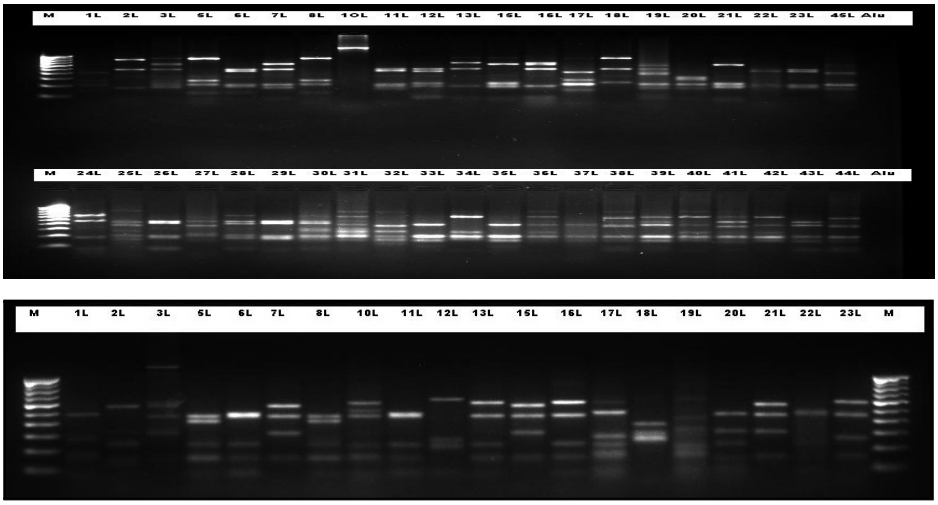


Figure.3. Pattern Restriction Analysis (PRA) of 16S rDNA by using a set of three restriction enzymes (*viz*., *Alu*I, *Msp*I and *Hae* III respectively).

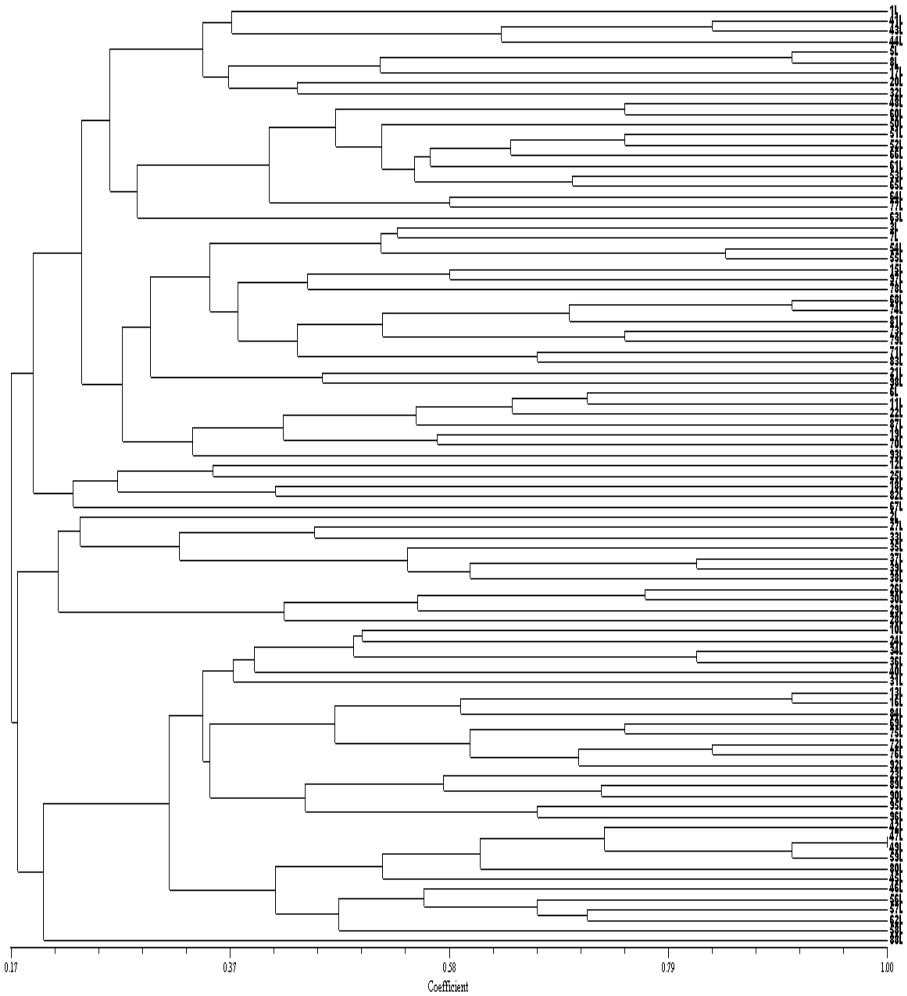


Figure. 4: Combined dendrogram of *Bacillus* isolates from IGP regions obtained by UPGMA method (unweighted pair group method using arithmetic average) based on RFLP with *Msp*I, *Hae*III and *Alu*I Restriction endonuclease (bar represents % similarity).

Conclusion:

The study area is located between 79°23' and 79°42'E, and 29°20' and 29°30'N. The altitude ranges between 1300 and 2600 m above sea level (243.89m asl). IGP region has very high fertile soil and yielding more than half of cereals across India, although soils of this region varies from alluvial to loamy, with high water holding capacity and neutral to alkaline pH range. In this region, we have found that though there is a great diversity among the isolates but the isolates having insignificant PGP activity, dominates over PGP producing *Bacillus* genera's which confirms the rationale. This dominance is more in Trans IGP region (central Punjab region) where the use of chemical fertilizer was surveyed to be more than Central (Cis) IGP regions. Inspite of the fact that the *Bacillus* had wide distribution, this genus showed wide variation in their population dynamics. Other workers from the different part of the world also correlated population dynamics of microbes with pH, moisture, organic content and fertilizers content of soils. Hence, it would be concluded that population of *Bacillus* genus, although good in number but insignificant PGP producers were found to be more than PGP producers, and their population dynamics showed that it declines in Trans IGP regions (Central Punjab) in comparison with Cis IGP regions

Project: Development of diagnostic kit (PCR based) for the Identification of Soil microbe (Fluorescent Pseudomonas)

PI : D. K. Arora
Co-PIs : Rajeev Kaushik
SRF : Subhash Yadav

Rationale

The genus *Pseudomonas* belongs to the γ subclass of the Proteobacteria and includes mostly fluorescent Pseudomonads as well as a few non-fluorescent species. Fluorescent *Pseudomonas* group represents: 1) phytopathogenic Cytochrome c oxidase-positive species, such as *P. cichorii*, *P. marginalis* and *P. tolaasii*; 2) non-phytopathogenic, non-necrogenic strains, such as *P. fluorescens*, *P. putida, P. chlororaphis*, *P. aureofaciens* and *P. aeruginosa* type species; 3) phytopathogenic necrogenic fluorescent *Pseudomonas* spp. without cytochrome c oxidase: *P. syringae* and *P. viridiflava*. Non-fluorescent *Pseudomonas* group contain *P. stutzeri, P. mendocina, P. alcaligenes* and *P. pseudoalcaligenes.* This project specifically focussed on fluorescent Pseudomonads because they are present in diverse type of environments, especially in the rhizosphere. Many studies revealed that these bacteria are able to improve plant growth and health and provide natural supressiveness of certain soils to reduce the impact of soil-borne diseases whereas others participate in the biodegradation of natural and man-made toxic chemical compounds.

The comparison of rRNA sequences is a powerful tool for deducing phylogenetic and evolutionary relationships among bacteria, archaebacteria, and eukaryotic organisms. These sequences have been derived previously by using various tools including oligonucleotide cataloguing, sequencing of clones, direct sequencing of RNA by reverse transcriptase, and sequencing of amplified product obtained by polymerase chain reaction (PCR). The present study expands on the use of DNA amplification technology for the study of rRNA sequences within eubacteria. The nucleotide sequences of various genes, especially those of small-subunit (SSU) rRNA has been widely used to identification and classification of microorganisms (Woese, 1987). The study of SSU rRNA sequences resulted in many findings, such as the *Archaea*, a group of prokaryotes that is separated from *Bacteria*. At present, this gene is most widely used and as its sequence is a mosaic of highly conserved regions interspersed with variable and hypervariable stretches which makes it most suitable candidate for PCR primer design to characterise Pseudomonads (Gürtler *et al.,* 1996; Stackerbrandt *et al.,* 1995). The 16S rRNA gene from *Pseudomonas* spp. contains 1492 nucleotide positions, of which 148 are variable and 65 positions of these are within three hypervariable regions. The "*Pseudomonas* hypervariable (hv) regions" were defined as: hv 1, *E. coli* 16S rRNA gene

sequence positions 71- 95; hv 2, *E. coli* 16S rRNA gene sequence positions 455 - 475; and hv 3, *E. coli* 16S rRNA gene sequence positions 998-1043 (Godfrey *et al.,* 2002; Moore *et al.,* 1996). These positions are located, respectively, within the regions V1: helix 6, V3: helix 18 and V6: helices P35-1 and P35-2 (Godfrey *et al.,* 2002, Neefs *et al.,* 1990). The regions hv1 is considered one of the most variable sequences in 16S rRNAs of bacteria across the phylogenetic spectrum and is useful for differentiating intrageneric lineages and discerning the type strains of some species of *Pseudomonas*. Godfrey and Marshall used primers designed from these regions to study the diversity of Pseudomonads isolates from different parts of the world. Moreover, the large database of 16S rRNA sequences is very useful for the analysis of environmental isolates and recognition of new sources of diversity.

In several strains that belong to the group of plant associated *Pseudomonas* species, expression of genes involved in the biosynthesis of secondary metabolites and extracellular enzymes is positively controlled by the GacS/GacA, a type of two-component system (reviewed by Heeb and Haas, 2001). This regulatory system consists of a membrane-bound sensor kinase protein (GacS) and a cytoplasmic response regulator protein (GacA). The current model proposes that GacS recognizes specific environmental stimuli and activates GacA, which in turn triggers the expression of specific genes (Appleby *et al*., 1996; Heeb and Haas, 2001; Pernestig *et al*., 2001). Several other elements are also involved in the regulation of certain genes, forming a complex regulatory cascade, with the GacS/GacA pair controlling secondary metabolite production at a higher hierarchy (Sarniguet *et al*., 1995; Whistler *et al*., 1998; Chancey *et al*., 1999; Blumer and Haas, 2000). For example, production of phenazine antibiotics, which are involved in the biocontrol activity and ecological competence of several *Pseudomonas* strains.

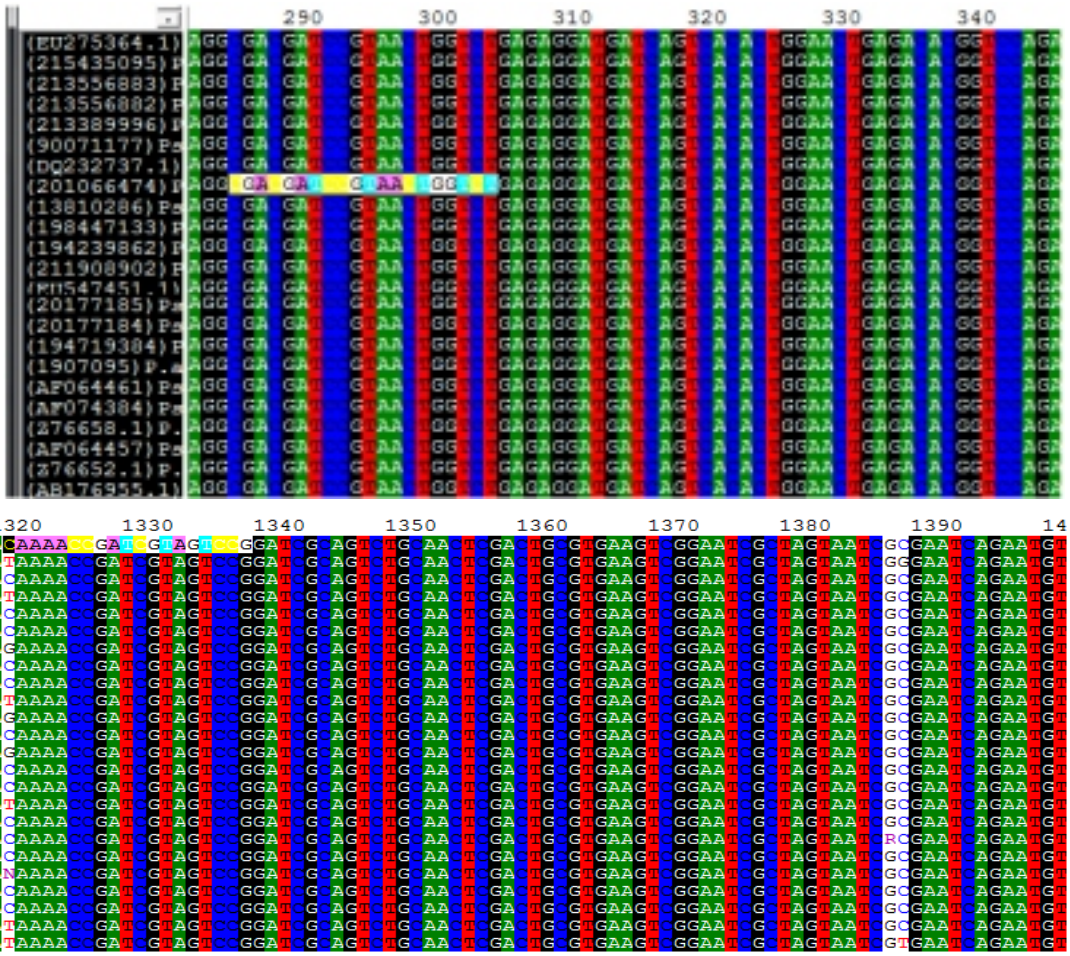


Fig. 1. Conserved regions in 16S rDNA region of different species of fluorescent *Pseudomonas*

Objectives

- Isolation of fluorescent pseudomonad from soils collected from different locations
- Biochemical and Molecular characterization of isolates
- Development of genus specific and species specific diagnostic kit (PCR based) for identification of fluorescent pseudomonads.

Methodology:

Primer design (genus and species specific): RDP release 8 (2009) was used with a total of 2,849 prokaryotic entries, 13 of which were classified as members of the genus *Pseudomonas*. Specific alignments of 16S rRNA sequences were retrieved by the SUB_ALIGNMENT routine from the RDP, and potential PCR primer target regions were manually determined. Their specificity was theoretically evaluated by the CHECK_PROBE routine of the RDP by using the total prokaryotic SSU rRNA sequence database as a target. Primer sequences were redesigned and tested in the same way, until optimal specificity was achieved. Novel 16S rRNA gene sequences were retrieved from GenBank with similarity searches with BLAST and were analyzed for matches with the designed PCR primers (Fig. 1).

The primer sequences were:

PSF1(5'-CGACGATCCGTAACTGGTCT-3')

PSR1 (5'-CGGACTACGATCGGTTTTG-3')

The amplification conditions were as follows: initial denaturation at 95°C for 5 minute followed by 35 cycles of 95°C for 30 s, 66.2°C for 30 s and 72°C for 1 minute and a final extension at 72°C for 10 minute.

- The target regions for PCR primers Ps-for and Ps-rev were identified at locations 289 to 308 and 1258 to 1275, respectively, of the *P. aeruginosa,* rRNA gene, which are homologous to locations 285 to 305 and 1320 to 1339 in the *Pseudomonas* 16S rRNA gene, respectively (Fig. 2). Theoretical hybridization targets for each primer were determined by extensively searching the 2,849 prokaryotic SSU rRNA gene sequences in RDP . Ps-for (the forward primer).

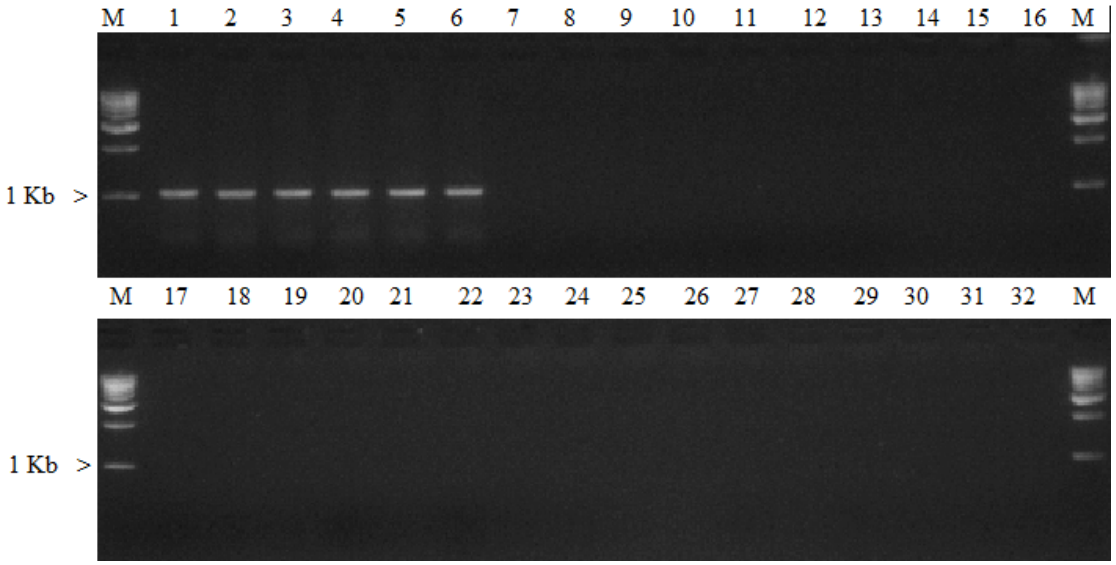


Fig. 2. 1: *Pseudomonas putida*; 2: *Pseudomonas fluorescens*; 3: *Pseudomonas plecoglossicida*; 4: *Pseudomonas fragii*; 4: *Pseudomonas mosselii*; 5: *Pseudomonas desmolyticum*; 6: *Pseudomonas aeruginosa*; 7: *Streptomyces viridochromogenes*; 8: *Streptomyces thermocaboxydus*; 9: *Streptomyces cinnabarinus*; 10: *Mesorhizobium ciceri*; 11: *Bacillus subtilis*; 12: *Bacillus megaterium*; 13: *Bacillus sporothermodurans*; 14: *Bacillus djibelorensis*; 15: *Bacillus humi*; 16: *Bacillus oleronius*; 18: *Bacillus pumilus;* 19: *Bacillus subtilis*; 20: *Bacillus polymyxa*; 21: *Bacillus megaterium*; 22: *Bacillus asahii*; 23: *Bacillus djibelorensis*; 24: *Bacillus senegalensis*; 25: *Paenibacillus* sp.; 26: *Lysinibacillus* sp.; 27: *Marinobacter* SL-9; 28: *Halomonas* sp.; 29: *Nitrinicola lecisaponesis;* 30: *Halomonas* sp.; 31: *Ochrobactrum* sp.; 32. *Micrococcus luteus.*

- Pseudomonas chlororaphis group specific PCR primer were designed from GacA (global activator of antibiotic and cyanide production) gene and validated in silico and in vitro.
- Pseudomonas chlororaphis subsp. aureofaciens strain 30-84 response regulator GacA (gacA) gene, complete cds

CGGTTAAGACAGGAAAGGGAACGCCTGGGGTTGTCGCAAAAGAAATTTGGTGAAGTTGGCGGCGTGGAA
GCCAATGCCCAAGGCAGATATGAAAATGGAGACCGTGTGCCCAAGGCGGATTATTTGTCCCGGGTGGCGG
CAAAAGGAGTGGATGTCCTTTATATAGTGACTGGCAGGCGGACCCCGACCAGG**GCCGATAACTTCAGCCA**
GAGCGAAGAGAAAATCGTCGTCAGTTATCGGGCTTTGTACAAGGAAGATCAGGACGCGATCCGGCACCTG
ACCCATACCCTTGCCGAGCTTTCGGTTTCCAGCCTCATGAAAAGAAAGGTGGAGCCCCGAGAGCCTTAGGG
CAGGGCGTTCGCAGCGCATCAACCATCCACCGGATACCCCTGGTGAACAAGTGCTTTTTATATGGTGTTTG
TCATTAGGTCAGCACGCTGCTTTTTTGCTAAGGTGTCCGGCAACCTATAAGACCAAATCGCGAGGTGTCTGC
TTGATTAGGGTGCTAGTAGTCGATGACCATGATCTCGTTCGTACAGGTATTACACGAATGCTGGCTGACATC
GATGGCCTGCAAGTGGTCGGTCAGGCCGAGTCAGGGGAGGAATCCCTGATCAAGGCCCGGGAATTGAAAC
CCGATGTGGTCCTCATGGACGTCAAGATGCCCGGGATCGGCGGTCTTGAAGCCACGCGCAAGTTATTGCGC
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TTTGCCGGCCAGCGTTACATCAGCCCGCAGATTGCCCAGCAACTGGTGTTCAAGTCATTCCAGCCGTCCAG
TGATTCACCGTTCGATGCCTTGTCGGAGCGCGAGATCCAGATTGCGCTGATGATTGTCGGCTGCCAGAAAG
TGCAGATCATCTCCGACAAGCTGTGCCTGTCTCCGAAAACCGTGAATACCTACCGTTACCGTATCTTCGAGA
AGCTCTCGATCAGCAGTGATGTCGAACTGACGTTGCTGGCGGTTCGCCACGGCATGGTCGATGCCAGCGCC
TGACAATGACCGACCCGTTTGATCCCAGT**GCTTTTCTTTCCACCTGCAG**

Forward 5' GCCGATAACTTCAGCCAGAG-3'

Reverse 5' GCTTTTCTTTCCACCTGCAG-3'

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
AF115381.1	Pseudomonas chlororaphis subsp. aureofaciens strain 30-84 respon	40.1	40.1	100%	0.050	100%	
XM_001380005.1	PREDICTED: Monodelphis domestica hypothetical protein LOC10001	36.2	36.2	90%	0.77	100%	G
AC208363.3	Gallus gallus BAC clone TAM32-19D6 from chromosome z, complet	34.2	34.2	85%	3.1	100%	
AC187771.4	Gallus gallus BAC clone CH261-117B22 from chromosome z, compl	34.2	34.2	85%	3.1	100%	
AL403632.3	Human DNA sequence from clone RP11-323H24 on chromosome 13	34.2	34.2	85%	3.1	100%	
CP001699.1	Chitinophaga pinensis DSM 2588, complete genome	32.2	223	90%	12	100%	
NC_012641.1	Homo sapiens calcium channel, voltage-dependent, beta 4 subunit (	32.2	32.2	80%	12	100%	
CP001657.1	Pectobacterium carotovorum subsp. carotovorum PC1, complete ge	32.2	216	95%	12	100%	
CR380955.2	Candida glabrata strain CBS138 chromosome I complete sequence	32.2	32.2	80%	12	100%	
XM_001963971.1	Drosophila ananassae GF21330 (Dana\GF21330), mRNA	32.2	32.2	80%	12	100%	G
CP001899.1	Geobacter lovleyi SZ, complete genome	32.2	58.5	80%	12	100%	
CP000968.1	Candidatus Korarchaeum cryptofilum OPF8, complete genome	32.2	32.2	80%	12	100%	
XM_001863661.1	Culex quinquefasciatus adult cuticle protein, mRNA	32.2	32.2	80%	12	100%	G
CP000826.1	Serratia proteamaculans 568, complete genome	32.2	111	95%	12	100%	
CU459958.2	CH251-67B4, complete sequence	32.2	32.2	80%	12	100%	
CU376411.3	CH251-520N11, complete sequence	32.2	58.5	80%	12	100%	
CP000855.1	Polynucleobacter necessarius subsp. asymbioticus QLW-P1DMWA-1,	32.2	62.4	85%	12	100%	
CU329703.2	CH251-131G1, complete sequence	32.2	58.5	80%	12	100%	
CU504771.4	PTB-812G14, complete sequence	32.2	32.2	80%	12	100%	
AC192753.2	Gallus gallus BAC clone CH261-67J20 from chromosome z, complet	32.2	32.2	80%	12	100%	
XM_415189.2	PREDICTED: Gallus gallus similar to KIAA2017 protein (LOC416818	32.2	32.2	80%	12	100%	U G
AY464052.1	Equine herpesvirus 1 strain V592, complete genome	32.2	32.2	80%	12	100%	
AC105613.1	Homo sapiens chromosome 5, BAC clone 114k9 (LBNL H94), compl	32.2	32.2	80%	12	100%	
AC144712.2	Danio rerio clone CH211-4G3, complete sequence	32.2	32.2	80%	12	100%	
AY665713.1	Equine herpesvirus 1 strain Ab4, complete genome	32.2	32.2	80%	12	100%	
AC125067.5	Mus musculus BAC clone RP23-399K1 from chromosome 12, compl	32.2	32.2	80%	12	100%	
AC115303.6	Mus musculus BAC clone RP24-188J6 from chromosome 1, complet	32.2	32.2	80%	12	100%	
CP000883.1	Colwellia psychrerythraea 34H, complete genome	32.2	111	90%	12	100%	
AC121318.17	Mus musculus chromosome 1, clone RP24-377P1, complete sequen	32.2	32.2	80%	12	100%	
XM_447186.1	Candida glabrata CBS138 hypothetical protein (CAGL0001188g) par	32.2	32.2	80%	12	100%	G

In vitro validation of Species specific PCR-primer for *Pseudomonas chlororaphis* designed from GacA gene

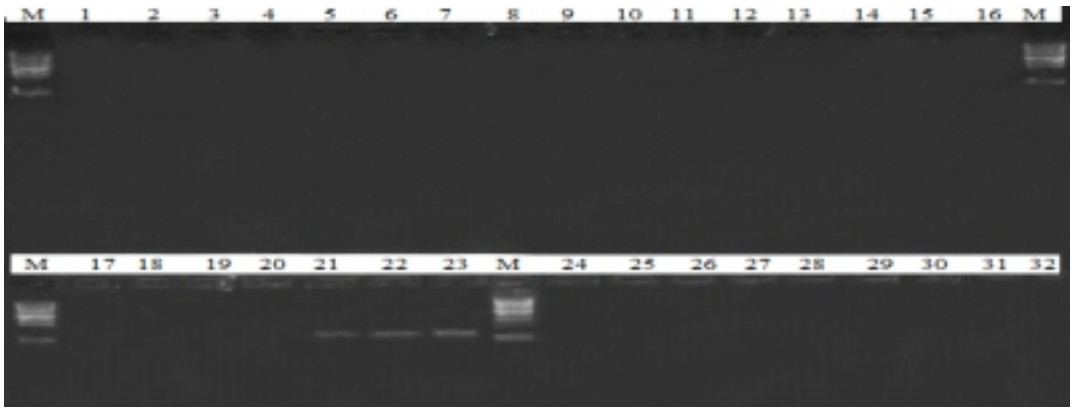


Fig. 1.5: *P. fluorescens*; 6: *P. putida;* 7: *P. fragii*; 8: *P. oleovorans*; 9: *P. plecoglossicida*; 10: *P. desmolyticum*; 11: *P. hydrophilla*; 12: *P. mendocina*; 13: *Bacillus subtilis*; 14: *Bacillus megaterium*; 15: *Bacillus fusiformis*; 16-20: *Bacillus drentensis,* 21-23: *P. chlororaphis*; 24-32: *Streptomyces* spp.

Conclusion:

The *Pseudomonas*-PCR protocol in conjunction with RFLP analysis, sequence determination, and phylogenetic analyses may be used to compare *Pseudomonas* population structures from a variety of ecosystems and provide further insight about the occurrence, potential roles, and possible unidentified subgroups of this genus in different ecosystems. In addition, it may represent a rapid assay for confirmation of members of the genus *Pseudomonas* (*sensu stricto*).

Project: Diversity analysis of microbes in extreme conditions

PI : D. K. Arora
Co-PIs : Rajeev Kaushik, Alok Kumar Srivastava, Sudhir Kumar and Mahesh Yandigeri
SRF : Harmesh Sahay, Sandeep Singh, Anuradha Rai, Manish Kumar, Sucheta Singh, Arvind Kumar Yadav, Sukumar Mesapogu

Rationale

Microbial diversity is the key to human survival and economic well being and provides a huge reservoir of resources which we can utilize for our benefit. Diverse microorganisms are essential to a sustainable biosphere like ability to recycle nutrients, produce and consume gases that affect global climate, destroy pollutants, treat wastes and they can be used for biological control of plant and animal pests. The study of microbial diversity is also important to solve new and emerging disease problems and to advance biotechnology, in few words, exploration, evaluation of microbial diversity are essential for scientific, industrial and social development.

In recent times, the microbes from extreme environments are focusing major attention to the scientific communities in recent years primarily due to the survival of microbes under extreme conditions. This would provide important clues on the origin and evolution of life, since many environmental conditions on earth closely resemble to those existing on other planets and secondly, these conditions also significantly influenced the world of microbial biodiversity. At present, only fraction of microbial life (less than 10%) is known and explored. The microbes from extreme habitats constitute important component of the vast unexplored biological potential. The limited studies on the diversity of the extreme organisms from varied habitats have indicated highly diversified population dynamics. Therefore, exploration of many more extreme habitats would be of great significance. Thirdly and last application indeed is their utilization in biotechnological applications. The robust biocatalysts and unique metabolic capabilities of extremophiles are the major points of attractions in developing processes towards bioremediation and other biotechnological applications. The value based molecules from microbes dwelling in unexplored and extreme habitats have recently prompted interest

among the scientific communities and industries.

The current inventory of the world's diversity is incomplete as vast number of microorganisms are unexplored. Scientists have identified about 1.7 million living species on our planet. Studies indicate that the 5,000 identified species of prokaryotes represent only 1 to 10% of all bacterial species. Therefore, we have only a small idea regarding the true microbial diversity in this world. For millennia, diverse microorganisms have yielded important biological materials useful to humans such as antibiotics, drugs, enzymes, herbicides, and growth promoters.

In India, it is even more relevant due to our enormous wealth of available biodiversity. The vast microbial diversity of the natural world, combined with ingenious methods to access the diversity, can provide us with abundant source of new and valuable products. Therefore, continued research is needed to describe and protect the unexplored resources for the preservation of natural ecosystems and the future benefit of mankind.

Objectives:

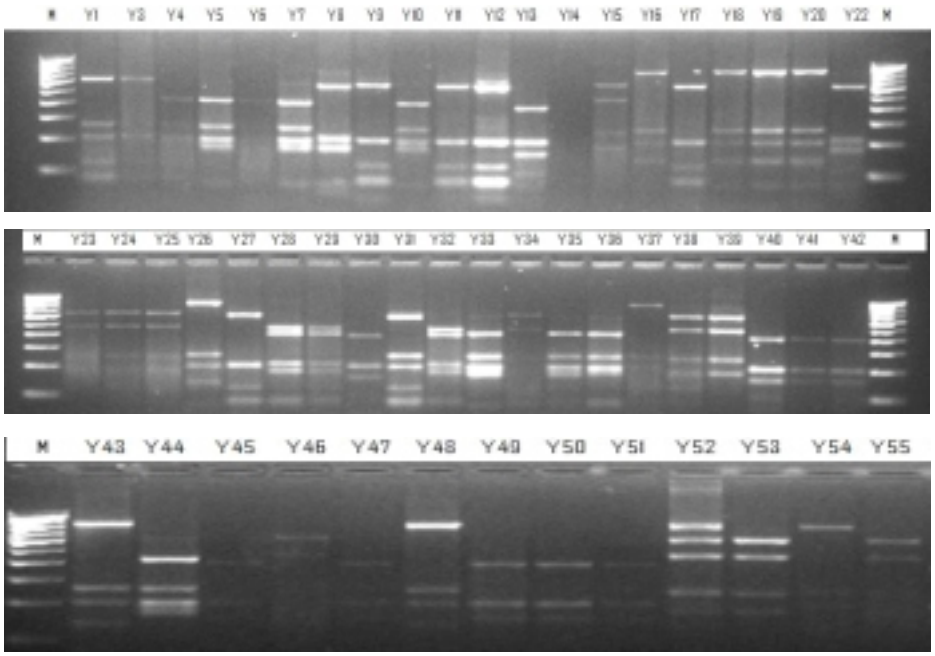
1. Microbial diversity analysis in extreme climates
2. Identification of osmolytes production by extremophilic bacteria
3. To look for production of enzymes (Amylase, Cellulase, CMCase, FPase, Xylanase & Protease) in thermophilic bacteria
4. Molecular characterization of Extremophilic organisms

Significant Achievements

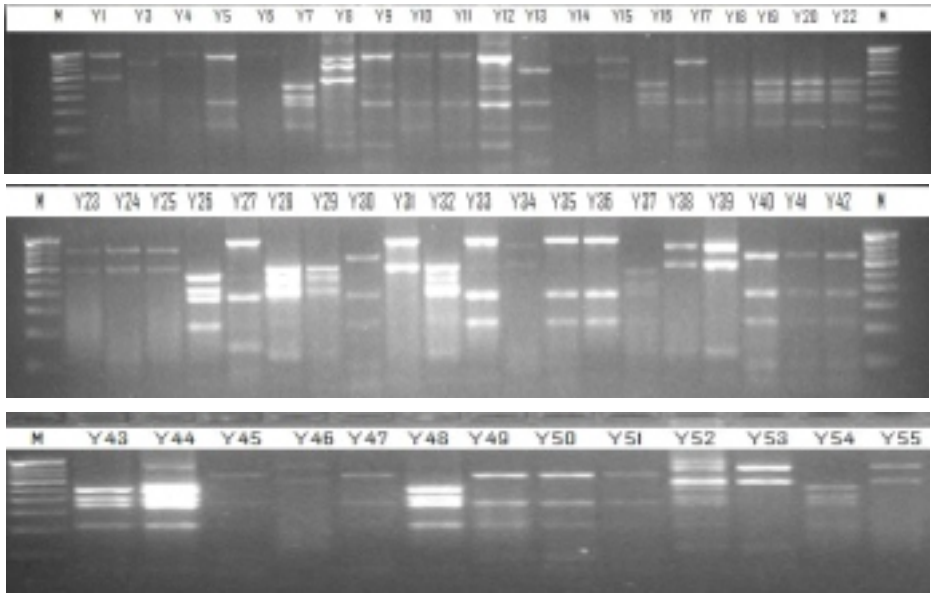
Diversity analysis of bacteria in Yumtang and Yumesamdong Hot spring, North Sikkim

- Aerobic, thrermophilic bacteria were isolated and characterized from water and sediment samples collected from, Yumtang and Yumesamdong Hot spring, India, having pH 6.5.
- The total number of microorganisms in the sediment and water samples was found to be $2x10^3$ cfu ml^{-1}.
- 53 morphotypes selected, 19 could grow at temperature 45°C and 11 at 60°C.
- Combined dendrogram based on ARDRA analysis revealed the existence of 22 clusters among the isolates (Fig. 1). The bacteria are in the process of identification.

16S rDNA PCR-RFLP analysis with *Alu*I



16S rDNA PCR-RFLP analysis with *Taq* I



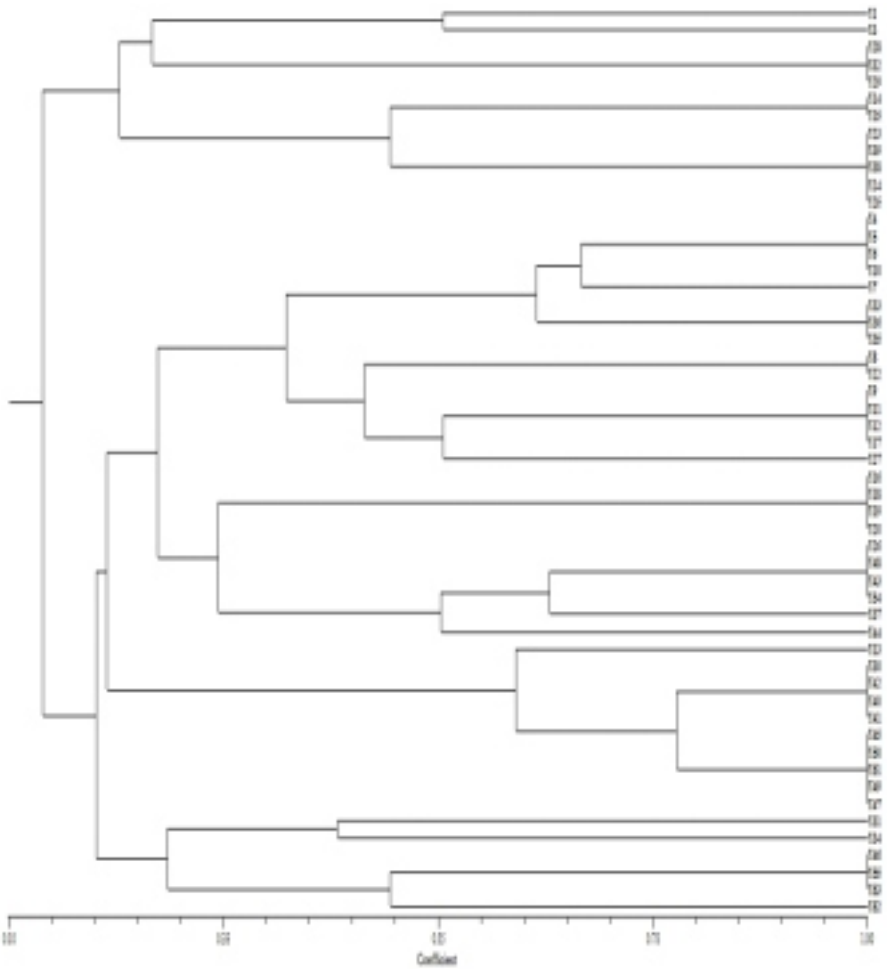
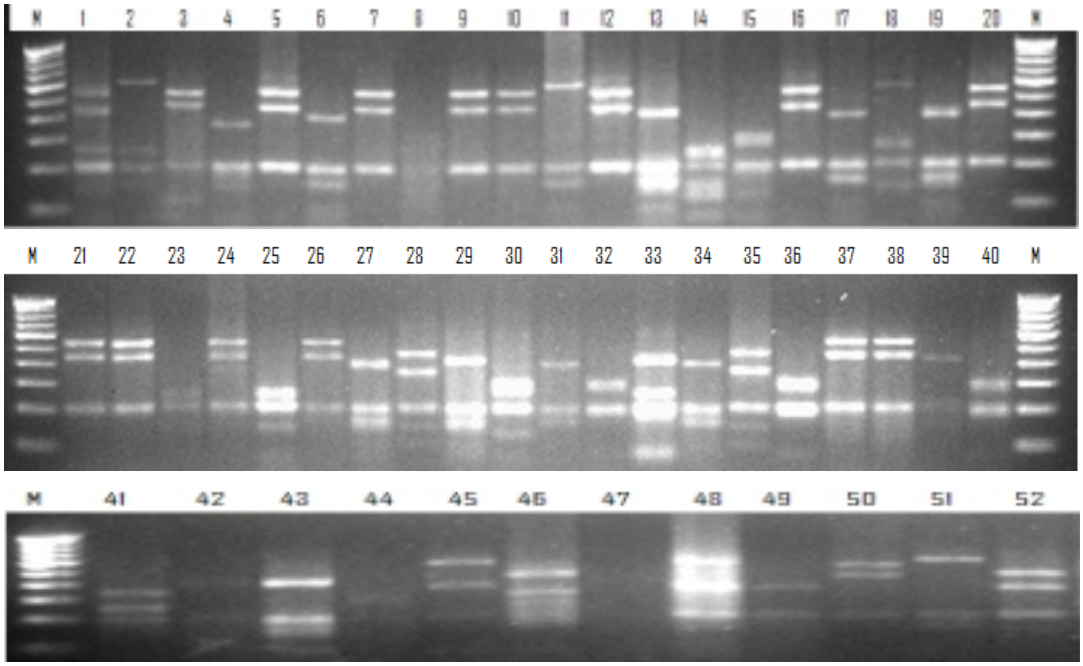


Fig. 1 Combined dendrogram showing similarity among the isolates based on RFLP analysis with *Alu* I and *Taq* I

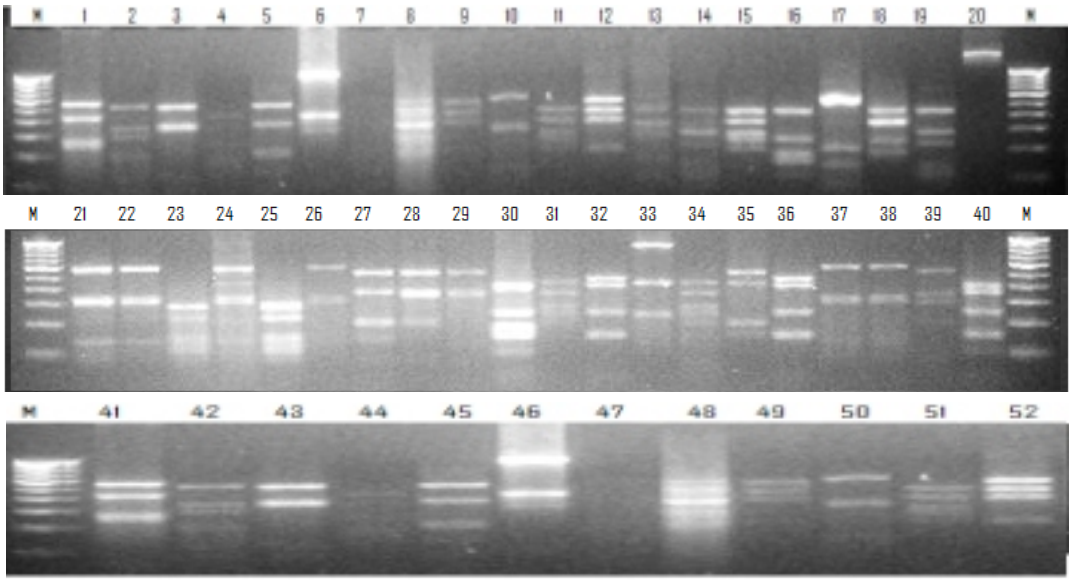
Diversity analysis of bacteria isolated from Gurudongar Lake (North Sikkim)

- In soil samples, the bacterial count ranged from 1.2 to 3.7 x $10^3 ml^{-1}$
- Total of 50 bacteria were picked that were able to grow at 4°C.
- All bacterial isolates showed production of copious amount of exopolysaccharide.
- Among the isolates, 12 bacteria were found to be psychrotolerant and could not grow beyond 15°C.
- 16S rDNA-RFLP analysis with *Hae* III and *Alu* I revealed greater diversity among the isolates.
- Combined dendrogram based on RFLP analysis revealed the existence of 16 clusters among the isolates. The bacteria are in the process of identification (Fig. 2).

16S rDNA PCR-RFLP analysis of bacteria isolated from Gurudongar Lake with AluI



16S rDNA PCR-RFLP analysis of bacteria isolated from Gurudongar Lake with HhaI



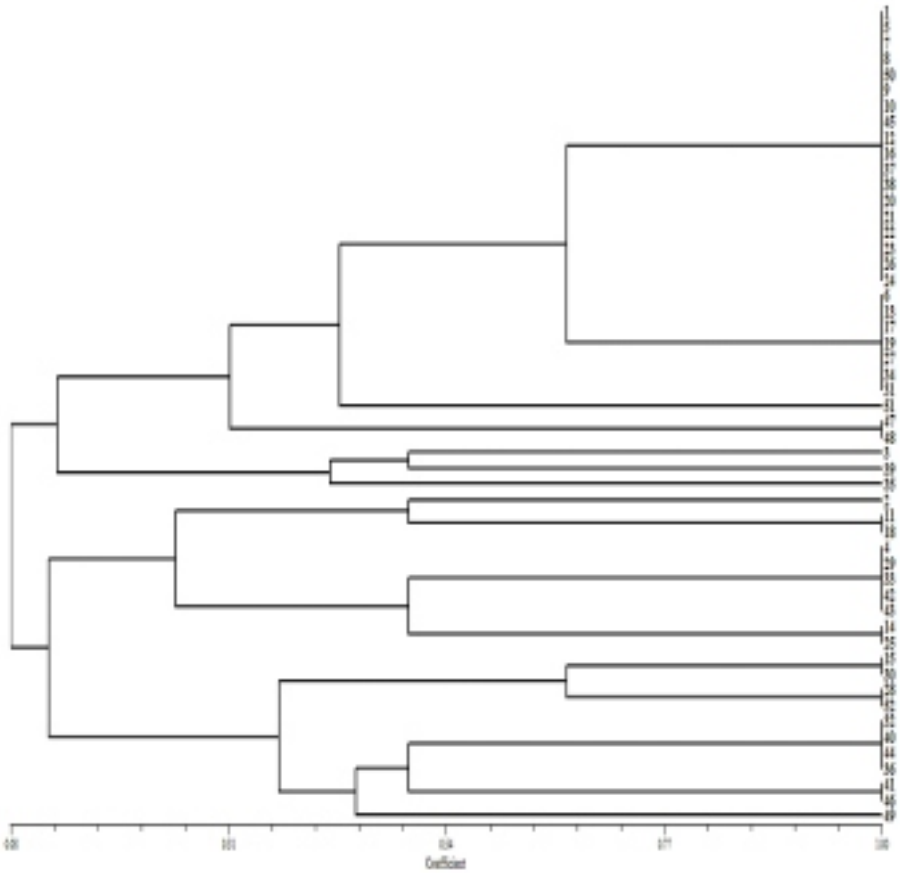


Fig. 2 Combined dendrogram showing similarity among the isolates based on RFLP analysis with *Alu* I and *Hha* I

Conclusion:

Isolation and identification of microbes from extreme environments could have biotechnological implications. Bacteria isolated from the hot springs and cold lakes showed greater molecular diversity. These isolates will be evaluated for their potential applications and further preserved for future consequences.

Project: Diversity of Actinomycetes from Indogangetic plains

PI : D. K. Arora
Co-PIs : Mahesh Yandigeri
SRF : Nityanand Malviya

Rationale

The actinomycetes are phenotypically diverse, gram positive bacteria containing more than 55% G+C content in their DNA They are found in most natural environments. These were originally considered to be an intermediate group between bacteria and fungi but now recognized as prokaryotic. Majority of the actinomycetes are free living, saprophytic bacteria found widely distributed in soil, water, and colonizing plants. Actinomycetes can be isolated from soil, water, and plant material. In soil, they are involved in the decomposition and mineralization cycles with the production of extracellular enzymes, such as cellulases, chitinases, and lignin peroxidases. Many species of actinomycteres also produce a wide variety of secondary metabolites, including antihelminthic compounds, antitumour agents, and antibiotics, which have been exploited in medicine and agriculture to cure various ailments.

Actinomycetes can be found almost in any substrate, although they prefer alkaline and neutral conditions in order to grow. The optimal pH range is between 7.0 and 8.0. Most of the actinomycetes grow at temperatures between 15 and 30°C. Actinomycetes show a compact, leathery appearance with dry surfaces and are reported as source of an earthy smell as they produce geosmin and 2-methyl isoborneol. These actinomycetes, including members of the genera *Streptomyces*, *Saccharopolyspora* and *Amycolatopsis*, which are highly adapted to survive under highly erratic and competitive soil environment. They are equipped with a wide array of enzymes for exploiting nutrients but also produce a broad range of bioactive metabolites of industrial and medical importance, e.g. compounds with antibiotic activity against fungal and bacterial competitors. Moreover, the exploration, characterization, maintenance, conservation and documentation of various categories of actinomycetes are also important with reference to industrial and agricultural sectors.

In India, Indo-gangetic plain (IGP) is considered to be most fertile ecoregion with wheat-rice cropping system being most prevalent. However, over the years there has been decline in fertility and productivity in these plains. Microbes are known to influence the crop rhizosphere by production of various metabolites and nutrients. Actinomycetes play role in rhizosphere by involving in various activities like biodegradation, biomineralization, biocontrol and plant growth promotion. They are equipped with a wide array of enzymes for exploiting nutrients but also produce a broad range of bioactive metabolites of agricultural, industrial and medical importance. Hence, this project has been formulated to isolate, utilize and conserve actinomycetes diversity of IGP in order to exploit the actinomycetes for agricultural productivity and human welfare.

Objectives:

- Isolation, characterization and identification of actinomycetes from Indo-gangetic plains.
- Molecular analysis of actinomycetes diversity in Indo-gangetic plains.
- Functional characterization of isolated strains using BIOLOG microbial identification system and conventional biochemical methods.

Significant Achievements:

Sampling area and isolation:

Among the Indogangetic plains, Dehradun and Lucknow belt were chosen for the study. Soil samples were collected from rhizospheric regions of various cultivated crops. Isolation of actinomycetes was carried out from the soil samples obtained from Dehradun belt and Lucknow belt regions (Table 1). Isolation of actinomycetes was carried out using various media and enrichment techniques. Colony forming units ranged from 14×10^3 to 32×10^3. A total of 192 morphotypes of actinomycetes were isolated using starch casein agar, glucose yeast extract malt extract agar, Actinomycetes isolation agar, soil extract agar and various ISP media from these regions.

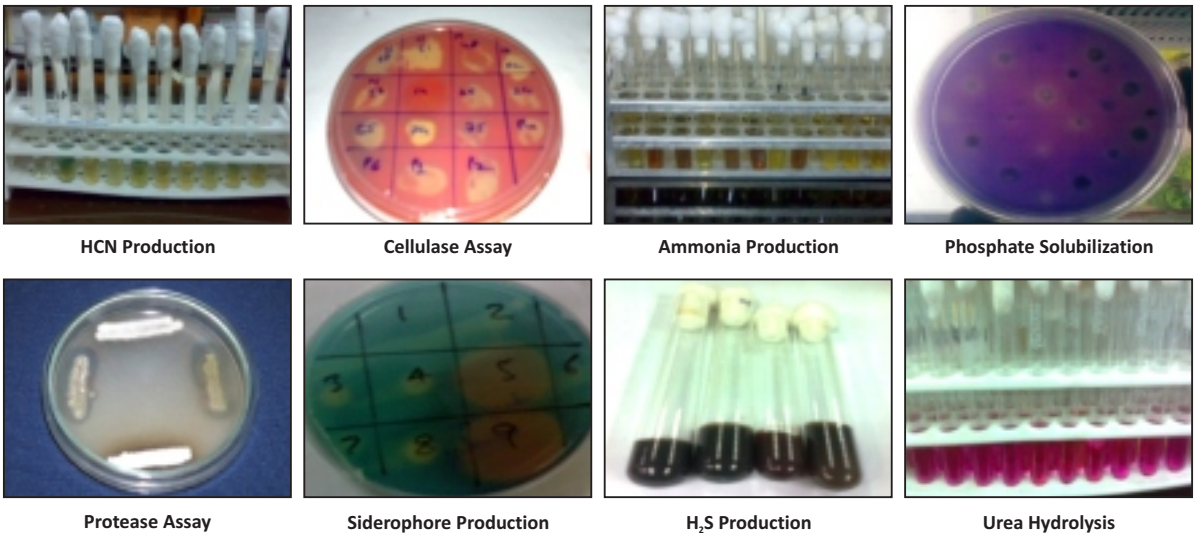


Fig.1. Biochemical characterization of actinomycetes from Indo-gangetic plains.

Table1. Geographical location and enumeration of actinomycetes population in IGP regions.

Location	Latitude and Longitude	Agro-climatic region and zone	Soil Type	pH	Total no. of colonies/ actinomycetes count	Geographical area (Sq km)
Dehradun	30.33°N 78.06°E	Northern indogangetic plain	Alluvial soils	7.5-7.8	35/23	3,088
Nainital	29°23°N 79°27°E	Northern indogangetic plain	loamy to sandy loam	6-6.5	29/14	11.73
Haridwar	29.96°N 78.16°E	Northern indogangetic plain	loamy to sandy loam	7-7.5	31/14	2,360
Almora	29.59718°N 79.6570°E	Northern indogangetic plain	loamy to sandy loam	6-6.5	32/19	3,082
Lucknow	26.50°N 80.50°E	Upper Gangetic plains-mid plains	Saline-alkaline alluvial	7.8-8.5	47/32	2,528
Sitapur	27.57°N 80.68°E	Upper Gangetic plains-mid plains	Saline, alluvial	7.5-7.8	36/23	5,743
Bahraich	27.58°N 81.6°E	Upper Gangetic plains-mid plains	Saline, alluvial	7.3-7.7	38/22	5,020

Morphological and biochemical characterization:

The isolates were characterized on the basis of morphological, physiological and biochemical characteristics and sugar utilization patterns. Out of 145 isolates, 50 were recorded as phosphates solubilizers, 13 siderophore producers, 83 ammonia producers, 28 HCN producers, 20 protease producers, 17 amylase producers, 61 cellulase producers, 46 hydrolysed gelatin, 44 hydrolyzed urea and 26 H_2S producers (Fig.1). Actinomycetes isolates were characterized for their salt tolerance ability and found that 140, 107, 81 and 41 isolates were resistant against 0.34, 0.69, 1.03 and 1.71 M concentration of NaCl respectively (Fig.2). This clearly shows that indogangetic plain harbours good number actinomycetes with plant growth promoting, biodegrading and salt tolerant abilities.

Molecular characterization:

Cultures were identified using classical approaches (Fig. 3) followed by 16S rDNA sequencing. Molecular characterization of all the isolated actinomycetes was carried out using 16S rDNA universal primers (pA and pH). Amplified Ribosomal DNA Restriction Analysis (ARDRA) technique was used to study the diversity of the actinomycetes from four mangrove ecosystems. 16S rDNA product of ~1400bp obtained from all the actinomycetes was subjected to restriction analysis using *Taq*I, *Msp*I and *Hae* III restriction endonucleases. The restriction profile obtained was used to construct the dendrogram using Jaccard's coefficient for assessing the phylogenetic relationship between the isolates.

The phylogenetic relationship indicated that isolate could be clustered into 48 groups (Fig. 4). The representative isolates have been subjected to sequencing for further identification. Cultures identified using 16S rDNA sequences were submitted to NBAIM culture collection namely, *S. viridodiastaticus*, *S. heliomycini*, *S. albogriseolus*, *S. griseorubens* and *Streptomyces macrosporeus* with accession numbers GU817410, GU817411, GU817412, GU817413 & GU817414 respectively.

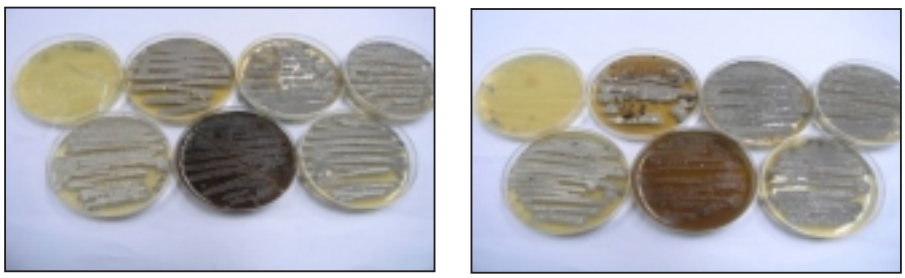


Fig.3. Morphological characterization of identified actinomycetes on ISP media using classical approaches.

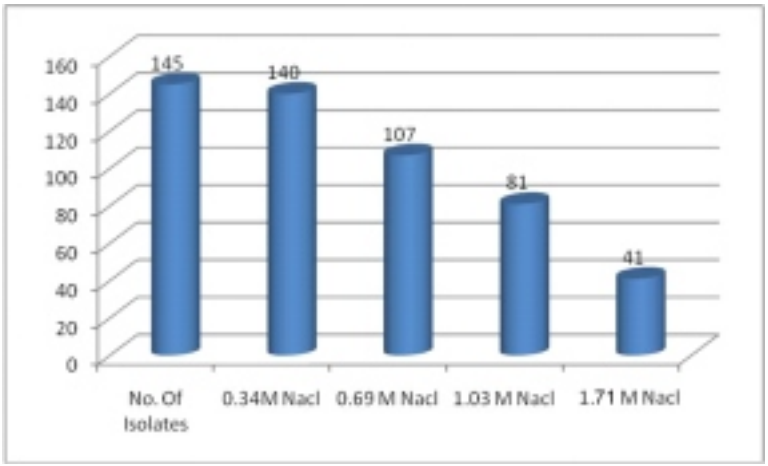


Fig. 2. NaCl tolerance ability of actinomycetes isolates from IGP regions of India. (Numbers on the bars represent the total number of actinomycetes resistant at respective concentration of NaCl)

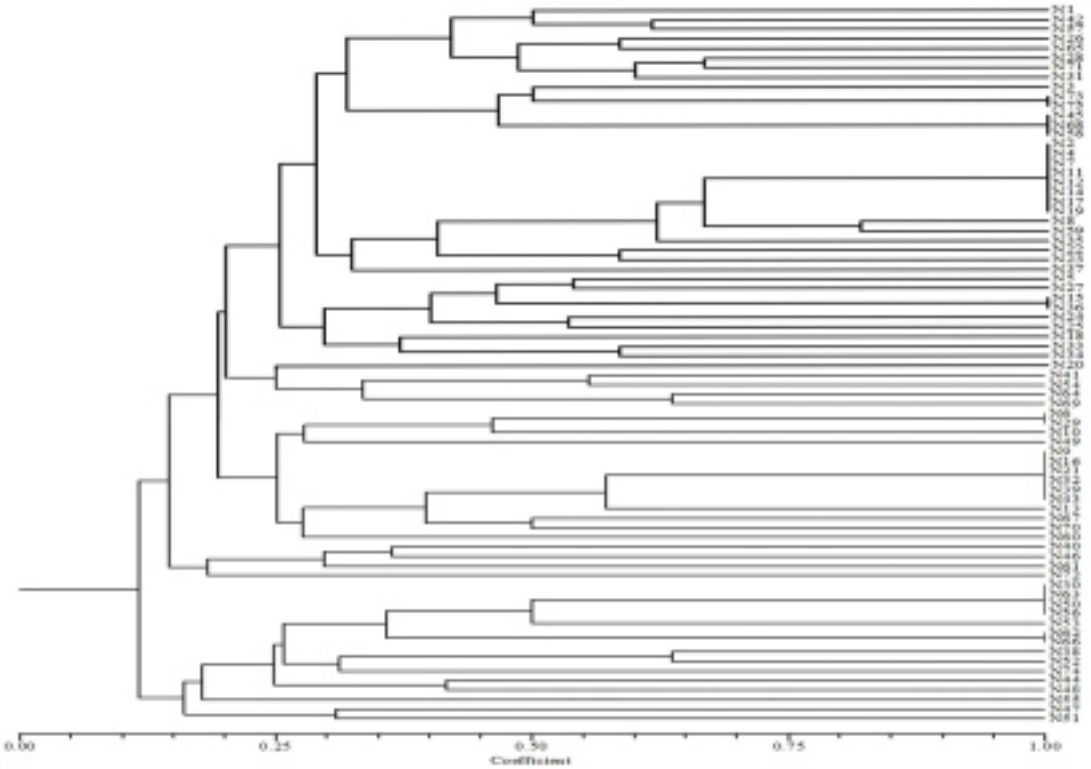


Fig. 4. UPGMA Dendrogram showing phylogenetic relationship among of the actinomycete isolates from Indogangetic plains.

Diversity Index:

Diversity Indices of actinomycetes from IGP regions were also calculated and tabulated on the basis of clustering using shannon index and simpson index (Table 2).

Table.2. Diversity index of actinomycetes isolated from Indogangetic Plains of India.

Regions	Shannon index (D)	Simpson index (1/D)	Species evenness
Lucknow- Sitapur-Bahraich	3.531	0.955	0.922
Dehradun-Nanital-Haridwar- Almora	1.87	0.534	0.429

Diversity indices were calculated for species richness and evenness on the basis of clustering. From the indices it was concluded that Lucknow belt had rich diversity and evenness of actinomycetes species in comparison to the Dehradun belt.

Conclusion:

A diverse group of actinomycetes were explored in Lucknow and Dehradun belt of Indogangetic plain. Actinomycetes obtained from these regions were found diverse in morphological and biochemical characteristics. From the studies it was concluded that Lucknow region has more diverse actinomycetes in terms of diversity (*Shannon index* 3.531) followed by Dehradun region (*Shannon index* 1.87). Nearly 28% of the isolates possessed salt tolerance ability indicating that indogangetic plains harbor good number of salt tolerant actinomycetes. Also results showed cultivated lands of central Punjab region had lesser diversity than Lucknow cultivated lands. Sufficient functional diversity was also observed from the various biochemical parameters. From the study, it can be inferred that Lucknow soils harbour good number of agriculturally important microbes because of which the fertility status of the Lucknow soils is maintained in comparison to the Punjab belt where chemical fertilizers are used in moderately good quantity. Among the actinomycetes population of *Streptomyces* genera was dominant in comparison to other genera. The results emanating from this study will have far reaching implications and backbone for future research and developments in agriculture sector.

Theme : Nutrient Management PGPR and Biocontrol

7

Title: Exploration, Collection and Characterization of some Agriculturally Important Biocontrol Agents Suitable for Disease Management

PI : D. K. Arora
Co-PIs : Alok Kumar Srivastava, Sudheer Kumar
SRF : Rajesh Kumar Singh

Rationale

Plant rhizosphere is a versatile and dynamic ecological environment of intense microbe plant interactions for harnessing essential micro and macro nutrients from a limited nutrient pool. Multiple types of biological interactions among living microorganisms and plants going inside soil community. Understanding these interaction will help in better designing of plant disease management strategies especially in case of soil borne pathogens. Biological control is one of such strategy which is getting greater attention in recent time due to low cost and eco-friendly nature. Additionaly, plant growth promoting rhizobacteria (PGPR) present in the rhizosphere at root surfaces and in association with roots, which help in improving the extent or quality of plant growth directly and or indirectly. Some of the most commonly found soil microbes belong to different fungal and bacterial antagonistic and function as antagonists of phytopathogens, thus protecting plants and reducing disease incidence in many different soil types. Together with other species, such as *Pseudomonas* spp., *Bacillus* spp., *Coniothyrium* spp., *Pythium* spp. etc., these highly interacting microbes have been extensively studied and commercialised mainly as biopesticides/ biofertilizers and soil amendments, all containing live cells. The current advances of the basis and the progress of biocontrol methods, the link between environmental factors and plant infection development, and the use of formulation technology in biological control of plant pathogens is the need of hour.

Objectives:

- Selection of antagonists for pathogens (*Fusarium* spp.).
- Screening and selection of potential antagonistic isolates for important field crops.
- Characterization of active principle responsible for antagonisms.
- Dosage standardization and delivery system.
- Determination of shelf-life of formulations.
- Mass multiplication of antagonists (*Trichoderma*).
- Field evaluation of potent bio control agents.

Methodology:

Molecular Characterization:

The PCR amplified 16S rDNA product (560 bp) was restriction digested for 3 hours at 37°C in 25 µl reaction mixture containing 10 U of one of the following restriction enzymes, *Alu*I, *Hae*III and *Mbo*I. Restriction digestion was then analysed by agarose electrophoresis (1.4%). A 100 bp ladder (Fermentas Inc.) was used as molecular size marker. The analysis was done at least twice with each enzyme. Calculation of the pair-wise coefficients of similarity was based on the presence or absence of bands. A cluster analysis with the UPGMA algorithm was performed with the NTSYSpc numerical taxonomy and multivariate analysis system (Raaijmakers and Weller, 2001).

BOX-PCR fingerprinting

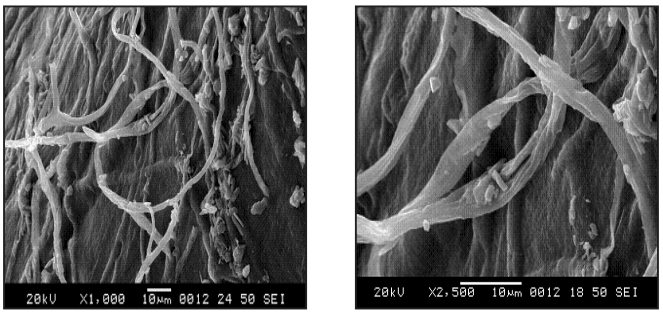
The single primer corresponding to BOX (BOXA1R, 50-CTA CGG CAA GGC GAC GCT GAC G-30) was obtained from Integrated DNA technology (Coralville, USA).

16S rDNA Sequencing:

The PCR amplified 16S rDNA were purified with a Quaquick purification kit (Qiagen Inc. Germany). The DNA sequence was double checked by sequencing both strands using primers pA and pH for forward and reverse reaction, respectively. The nucleotide sequences were di-deoxy cycle sequenced with fluorescent terminators (Big Dye, Applied Biosystems) and run in 3130xl Applied Biosystems ABI prism automated DNA sequencing.

Significant Achievement:

Fourteen *Bacillus* and fourteen *Pseudomonas* spp. strains selected on the basis of different PGPR activity and cell-wall degrading enzymes were evaluated against different *Fusarium* pathogens *viz., Fusarium udum, Fusarium solani, Fusarium oxysporum* f. sp. *ciceri* and *Fusarium lycopersici. In vitro* and pot experiments showed that only 4 isolates were giving effective to restrict the growth of *Fusarium* spp. which were further evaluated at greenhouse and field conditions. All the 28 isolates showed positive result in the principle studies in antagonistic as well as PGP activity. All these selected isolates were further characterized on the basis of molecular studies and finally 14 *Bacillus* species were identified by 16S rDNA sequencing (Fig. 1, 2 and Table 1)



***Fusarium oxysporum f.* sp. *ciceri* (Pathogen)**

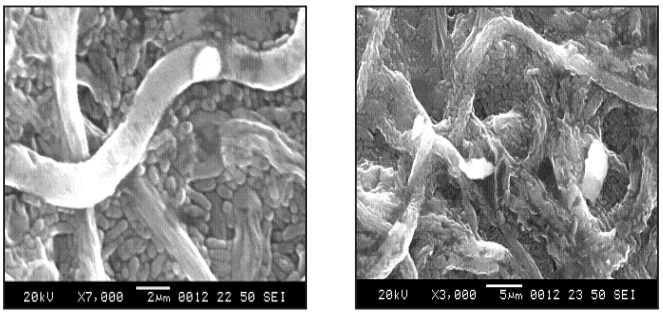
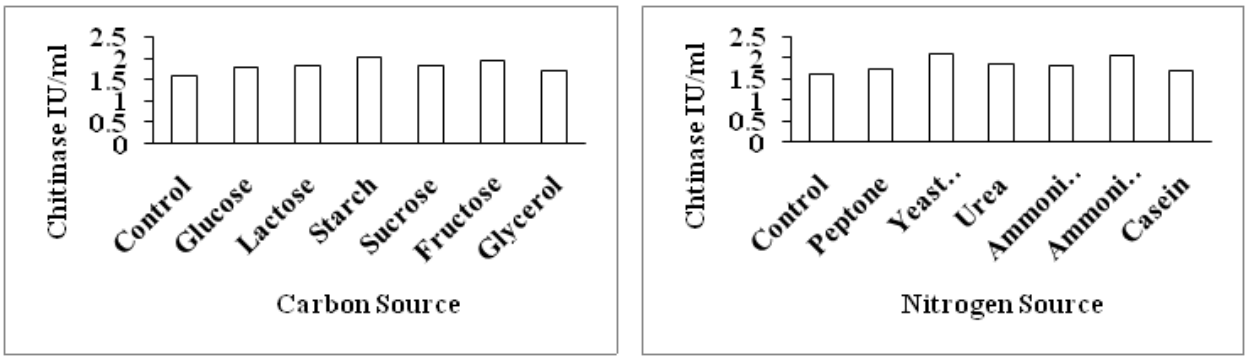


Fig. 1. Scanning Electron Microscopy of screened selected bacterial biocontrol agent against *Fusarium oxysporum f.* sp. *ciceri* (Pathogen) interaction after 7 days.

Optimization of parameters for chitinase production:



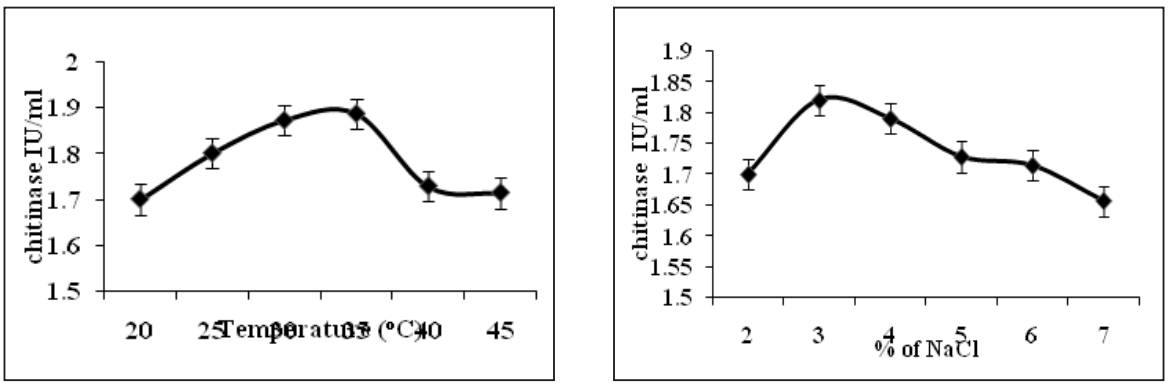


Fig. 2. Effect of various carbon and nitrogen source on chitinase production by *Lysinibacillus fusiformis* (B-13)

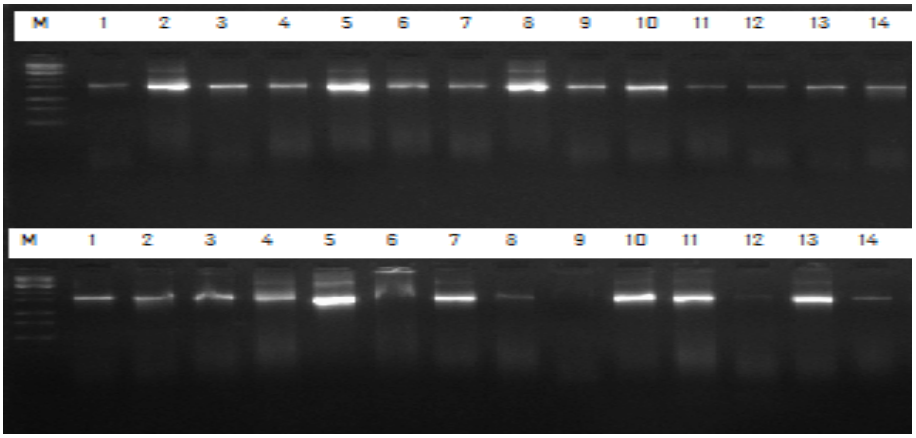


Fig. 2. PCR amplification of 16S rDNA

Table: 1. Based on the sequencing of 16S rDNA and BLAST analysis the isolates were identified as:

S. No	Species	Similarity %	Accession No.
B-1	*Lysinibacillus fusiformis*	98%	EU430993
B-2	*Lysinibacillus* spp.	97%	FJ237498
B-3	*Bacillus sphaericus*	96%	DQ833578
B-4	*Bacillus* spp.	98%	GU566326
B-5	*Bacillus subtilis*	99%	FJ393325
B-6	*Bacillus amyloliquefaciens*	99%	GU181235
B-7	*Bacillus cereus*	97%	EU161996
B-8	*Bacillus* spp.	98%	GU566326
B-9	*Lysinibacillus fusiformis*	98%	EU430993
B-10	*Bacillus cereus*	97%	EU161996
B-11	*Bacillus amyloliquefaciens*	99%	GU181235
B-12	*Bacillus sphaericus*	96%	DQ833578
B-13	*Lysinibacillus fusiformis*	98%	EU430993
B-14	*Lysinibacillus* spp.	97%	FJ237498

Conclusions:

Increase in public concern about the environment has increased the need to develop and implement effective biocontrol agents for crop protection. An effective PGPR and PGPF will be developed for disease control only after understanding its performance in the environmental conditions. In nature agriculture crops are exposed to diverse environmental conditions and change of seasons, which alter the microclimatic conditions existing around the infection court. A thorough knowledge on the mechanisms and performance related to disease control will help in the selection of bacterial isolates that suits to produce a reliable commercial product.

Project: Exploration, collection and characterization of some agriculturally important biocontrol agent suitable for disease management
Subtitle: Evaluation of Oxalate Oxidase gene and its potential to control *Sclerotinia sclerotiorum*

PI : D. K. Arora
Co-PIs : Alok Kumar Srivastava, Sudheer Kumar
SRF : Shivani Yadav

Rationale

Sclerotinia sclerotiorum is a necrotrophic, phytopathogenic, filamentous ascomycetes fungi. It is recognized as an omnivorous plant pathogen with broad host range and worldwide distribution. Over 400 species of plants are susceptible to this pathogen. The majority of these hosts are dicotyledonous, although a number of agriculturally significant monocotyledonous plants are also parasitized by this pathogen. The sclerotium of *S. sclerotiorum* is a pigmented, asexual, multicellular, and firm resting structure composed of condensed vegetative hyphal cells which become interwoven and aggregate together, and it is capable of surviving years in soil.

S. sclerotiorum acidifies its ambient environment by producing oxalic acid. This production of oxalic acid during plant infection has been implicated as a primary determinant of pathogenicity in *S. sclerotiorum*. Oxalic acid also necessary for activity of many hydrolytic enzymes including polygalacturons. Oxalic acid chelates calcium ions and resulting in a destabilization of pectate polymers, allowing increased access and sensitivity to pathogen-produced pectolytic enzymes. This gene is also responsible for the conversion of oxalic acid and oxygen to carbon dioxide and hydrogen peroxide. Keeping the importance of this gene, the present study was planned to evaluate the potential of oxalate oxidase gene to control *Sclerotinia sclerotiorum.*

Objectives:

- Isolation and characterization of antagonistic fungi (against *S. sclerotiorum*) from vegetable rhizosphere.
- To screen potential isolates for oxalate oxidase gene against plant pathogen *S. sclerotiorum* by using oxoX primer
- To develop a potential biocontrol agent by transforming oxalate oxidase gene into *Coniothyrium minitans*

Primer used:

oxo X GF (5'-ATCTCAGACCATCTCCCCACTTGC-3')
oxo X GR (5'-CGTCACATCAATGGACCTTGTCG-3')
oxoX-C F (5'-AGAACGCAGACACGCTTG -3')
oxoX-C F (5'-AATGCCTTGCTCTTGTTCA-3')

Significant Achievements:

- Out of total 35 fungal isolates, 10 isolates were found potential antagonists against *S. sclerotiorum*. To determine probable mechanism of antagonism, potential antagonists were screened for their ability to produce oxalate oxidase using minimal medium containing 1% oxalic acid.
- Growth rate of fungal isolates on minimal medium was observed to be very slow compared to growth on PDA medium. Only two isolates were able to grow on minimal medium containing 1% oxalic acid.
- Genomic DNA was amplified with primer OXO to detect the presence of oxalate oxidase gene in potential isolates (Fig. 1).
- Amongst thirty five fungal isolates, only two

fungal strains have shown the amplification of oxalate oxidase gene.

- Screening of remaining fungal isolates for the presence of oxalate oxidase gene is in progress.

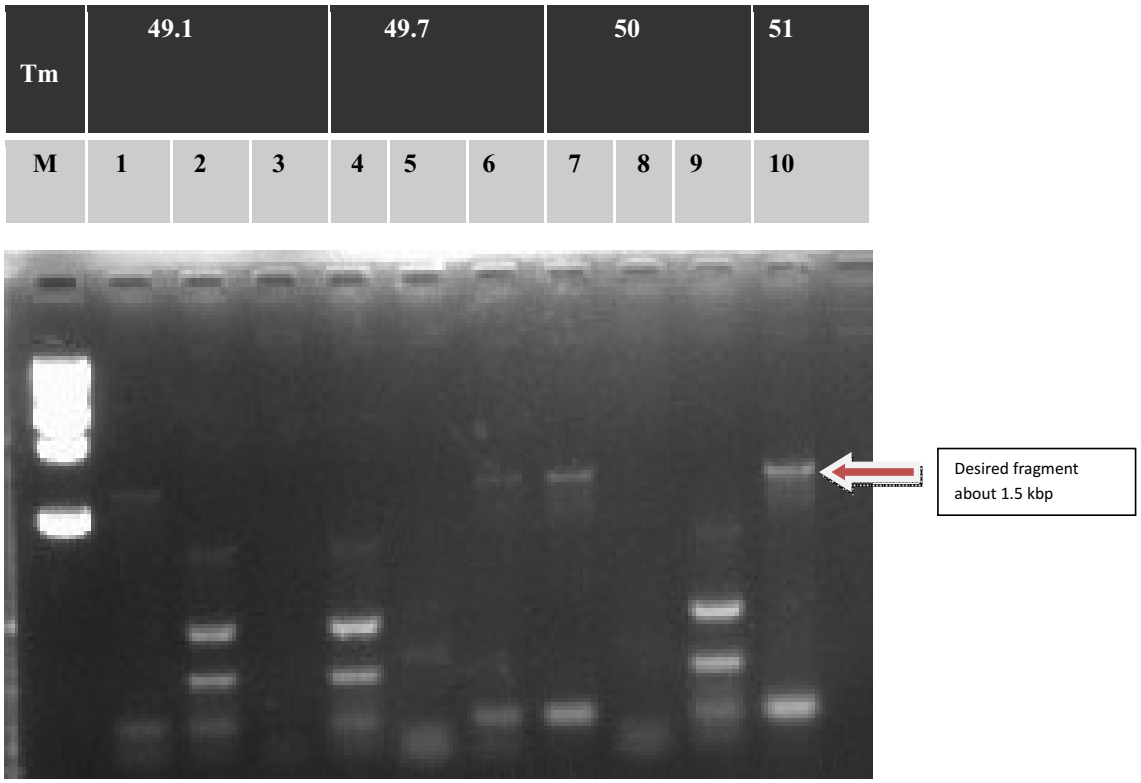


Fig. 1. Gradient PCR for validating oxoC primer

Conclusion:

Results of screening and identification of *oxo* gene in fungal biocontrol agents screened are encouraging, two fungal isolates are found positive for *oxo* gene.

Project: Evaluation of endophytic fungi for growth promotion and biocontrol

PI : Alok K Srivastava
Co-PIs : Sudheer Kumar
SRF : Shikha Gangwar, Supriya Srivastava

Rationale

Endophytes are microorganisms that live within host plants for at least part of their life and do not cause apparent symptoms of diseases. In general, beneficial endophytes promote host plant growth, increase plant nutrient uptake, inhibit pathogen growth, reduce disease severity and enhance tolerance to environmental stresses. These microorganisms play important roles and offer environmentally-friendly methods to increase productivity by reducing chemical inputs used to manage various diseases. The endophytes are potential candidates for genetic modification with useful genes, which could be used to impart additional traits. Endophytes may benefit host plants by preventing pathogenic organisms from colonizing them. Extensive colonization of the plant tissue by endophytes creates a "barrier effect", where the local endophytes outcompete and prevent pathogenic organisms from taking hold. Endophytes may also produce chemicals which inhibit the growth of competitors, including pathogenic organisms. The

presence of fungal endophytes has been shown to cause higher rates of water loss in leaves.

Objectives:

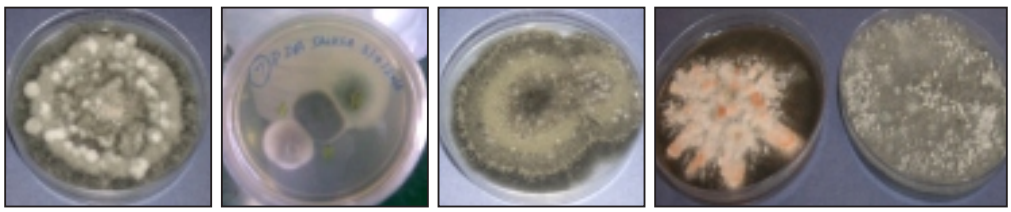
- Isolation of endophytic fungi
- Identification and characterization of endophytic fungi
- Molecular characterization of endophytic fungi
- Determine endophytic microbial diversity

Significant Achievements

- Plant parts *viz.*, leaves and root from different vegetable crops were collected from the field and examined for the isolation of fungi as endophyte (Table 1). The samples were washed thoroughly with water, and later on surface sterilized by using NaOCl and 70% methanol. The surface sterilized plant parts were crushed in sterile saline buffer and aliquots (0.2 ml) plated on different fungal media. Six distinct fungal isolates growing as endophyte were obtained and maintained.
- Total 110 isolates of endophytic fungi were isolated from different plants from Indo-gangetic plains (Table 1). The isolates were maintained at 28±2°C.
- The isolates were characterized on the basis of morphology, growth characteristics (Fig. 1) and production of various metabolites including IAA, siderophore, ammonia, and HCN etc.
- The isolates were further characterized by molecular methods. The genomic DNA of all the isolates were obtained using lysis buffer. ITS-1 and ITS-4 region of ribosomal DNA was amplified using universal primer which yielded a fragment of size about 550 bp.
- The variability within the ITS amplified regions is being investigated by restricting this fragment with restriction enzyme *Mbo*I. However, no substantial polymorphic pattern among the isolates was found by using ITS with restriction enzyme on 2.5% agarose.
- The 550 bp ITS product of 12 isolates were further purified and sequenced on ABI cycle sequencing using Sanger's sequencing technique. The sequence was aligned using BLASTn for identification (Table 2) and submitted to NCBI database.

Table 1. List of Plants explored for isolation of endophytes

S. No.	Botanical Name (Host Plant)	Common Name	Number of Isolates
1.	*Capsicum* spp.	Chilli	3
2.	*Amaranthus*	Chaulai	2
3.	*Abelmoschus esculantum*	[illegible]	5
4.	*Luffa acutangula*	Toari	2
5.	*Cucumis utilissimus*	cucumber	3
6.	*Cummis sativas*	Kakari	2
7.	*Lycopersicon esculatum*	Tomato	3
8.	*Citrus aurantifolia*	lime	7
9.	*Legimera cineria*	Bottle guard	3
10.	*Solanum lycopersicum*	Tomato	10, 9
11.	*Solanum lycopersicum*	Tomato	14
12.	*Azardirachta indica*	Neem	2
13.	*Euphoria pekinesis*	Euphorbia	2
14.	*Oscimum sanctum*	Tulsi	6
15.	*Helianthus annuus*	Sunflower	1
16.	*Zea mays*	Maize	2
17.	*Sesamum indicum*	Til	3
18.	*Oryza sativa*	Rice	2
19.	*Cajanus cajan*	Pigeon pea	1



Fungal endophytes isolated from *Luffa acutangula*

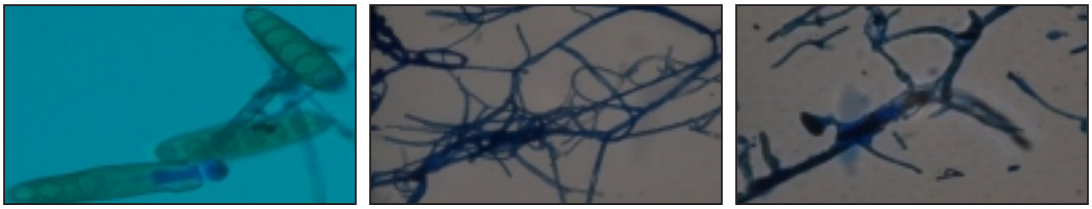


Fig. 1. Morphological characters of Isolate No. 7 and 21

Table 2. Similarity matrix and phylogenetic affiliation of endophytic isolates

S. No.	Host plant	Phylogenetic Affiliation	% similarity
1.	*Euphoria pekinesis*	Fungal endophyte isolate	-
2.	*Amaranthus*	Uncultured endophyte fungus	99%
3.	*Luffa acutangula*	*Macrophomina phasalina*	100%
4.	*Zea mayes*	Uncultured endophyte fungus	99%
5.	*Lycopersicon esculatum*	*Cerotobasidium* sp.	84%
6.	*Citrus aurantifolia*	Fungal endophyte sp.	99%
7.	*Colocasia esculenta*	*Colletotrichum* sp.	98%
8.	*Curcuma domestica*	Uncultured endophyte fungus	99%
9.	*Oryza sativa*	*Dioscorea polysiachya*	99%
10.	*Sesamum indicum*	Uncultured endophyte	99%
11.	*Cajanus cajan*	Fungal endophyte	93%

Conclusion:

A total 110 fungal isolates were obtained as endophytes from vegetables commonly grown in IGP region. Morphological characterization of the isolates was carried out. The potential isolates were characterized through ITS sequencing and further utilized for their possible role in growth and vigour of plant.

Project: Harnessing Arbuscular Mycorrhiza for Biofertilization in Horticultural Crops

PI : G V Thomas
So-PI : Alok K Srivastava
SRF : Pratiksha Singh

Rationale

Arbuscular Mycorrhizal (AM) fungi (or Vesicular-Arbuscular Mycorrhizal, VAM fungi), belong to the Phylum Glomeromycota are symbionts with terrestrial plant roots. Artificial inoculation of arbuscular mycorrhizal (AM) fungi has potential benefits in not only for sustainable crop production but also for environmental conservation. However, the difficulty of inoculum production due to the obligate biotrophic nature of AM fungi has been the biggest obstacle to putting inoculation into practice. Nevertheless, several companies have sought to produce inoculum of AM fungi. Mycorrhizal fungi are species of fungi that intimately associate with plant roots forming a symbiotic relationship with the plant providing sugars for the fungi and the fungi providing nutrients such as phosphorus to the plants. Mycorrhizal fungi can absorb, accumulate and transport large quantities of phosphate within their hyphae and release to plant cells in root tissue. It is now generally recognized that they improve not only the phosphorus nutrition of the host plant but also its growth, which may result in an increase in resistance to drought stress and some diseases. Therefore, AM fungi offer a great potential for sustainable agriculture, and the application of AM fungi to agriculture has been developed. In fact, in some countries the AM fungal inocula have been commercialized.

Objectives:

- Collection of Arbuscular Mycorrhizal cultures from different centers.
- Molecular characterization of Arbuscular Mycorrhizal cultures.

Significant Achievements:

Arbuscular mycorrhizal fungal structure in roots is usually not observed without appropriate staining. Freshly collected root samples was washed gently and be free from soil particles. Ultrasonic treatment is effective to disperse soil particles closely adhered to roots. Roots were treated with 10% KOH solution for 30 min to 1-2 h in a hot bath, depending on thickness of root structure. Treated roots were washed with water and treated with 2% HCl solution. Acidified root samples was stained with 0.05% trypan blue (or acid fuchsin) in lactic acid for 10-15 min in a hot bath or for a few hours without heating. The roots were destained with lactic acid or lacto-glycerol and observed using microscopic observation. The stained roots was observed first under a dissecting microscope with transmitted illumination and then observed under a compound microscope (Fig. 1).

Table 1. Crops used for the isolation of arbuscular mycorrhizal fungi and root colonization

Crop	Root colonizations (%)
Potato	41
Maize	48
Sunflower	45
Chick pea	32
Grasses	32
Rice	31
Wheat	42

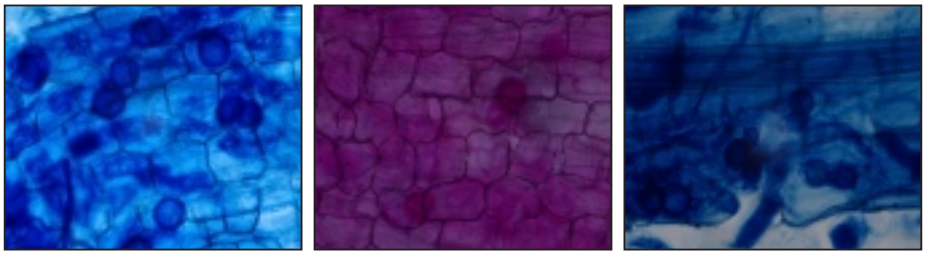


Fig. 1. Microscopic observation for the isolation of VAM fungi from different crops.

Conclusion:

Commercial inoculum production of AM fungi has been increasing during the past decade, although the future prospect of the business is still uncertain. An understanding of the physiology and ecology of the association and host specificity (which cultivars mostly likely to be benefited from which strain of mycorrhiza) under different agro-climatic conditions will help in screening and selection of the most efficient fungal endophytes. Out of twenty crops, only eight crops was selected for the studies of VAM fungi and only maize plants shows maximum mycorrhizal colonization than sunflower, wheat and potato. After screening, we will go further for the identification and molecular characterization of these fungi.

Theme :
Agrowwaste Management, Bioremediation and PHT

8

Project: Assessing structural and functional shifts in soil microbial communities of paper mill effluent contaminated soils and utilization of microflora for crop growth promotion in these soils

PI : Rajeev Kaushik
Co-PIs : D. P. Singh
SRF : Binu Mani Tripathi, Priyanka Kumari, Anamika Srivastava

Rationale

Pulp and paper mill is the major industry in our country. The heavy demand for the paper helps in steady expansion of paper industries. Since early fifties, the number of paper pulp mills in India has increased from 17 to more than 406 in 2008, with simultaneous increase in paper production from 0.13 to 1.9 million tons per annum (Singh and Thakur, 2006). Pulp and paper mills are utilizing huge amount of lignocellulosic plant components along with various chemicals and thus regarded as major polluting industries. Irrigating crops with pulp and paper mill effluent is a cheap and attractive alternative for discharge of effluent to natural waterways. Pulp and paper mill effluent contains several elements including important plant nutrients such as nitrogen (N), phosphorus (P) and potassium (K), which contribute to higher crop yields when applied to nutrient deficient soils. Other elements (magnesium, sodium, chlorides, sulfur) and organic compounds (chlorinated lignins, phenolic derivatives) that are common in pulp and paper mill effluent can cause toxicities and nutrient imbalance in plants. The tendency of certain elements (especially Na) to accumulate in pulp and paper mill effluent irrigated soils affects soil structure, increases soil salinity, resistance to root expansion and reduces water percolation and soil aeration. Furthermore, the addition of such a "mixed bag" of compounds may induce changes in physiochemical properties of the soil and also the significant shifts in structure and function of the associated microbial community, which in turn may ultimately affect the soil viability for agriculture purposes.

Objectives

- To assess the functional and structural shift in culturable soil microbial population as a result of long term irrigation of pulp and paper mill effluent.
- Diversity analysis of unculturable microflora in pulp and paper mill effluent contaminated soils.
- Characterization and utilization of selected microbial isolates for plant growth promotion in effluent degraded soil.

Significant Achievements

- The *Streptomycetes* population was significantly higher (120 X 10^5 cfu g^{-1} dry weight soil) in Effluent Irrigated Field (EIF) soils compared to Water Irrigated Field (WIF) counterpart (48 X 10^3 cfu g^{-1} dry weight soil). The 55 isolates (29 from WIF and 26 from EIF soils) gave whole-organism hydrolysates rich in *LL*-A_2pm thereby confirming that they were members of the genus *Streptomyces*, were selected for further analysis.
- Significant variation was observed among the isolates (from WIF or EIF soils) with respect to production of xylanase and cellulase. Significant variation was rule with regard to numerical dominance of producer of these enzymes among the two soil sources (EIF and WIF). Xylanase producers were more in EIF soils (12 from EIF and

4 from WIF) with maximum production by isolate NBE43 from EIF soils. Likewise, producers of cellulase, more in EIF soils (11 from EIF and 3 from WIF) with maximum production by NBE51 from EIF (Table 1).

- The percentage of isolates exhibiting plant growth promoting attributes among the EIF and WIF soils also showed significant variation. Indolic compounds and siderophore producers were more in EIF soils, whereas higher phosphate solubilization was observed in WIF soils (Table 1).

Table 1. Production of extracellular enzymes (IU) by *Streptomyces* isolates from WIF and EIF

Isolate[a]	Xylanase	Cellulase	Indolic compounds production(µg mg^{-1} protein)	Siderophore production	P-solubilization
NBE3	193.53	ND[b]	128.17	ND	ND
NBE4	ND	ND	ND	+	ND
NBE6	168.59	ND	121.52	++	ND
NBE7	ND	33.81	399.63	+++	ND
NBE9	201.20	ND	ND	ND	ND
NBE12	194.24	ND	55.50	ND	ND
NBE13	77.46	ND	369.74	++	++
NBE14	214.39	54.68	496.72	++	ND
NBE23	142.45	ND	627.10	+++	++
NBE24	ND	28.78	ND	ND	ND
NBE27	ND	ND	408.49	++	ND
NBE28	139.57	44.12	ND	ND	ND
NBE35	ND	43.17	186.67	ND	ND
NBE40	ND	24.70	603.94	+++	ND
NBE43	283.69	66.43	ND	ND	ND
NBE45	ND	49.40	423.61	++	ND
NBE50	220.62	ND	283.54	ND	ND
NBE51	ND	74.58	ND	+	ND
NBE55	202.88	ND	ND	ND	ND
NBE56	ND	73.38	362.92	ND	ND
NBE57	213.43	53.96	ND	ND	ND
NBC5	ND	ND	84.88	ND	++
NBC11	ND	28.78	114.27	ND	+++
NBC15	ND	ND	ND	ND	++
NBC25	ND	ND	24.08	ND	++
NBC29	82.49	ND	ND	+	ND
NBC32	45.08	36.45	ND	++	+++
NBC33	ND	ND	ND	ND	ND
NBC38	ND	40.77	ND	ND	+++
NBC39	ND	ND	226.01	++	ND
NBC41	201.20	ND	ND	ND	ND
NBC46	100.48	ND	ND	ND	ND
NBC49	ND	ND	ND	ND	++
NBC54	ND	ND	ND	++	ND
LSD (p=0.01)	13.40	9.62	24.11	-	-

[a] Isolates designated NBC are from WIF and NBE from EIF , [b] ND: Not Detected, [c] +: weak reaction; ++: intermediate reaction; +++: strong reaction

- The result of clustering analysis based on use/non-use of 95 substrates studied by the BIOLOG™ system. At a 58% similarity level, all Streptomyces isolates were grouped into two major groups (A and B). D-melezitose, D-melibiose, β-methyl-D-galactoside, 3-methyl glucose, α-methyl-D-glucoside, β-methyl-D-glucoside, α-methyl-D-mannoside, D-raffinose, salicin, 2'-deoxy adenosine and L-fucose were not utilized by any of the isolates of group A whereas D-fructose-6-phosphate and α-D-glucose-1-phosphate were not utilized by the isolates of group B. Only one carbon source, D-ribose, was used universally by all the isolates and sedoheptulosan was the only substrate not utilized by any isolate from both groups. The substrate usage patterns for the isolates tested revealed a broad variability. Isolate NBE13 from group A used the fewest substrates, 10/95, about 9.5% of the total number of substrates tested. Whereas two isolates NBE7 and NBE35 from group B used the most (78/95), about 78.94% of the substrates.
- Restriction digestion of 16S rRNA gene using three endonucleases (Dde I, Mbo I and Taq I) yielded 7 to 9 distinct restriction patterns for each enzyme. About 2 to 8 restricted fragments of varying sizes were common to each of the restriction patterns. Cluster analysis of combined 16S rRNA gene restriction pattern based on Jaccard's similarity index, grouped all the 55 isolates under 14 distinct groups with similarity percentage ranging from 17 -100% (Fig. 1). Majority of the isolates were under group I (43% of the total number), while the remaining 57% isolates shared rest of groups. ARDRA cluster I, III, VI and VIII had isolates from both WIF and EIF. ARDRA cluster II, IV, VII, X, XI, XIII and XIV had isolates only from EIF, where as cluster V, IX and XII had isolates only from WIF.

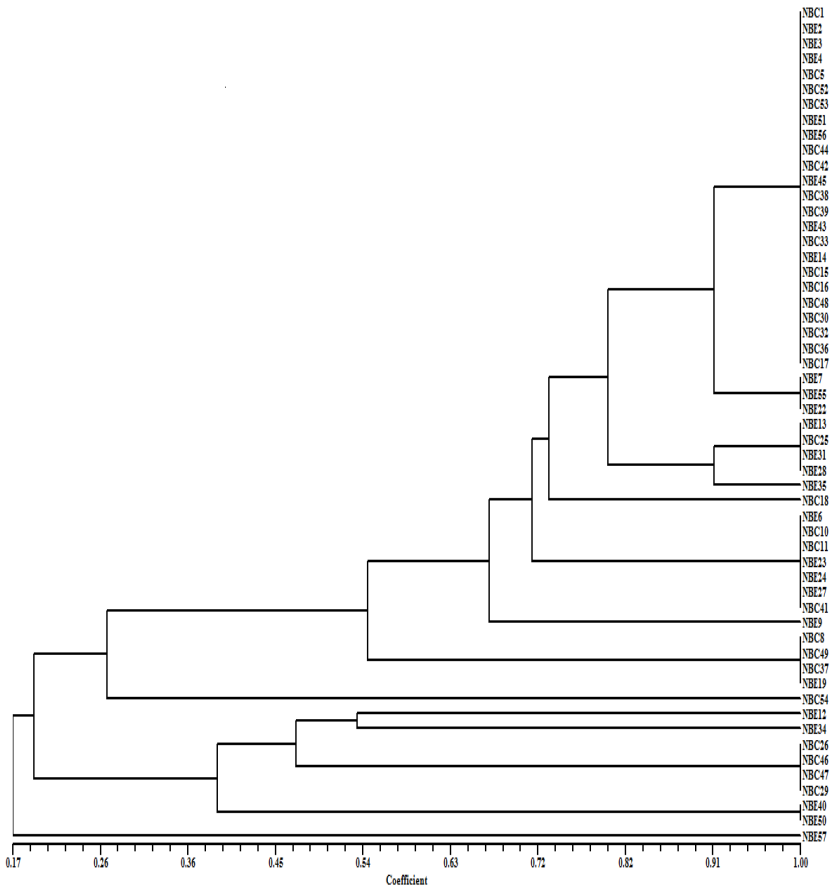


Fig. 1. Dendrogram with clustering of 55 isolates of *Streptomyces* from WIF and EIF generated from restriction of 1,500 bp 16S rRNA gene amplicon by three different restriction enzymes

- For sequence analysis, a total 14 isolates was chosen in such a way that each of the isolates represents a group with similar ARDRA pattern as generated by three different restriction enzymes. The representative isolates from all 14 clusters were identified as *S. variabilis*, *Streptomyces* spp. *S. glaucescens*, *Streptomyces* spp., *S. viridochromogenes*, *S. cinnabarinus*, *S. aburaviensis*, *S. viridis*, *Streptomyces* spp., *S. xylophagus*, *S. macrosporeus*, *S. thermocarboxydus*, *Streptomyces* spp., and *S. albogriseolus*.
- It is evident from different diversity index that EIF harboured high *Streptomycetes* diversity (H=2.098 and 1/D=6.259) relative to WIF (H=1.419 and 1/D=2.870).The evenness of the community structure was higher in EIF (E=0.874) compared to WIF (E=0.729) soils (Table 2).
- DNA was recovered from both WIF and EIF soil samples. *Streptomycetes* community fingerprints were generated from two replications of each of the sample, and reproducible DGGE profiles of 16S rRNA gene were obtained. The DGGE profiles were found to be very similar among both the soils (WIF and EIF). No difference (presence or absence of bands) could be observed visually when the two patterns were compared (Fig.2), indicating that the predominant unculturable *Streptomycetes* community found in these soils does not vary regarding the effluent treatment.

Table 2. Diversity index of *Streptomyces* isolates based on ARDRA profile and carbon-substrate pattern

	Diversity indices[a]		
Sites	**Shannon index of diversity (H')**	**Margalef index (R)**	**Pielou index (E)**
WIF	1.419	0.729	2.870
EIF	2.098	0.874	6.259

[a] The number of isolates showing similar ARDRA profile are grouped and used for estimating diversity indices.

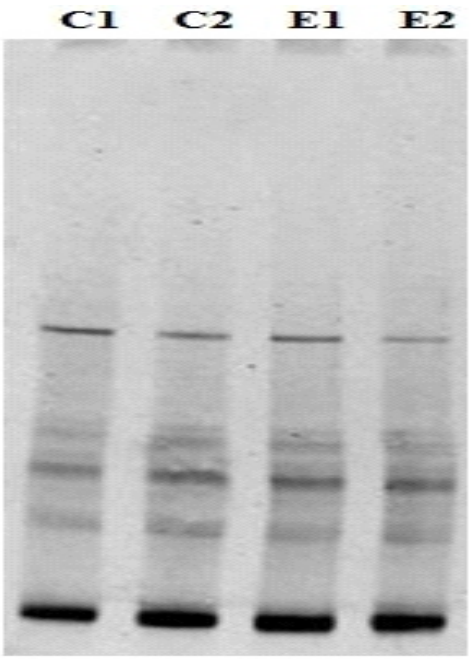


Fig. 2. DGGE fingerprints of *Streptomyces* specific 16S rRNA gene fragments amplified from soil DNA templates obtained from WIF and EIF: (lanes C1, C2) - WIF, (lanes E1, E2) – EIF

Conclusion

This study indicated that pulp and paper mill effluent contamination did not have a significant effect on the total genetic diversity of streptomycetes but affected physiological and metabolic status, so that the number of streptomycetes isolates capable of responding to laboratory culture and their taxonomic distribution were altered. Thus, it appears that plate counts may be a more appropriate method for determining the effect of effluent contamination on streptomycetes than culture-independent approaches. *Streptomyces* isolates in this study could be further exploited for commercial production of enzymes of biomass degradation as well as promoting plant growth in such soils.

Theme : Microbial Management of Abiotic Stress

9

Project: Development of microbial consortium for alleviation of salt and drought stress for growth and yield of wheat

PI : D. K Arora
SRF : Shweta Tiwari, Rameshwar Tiwari, Pratibha Singh

Rationale

Abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide. The inanimate components of the environment associated with climatic, edaphic and physiographic factors that substantially limit plant growth and survival. Categorically, there are a number of abiotic stresses such as extremes in temperature, drought, salinity, heavy metals and radiation which have detrimental effects on plant growth and yield. It has been claimed that abiotic stress causes the most intense crop losses in comparson to any other factor and most of the major crops are reduced in their yield by more than 50% from their potential yield. The project therefore addresses the application of microbial consortium for the alleviation of salt and drought stress in wheat crop.

Objectives

- Isolation of microorganisms from rhizotic zones of cereal crop (wheat) grown under salt stress.
- Selection of salt tolerant bacteria.
- Biochemical characterization of selected microorganisms.
- Evaluation of selected micro-organisms in the rhizosphere of cereal crop (wheat) (Green house studies).
- Development of consortium of microorganisms that can alleviate the effect of salinity and improve the growth and yield of cereal crop (wheat).
- Field evaluation of consortium of microorganisms for improvement of wheat growth and yield.
- Osmoprotectant studies on salt tolerant bacteria

Significant Achievements

- A total of 113, 57, 53 and 110 isolates were obtained from saline regions of Chilka Lake, Goa mangroves, drought affected areas of Rajasthan (Bikaner and Jaisalmer) and Kutch region of Gujarat, respectively.
- Sambhar salt lake isolates with PGP traits (IAA, Ammonia, ACC deaminase, Siderophore and P-Solubilization) and extracellular enzyme activity showed maximum tolerance (up to 20% NaCl) (Fig. 1).
- Evaluation of potent isolates (S-121 *Bacillus pumilus,* AS-40 *Pseudomonas mendocina,* AS-18 *Arthrobacter* spp., SL-11 *Nitroncola lacisaponensis,* SL-9 *Halomonas* spp.) in wheat showed better crop growth and stress tolerance crop responses at different salt concentrations (Green house studies).
- Rhizosphere extracts from plants bacterized with SL 11 and AS 121 showed maximum seed germination in comparison to control and other treatments (Fig. 2)
- *Nitrinicola lacisaponensis* (SL-11 isolate) showed the best result in amplification of osmoprotectant gene choline dehydrogenase.

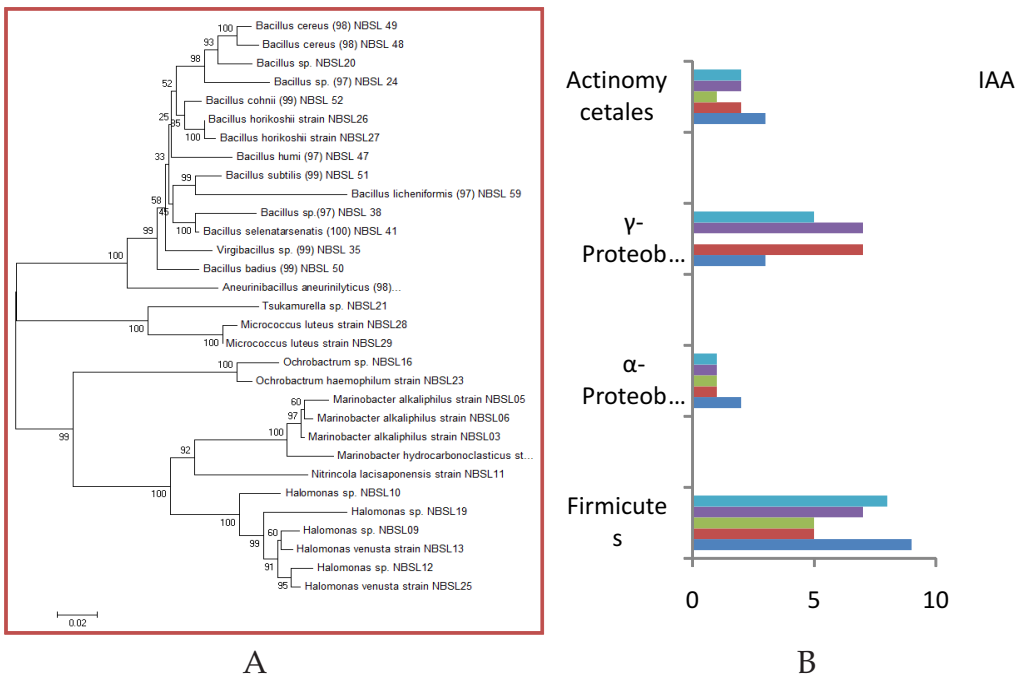


Fig. 1A. Neighbour-joining phylogenies constructed by MEGA for Sambhar salt lake isolates; **B.** PGP attributes of different isolates

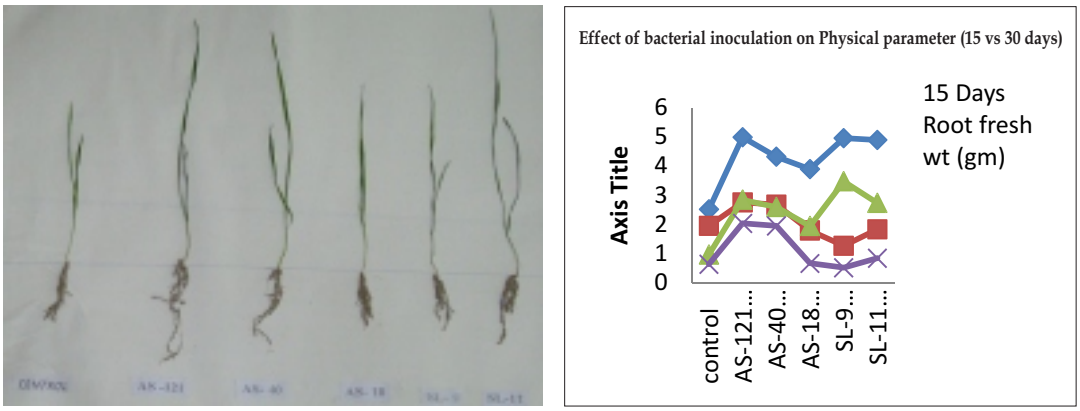


Fig. 2. Plant growth parameters of wheat inoculated with salt-tolerant bacteria

Conclusion

Salt tolerant microbes which can tolerate upto 15 -20% NaCl could be utilized to alleviate the effect of salt stress for growth and yield of wheat crop. Isolate AS-121 *Bacillus pumilus* and SL 11 *Nitrinicola lacisaponensis* has a great potential as it possess all the attributes of physical, biochemical and PGP traits

Utilization of Actinomycetes to Alleviate Salt Stress for Cereal Crops

PI : Mahesh Yandigeri
Co-PI : Kamlesh Kumar Meena
SRF : Manish Roy, Divya Singh

Rationale:

Agricultural productivity is severely affected by soil salinity because salt levels that are harmful to plant growth affect large terrestrial areas of the world. The damaging effects of salt accumulation in agricultural soils have influenced ancient and modern civilizations. It is estimated that 20% of the irrigated land in the world is presently affected by salinity. In India about 10 million ha of arable land is salt affected and approximately 68% is affected with drought. Increased salinity and drought in soil is harmful to both microbes and crops. Microbes have been implicated in alleviation of effects of abiotic stresses by various mechanisms like production of osmolytes, sugars, sugar alcohols, exopolysaccharides etc. Such microorganisms not only alter the environment around the rhizosphere of crops but also maintain the ratio of various nutrients.

Actinomycetes are found in neutral to saline soils. Most of actinomycetes are tolerant to alkaline conditions and in alkaline soils, 95% population may be actinomycetes. Most of the actinomycetes possess inherent capacity to tolerate salt stress (especially *Streptomycetes* genera, *Nocardiopsis* sp., *Saccharomonospora* sp.) by synthesis of the compatible solutes like alanine, proline, glycine betaine and -glutamine in response to stresses. It is also known that actinomycetes are known produce antibiotics and secondary metabolites of importance. They are known to inhibit many plant pathogens and some are known to produce plant growth promoting substances. Thus, keeping these points in consideration, an attempt was made to utilize actinomycetes to alleviate the salt and drought stresses and increase the crop yields under salt and drought affected soils.

Objectives:

1. Isolation and screening of actinomycetes from different salt and drought affected area of India for salt tolerance.
2. Characterization of the isolates for the accumulation of sugars, sugar alcohols, amino acids and other osmolytes.
3. Evaluation of the actinomycetes isolates under pot/field experiments and study of plant microbial interactions during salt stress.
4. Development of consortia of actinomycetes cultures to alleviate the salt stress for wheat and other millets.

Significant Achievements:

Soil sampling was performed from salt affected regions of Eastern Uttar Pradesh covering Mau Nath Bhanjan (25°56' N, 83°33'E) and Kanpur district belt (26°53' N, 79°37' E) (Kanpur, Auriya, Fatehpur and Mainpuri). 33 soil samples were collected through visual interpretation and with systemic and random sampling techniques from 0-15 cm deep rhizospheric soil of wheat harvested arable land. In-situ pH measurement was performed to obtained accurate pH measurement. Soil samples were analysed for pH, electrical conductivity and organic carbon (%). Electrical conductivity of soil samples ranged from 0.53 to 13.33 dS/m, pH in the range of 7.2 to 11.8 and organic carbon (%) from 0.075 to 11.55. All 33 soil sample analyzed for the physiochemical attributes with respect to the available organic carbon content deliberated the available organic carbon in the presence of saline condition.

Screening for salt tolerance:

Actinomycetes isolated from Uttar Pradesh have been subjected to screening for salinity tolerance using different salts with increasing concentrations. Actinomycetes isolated from the Uttar Pradesh (Mau and Kanpur districts) saline soils were allowed to grow on the glucose yeast extract and malt extract broth and medium amended with $MgSO_4$, KCl, KNO_3 and KH_2PO_4 from 0 to 10 percent of each salt. Actinomycetes were inoculated directly on plates. Further actinomycetes were also raised in broth for 4 days and further plated on solid medium at same concentration of salt.

Among 88 isolates, a total of five isolates recorded growth on 16% NaCl concentration (Fig. 1), 35 isolates at 10% KCl and 49 isolates at 10% $MgSO_4$ (Fig. 2). Remarkably none of the isolates from saline soils of Uttar Pradesh had shown growth on K_2HPO_4 and KNO_3 at any concentrations. The promising isolates of

actinomycetes showing tolerance to salt at high concentration will be further evaluated for osmolyte(s) production under salt stress.

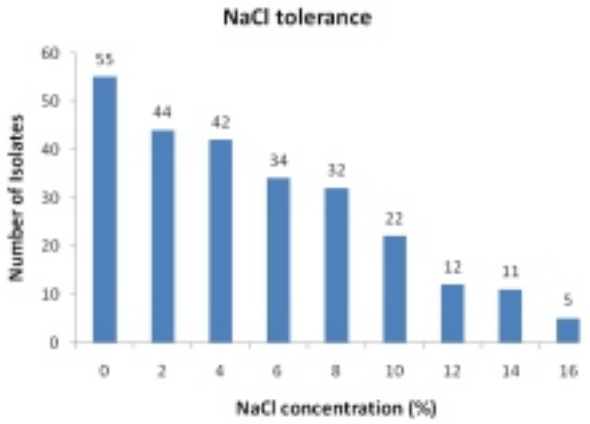


Fig. 1 Graph showing the NaCl tolerance by Actinomycetes Isolated from Kanpur Region of Uttar Pradesh

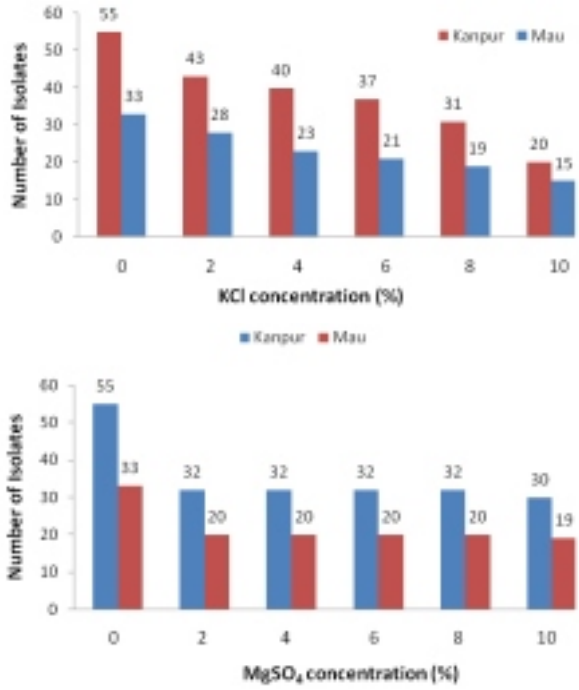


Fig. 2 Graph showing the KCl and $MgSO_4$ tolerance of Actinomycetes isolated from Kanpur and Mau Region (U.P.)

Exploratory survey and soil sampling survey was carried out for the salt affected regions of Bikaner, Jaisalmer districts of Rajasthan for the isolation of salt tolerant actinomycetes. Based on the visual symptoms of soil the soil samples were collected 0-15cm deep from rhizospheric, non rhizospheric zone and surface of the soils. The pH of the soil samples was measured by diluting the soil samples with double distilled water in the ratio of 1:1. pH of soil samples ranged from 7.5 to 9.5. The soil samples were used directly or with different enrichment techniques followed by serial dilution and plating for the isolation of diverse genera of actinomycetes. The enrichment techniques included heat treatment at 80°C for 15 min, Phenol (1.5%) treatment for 30 min, SDS(0.05%) for 30 min, $CaCO_3$(0.1%) for 5 days. Serially diluted enriched soil samples were plated on different media (Actinomycetes isolation agar, Starch casein agar, humic acid vitamin agar, ISP 3 and ISP 4). After 7 days of incubation, no significant actinomycetes colonial growth was observed on ISP 3, ISP 4 media and heat treated soils. A total of 107 morphotypes of actinomycetes were isolated using different media and enrichment methods. The colony forming unit was observed to be in the range of 0 to 70 X 10^3. The soil samples which were directly plated without any enrichment did show the growth of actinomycetes, the colonies on the plates was difficult to enumerate due to the bacterial and fungal contaminants.

Isolation and screening of actinomycetes for drought tolerance:

Exploratory soil sampling survey was carried out in the drought affected regions of Bikaner and Jaisalmer districts of Rajasthan for the isolation of drought tolerant actinomycetes. A total of 24 soil samples and 22 roots samples were collected. The plant root samples were surface sterilized then crushed with 1 ml distilled water followed by serial dilution and plating for the isolation of diverse genera of endophytic actinomycetes. Serially diluted sterilized root samples were plated on different media namely actinomycetes isolation agar, starch casein agar, humic acid vitamin agar, ISP 3 and ISP 4. After 7 days of incubation, no significant actinomycetes colonial growth was observed on ISP 3, ISP 4 media. The soil samples were used directly or with different enrichment techniques followed by serial dilution and plating for the isolation of diverse genera of actinomycetes. The enrichment techniques included heat treatment at (80°C for 2 h, 120°C for 1 h 110°C for 1 h and 40°C and 85% RH for overnight), Phenol (1.5%) treatment for 30 min, SDS (0.05%) for 30 min and $CaCO_3$ (0.1%) for 5 days. A total of 45 morphotypes of endophytic and 97 rhizospheric actinomycetes were isolated using different media and enrichment methods. The colony forming unit

was observed to be in the range of 0.1 to 9 $X10^3$. The soil samples which were directly plated without any enrichment did show the growth of actinomycetes, the colonies on the plates was difficult to enumerate due to the bacterial and fungal growth. Morphological characterization of endophytic and rhizospheric actinomycetes was carried out based on colour of aerial and substrate mycelia, pigmentation on media and acid-fast staining. Screening for drought tolerance was carried out using poly ethylene glycol 6000 (PEG 6000). Out of 45 isolates, screening for 27 isolate has been completed using PEG 6000 (Fig. 3). Growth kinetic analysis of endophytic actinomycetes isolates at varying concentration of PEG is under process. Among 45 endophytic actinomycetes, 27 isolates were screened up to 25% PEG (ranging from 5-25%). Two isolate were able to tolerate up to 15% PEG stress, whereas 7 isolates were found to be drought sensitive even at very low concentration of PEG 6000 (5%). These resistant isolates will be further screened and tested for the production of secondary metabolites and biomolecules including the osmolytes produced under stressed conditions.

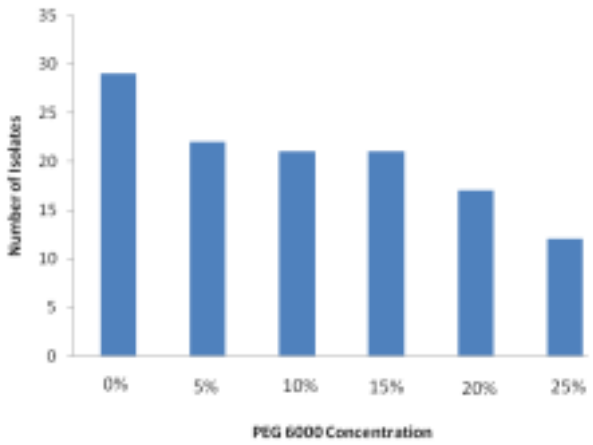


Fig. 3 Graph showing the number of drought tolerant isolates screened with PEG 6000

Conclusions:

The determination of the soil physiochemical properties reveals that the experimental regions were majorly affected by the salts due to the accumulated salts at the surface and the subsurface. The increased level of soil pH, Potassium, nitrogen and Electrical conductivity also refers that the soil were highly affected by salt accumulation.

Among the various enrichment methods used for isolation of actinomycetes $CaCO_3$ treatment was found better in comparison to the SDS and phenol treatments, whereas heat treatment and the media like ISP3 and ISP4 did not yield good results. Also for screening of actinomycetes for salt tolerance, Sodium chloride, Potassium chloride and Magnesium sulphate salts yielded better results. It shows the passive transportation throughout the cell wall majorly induced by sulphate and chloride ion concentration in spite of phosphate and nitrate ions. Few of the isolates had shown growth on all three above salts, indicating the isolates are highly salt stress tolerant. In future these isolates will be tested for the production of secondary metabolites and biomolecules including the osmolytes produced under stressed conditions. Isolates which can perform better will be utilized further for the development of a consortium that can be used to alleviate salt stress under saline soils. The drought resistant endophytes obtained from the study will be utilized for field experiment and tested for alleviation of drought stress among the cereals and millets.

Theme : Microbial Genomics

10

Project: Complete Genome Sequencing of *Mesorhizobium ciceri* Ca 181

PI : D. K. Arora
Co-PI : Rajeev Kaushik, Alok Kumar Srivastava
SRF : Ram Nageena Singh, Anu Sharma, Preeti Shahi, Raghvendra Pratap Singh

Rationale

The genomic revolution of the 1990s has yielded almost a thousand sequenced microbial genomes. More recently, the explosion of random community genomics, or metagenomics, where DNA is sequenced directly from environmental samples has provided insights into microbial communities. In the traditional approach, DNA is cloned into BACs, or small plasmids, and dideoxy chain termination sequencing ("Sanger sequencing") is used to determine the sequences. In the alternative approach, DNA is sequenced without cloning, using one of the so-called next-generation sequencing techniques, usually pyrosequencing. Both approaches have advantages and disadvantages. Pyrosequencing has much higher throughput and a lower error rate per base sequenced compared to Sanger sequencing, but those errors are biased toward certain mistakes.

Mesorhizobium ciceri ca181 was selected for whole genome sequencing, as it is a nodule forming chick pea rhizobia with very high specific qualities like, efficient nitrogen fixation and shows good nodulation competitiveness and performed well at different locations in different agro-climatic regions and soil types in All India Coordinated trials. The whole genome sequencing of this bacterium will unveils the specific properties of it which is encoded by genes that works in the coordinated form of specific metabolic pathways. After the completion of gene prediction and annotation, we will understand the reason of uniqueness of this bacterium, this will come in the form of new genes, operons and proteins.

Objective:

- Complete Genome sequencing of *Mesorhizobium ciceri* Ca181

Significant Achievements:

- Genome Sequencing of *M. ciceri* ca181 was done by Next Generation 454 pyrosequencing technology (Fig. 1). About 849 million base pairs sequence data was generated in the form of 2684068 (2.68 million) sequence reads. This huge sequence data was assembled through De novo Genome Assembler (Newbler Assembler) software. Total of 64 contigs were formed having average length of 112361 (0.11Mb) base pairs. Largest contig is having 577250 (0.57 Mb) base pairs. Complete assembled genome is about 6.7 MB with (about 8.0 Mb assumed) estimated genome size, and 70X coverage depth.

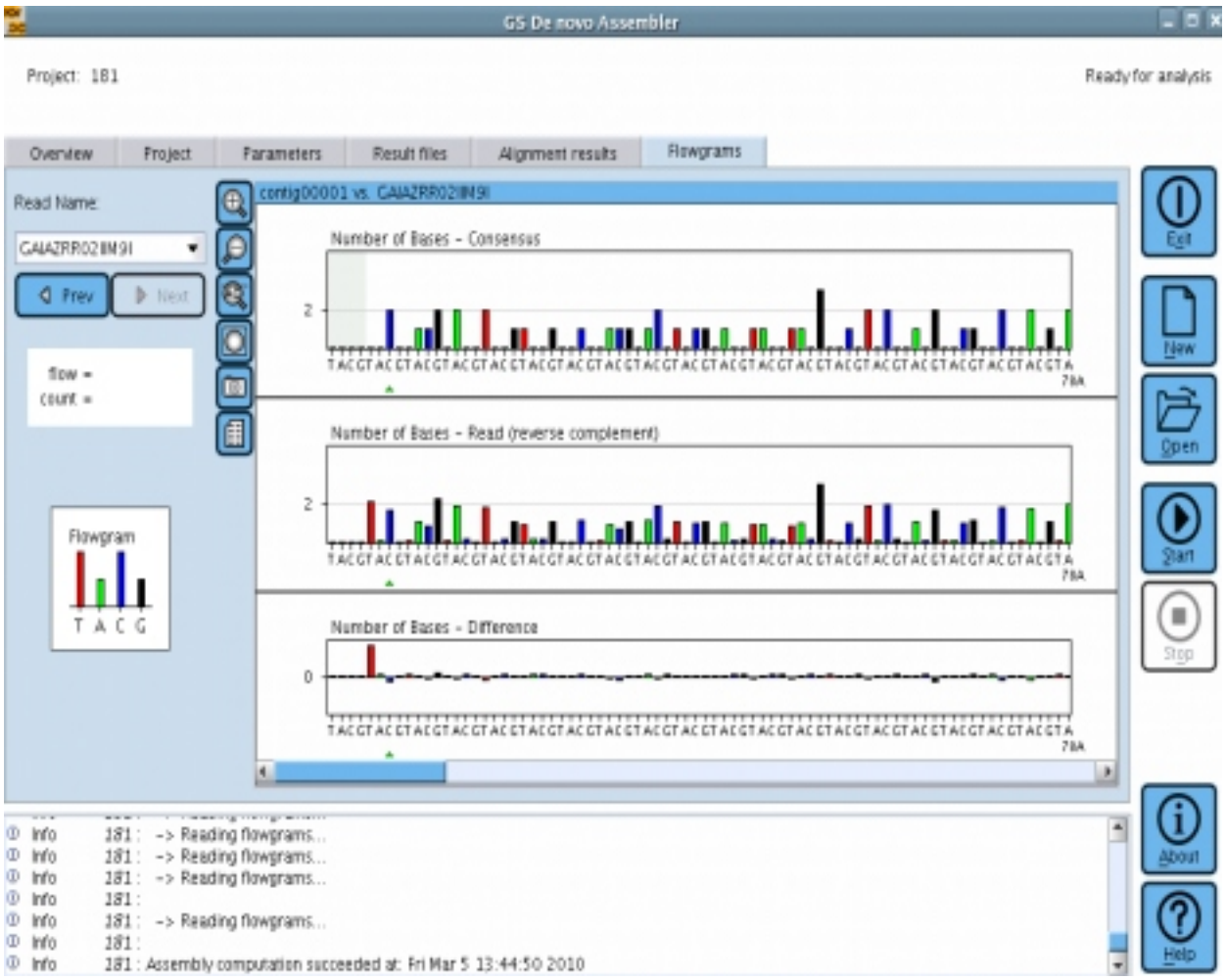


Fig. 1. Consensus read of the sequence from 454 Pyrosequencing

Summary: Genome Assembly

Number of bases	**849829976 (849.8 Mb)**
Number of Aligned Bases	**841891311 (841.8 Mb)**
Number of reads used in the assembly	**2684065 (2.68 Million)**
Number of reads assembled	**2631323 (2.63 Million)**
Total number of Contigs formed	**64**
Total number of large contig	**57**
Largest Contig Size	**577250 (0.5 Mb)**
Average contig size	**112361 (0.11Mb)**
Expected Genome Size	**6.7 MB**
Coverage depth	**70X**

- There are some online analysis software tools available for the prediction of genes and operons present in the sequence contigs. These softwares worked on specific algorithms to search the genes between start codon and stop codon. All the Assembled contigs were used for the gene prediction and operon search against available database using *FGENESH-B* online bacterial gene prediction software (developed for the gene prediction in *M. loti*) (Fig. 2).

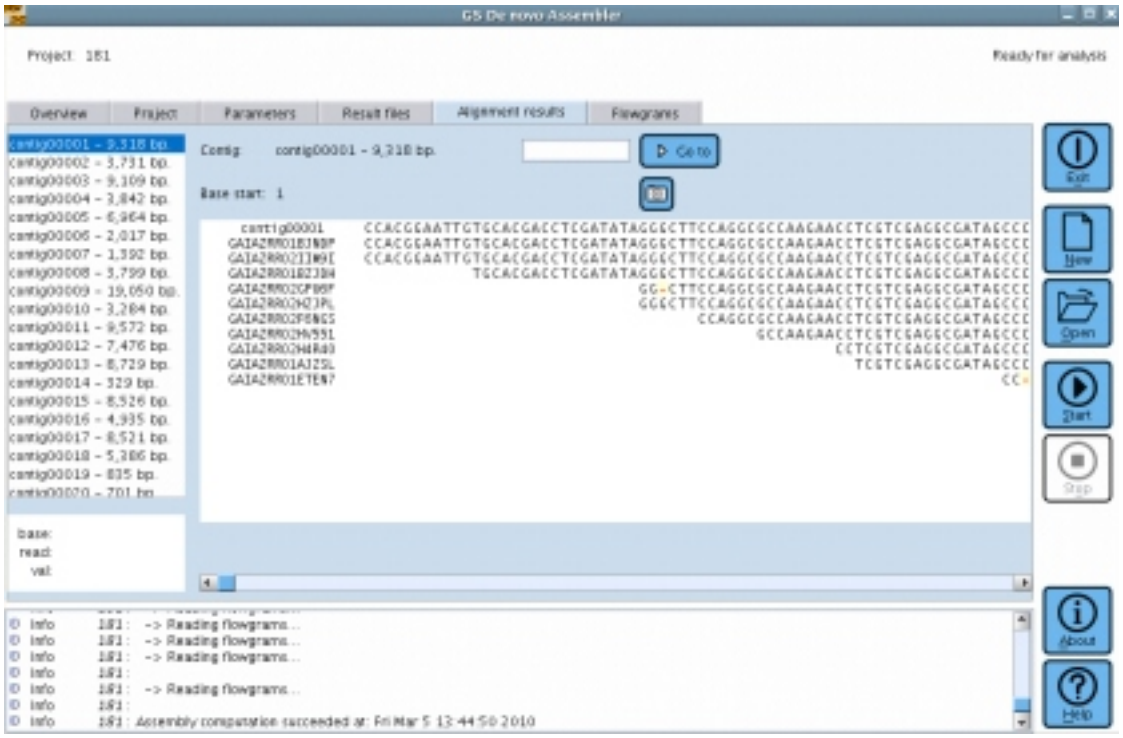


Fig. 2. Contigs formed after the De novo Assembly from GS De novo assembler

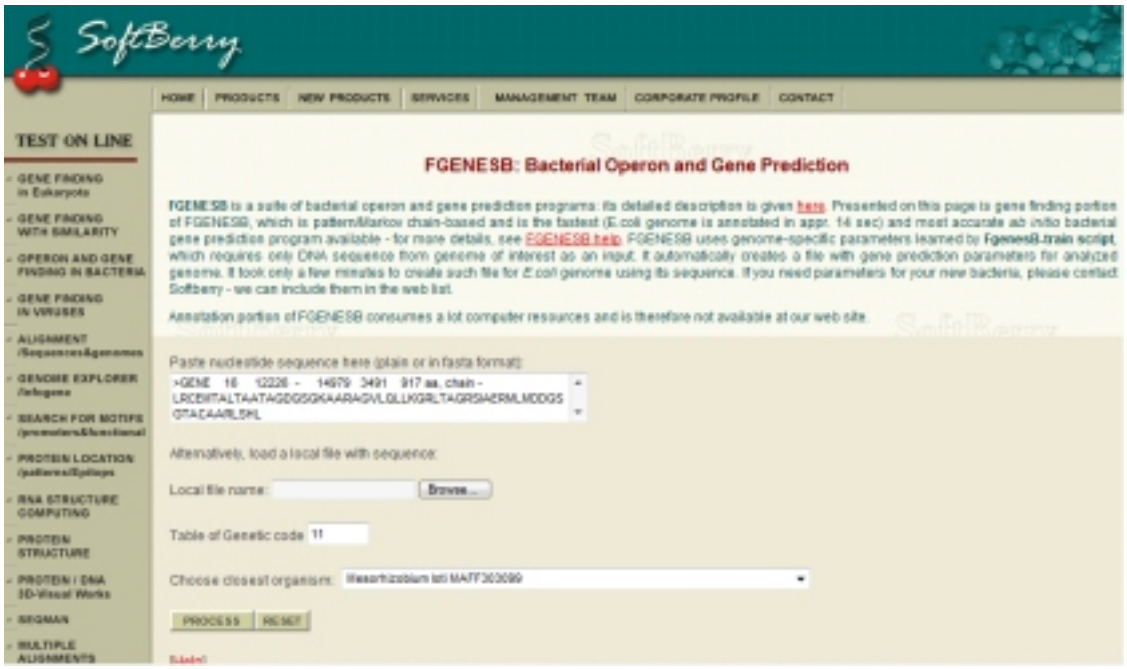


Fig. 3a. Prediction of Genes through FGENES-B online program

- A total of 6461 genes have been predicted out of 64 contigs used for genes and operon prediction through *FGENESH-B*. (Gene prediction program specific for gene prediction in bacterial genome) (Fig 3a).
- Gene annotation is the categorization of predicted genes which involves search of functional domains and super-family of particular gene and protein.
- 350/6461 genes were blasted against NR Protein database of NCBI installed on local server for functional attributes.
- BLASTP program were used with customized parameters.
- Blast output result was tabulated on the basis of (% Identity: more than 30 %).

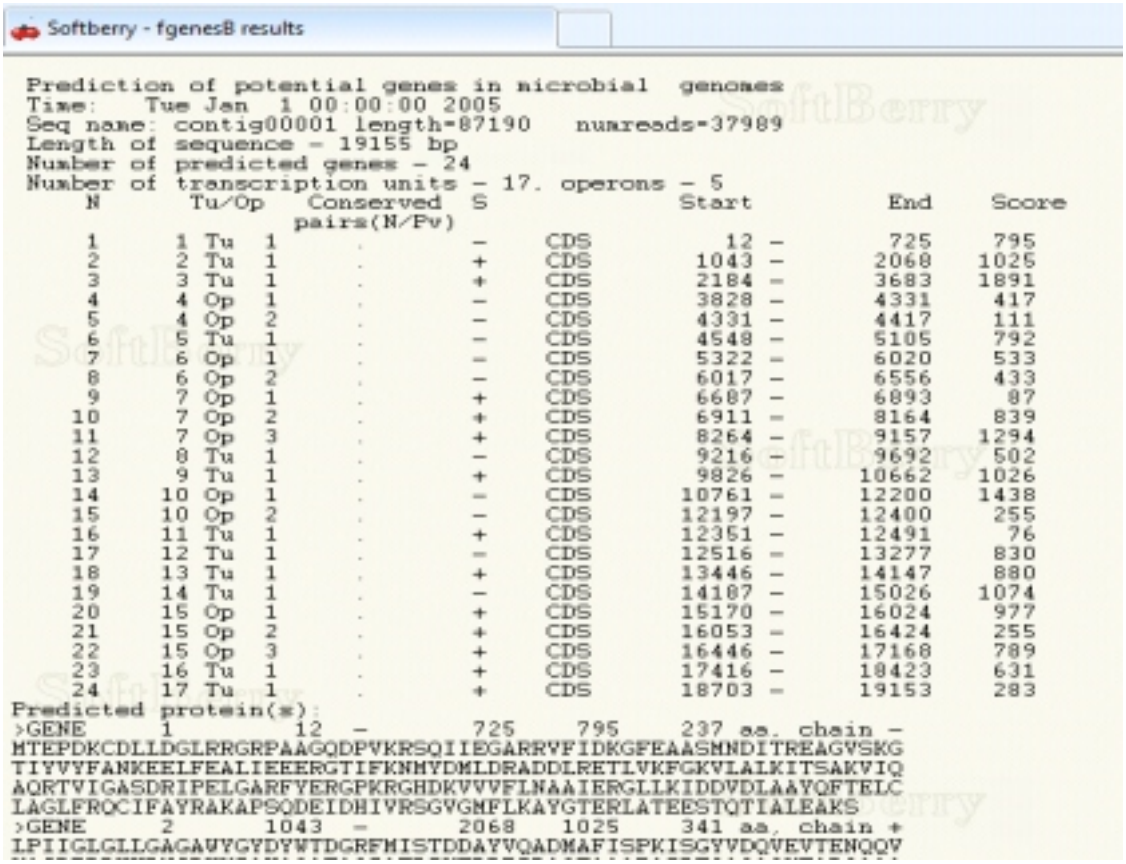


Fig. 3b. Prediction of Genes through FGENES-B online program

Summary: Gene Annotation

Contigs used for gene prediction	64
Number of genes predicted	6461
Total number of transcriptional units	4129
Total number of Operons	1512
Genes used for Protein prediction	350

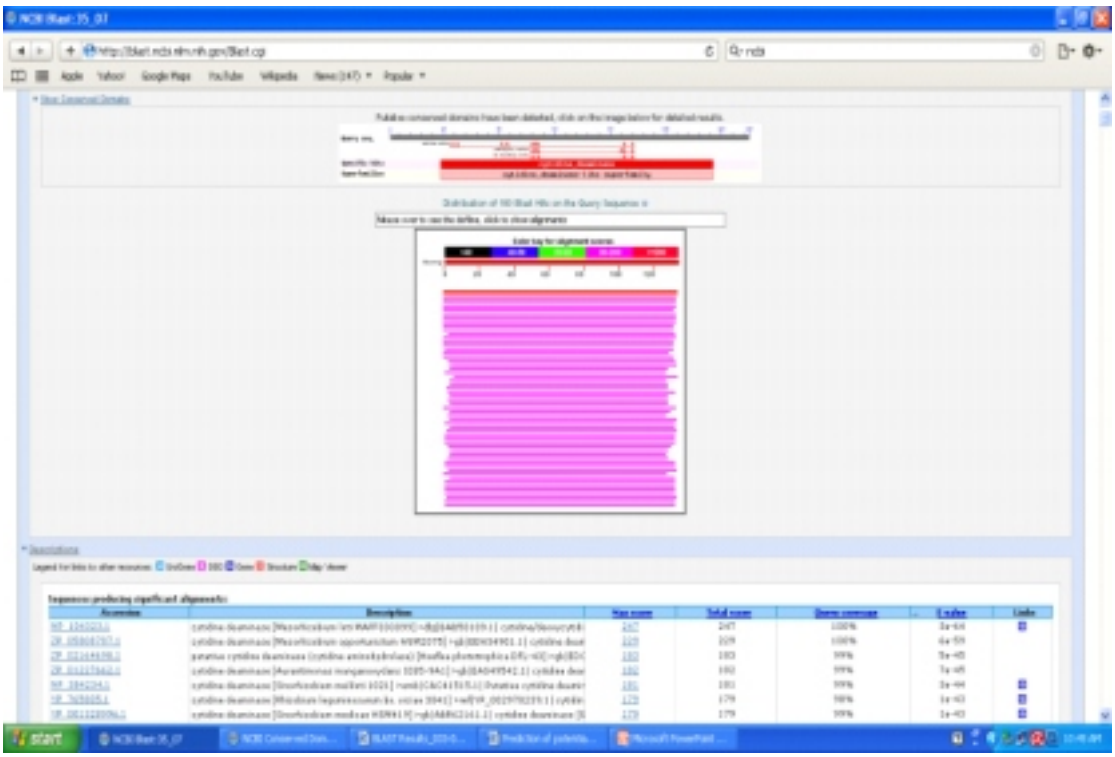


Fig. 4. Annotation of genes and result of BLASTP

Sequencing of Mega Plasmids:

They are extra chromosomal molecules present besides the chromosome in many bacterial sp. Some of them have high molecular weight from 90 to 1600 kb. In *Rhizobia* sp. they play very important role in host specificity and other biological function. 2000 short plasmid (0.5- 2.0 kb inserts) clones have been generated by shearing the sym Plasmid. Sequencing of 0.5 to 2.0 kb inserts was done by shotgun method (Sanger method). 1344 reads have been generated from the sequencing of 672 clones with end to end sequencing (Fig 5a, 5b).

Fig. 5a. BLAST similarity result of Mega Plasmid in *M. ciceri* ca181

Total number of sequenced clones	**672**
Total number of reads (Forward & reverse)	**1344**
Total number of contigs	**150**
Number of genes predicted	**150**
Remaining reads for contigs	**960**
Remaining clones for sequencing	**288**

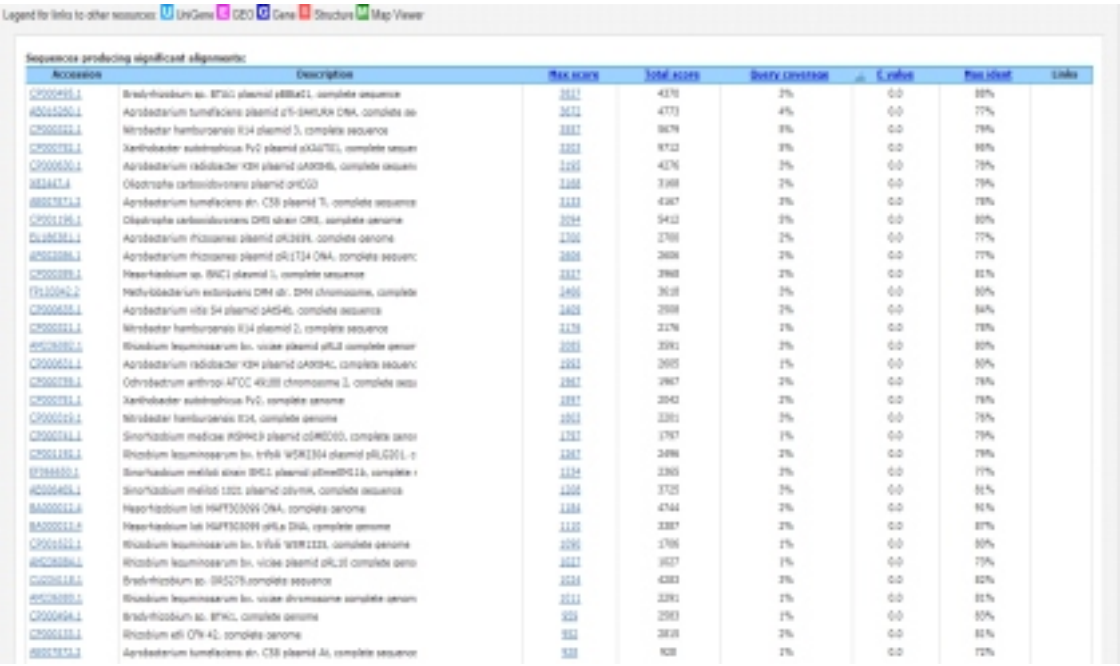


Fig. 5b. BLAST similarity result of Mega Plasmid in *M. ciceri* ca181

After assembly, the genome was search for the similarity in genome database available at NCBI Genome browser. It appeared that *M. ciceri* ca181 was 35% similar from its closest organism *M. loti* (Fig 6).

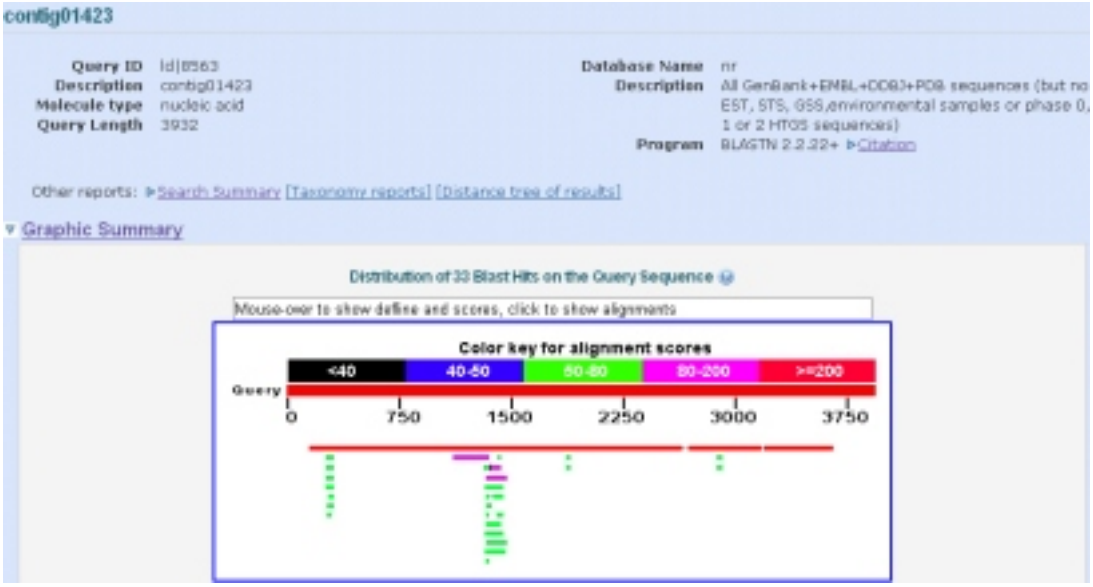
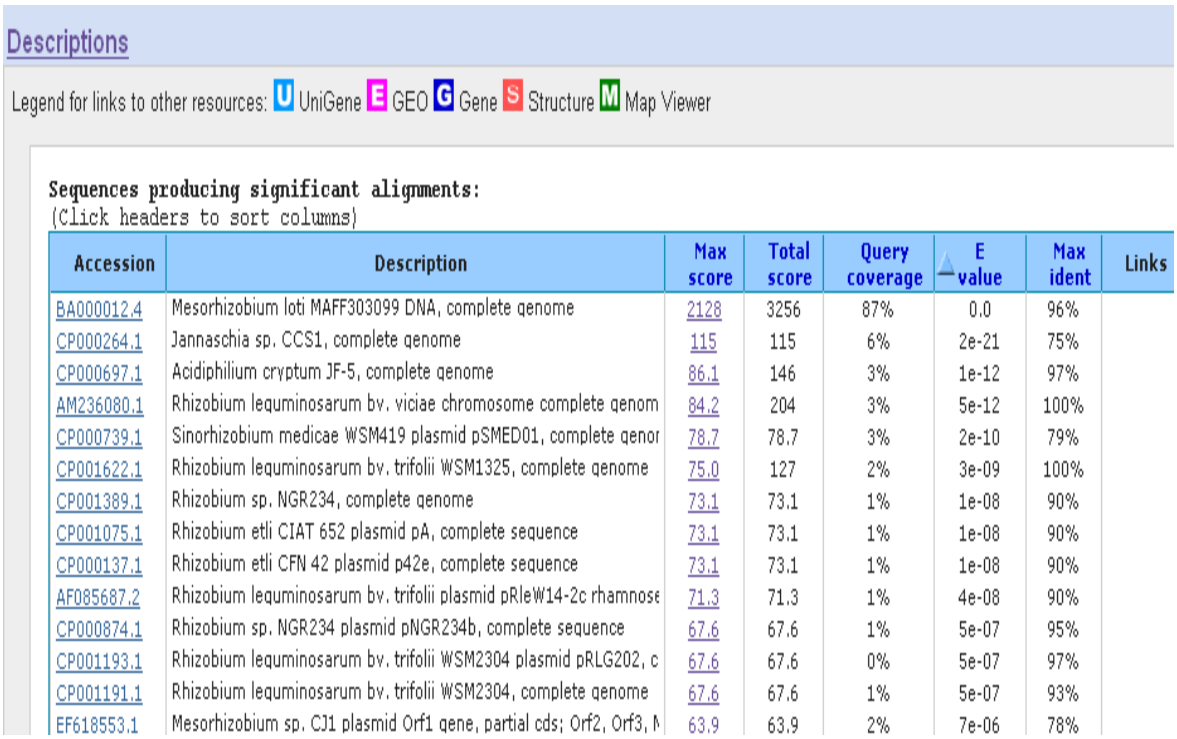


Fig. 6a. Blast Similarity result of contigs



Descriptions

Legend for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
BA000012.4	Mesorhizobium loti MAFF303099 DNA, complete genome	2128	3256	87%	0.0	96%	
CP000264.1	Jannaschia sp. CCS1, complete genome	115	115	6%	2e-21	75%	
CP000697.1	Acidiphilium cryptum JF-5, complete genome	86.1	146	3%	1e-12	97%	
AM236080.1	Rhizobium leguminosarum bv. viciae chromosome complete genom	84.2	204	3%	5e-12	100%	
CP000739.1	Sinorhizobium medicae WSM419 plasmid pSMED01, complete genor	78.7	78.7	3%	2e-10	79%	
CP001622.1	Rhizobium leguminosarum bv. trifolii WSM1325, complete genome	75.0	127	2%	3e-09	100%	
CP001389.1	Rhizobium sp. NGR234, complete genome	73.1	73.1	1%	1e-08	90%	
CP001075.1	Rhizobium etli CIAT 652 plasmid pA, complete sequence	73.1	73.1	1%	1e-08	90%	
CP000137.1	Rhizobium etli CFN 42 plasmid p42e, complete sequence	73.1	73.1	1%	1e-08	90%	
AF085687.2	Rhizobium leguminosarum bv. trifolii plasmid pRleW14-2c rhamnose	71.3	71.3	1%	4e-08	90%	
CP000874.1	Rhizobium sp. NGR234 plasmid pNGR234b, complete sequence	67.6	67.6	1%	5e-07	95%	
CP001193.1	Rhizobium leguminosarum bv. trifolii WSM2304 plasmid pRLG202, c	67.6	67.6	0%	5e-07	97%	
CP001191.1	Rhizobium leguminosarum bv. trifolii WSM2304, complete genome	67.6	67.6	1%	5e-07	93%	
EF618553.1	Mesorhizobium sp. CJ1 plasmid Orf1 gene, partial cds; Orf2, Orf3, M	63.9	63.9	2%	7e-06	78%	

Fig. 6b. Similarity Blast result of contigs

Conclusion:

These results show potential that after complete analysis of the genome, it will give some new and unique genes and processes involved in the specificity of this organism. The study is incomplete to draw final conclusion. However, the identification of few sequences with unknown function could be interesting to further work on because it does not have any match in the database.

Project: Microbial Genomic Resource Repository

PI : D. K. Arora
Co-PI : Rajeev Kaushik, Alok K Srivastava, Sudheer Kumar
RA : Ram Nageena Singh, Sukumar Mesapogu, Manoj Kumar Singh
RSRF : Madhusudan Panchal, Neha Srivastava, Achala Bakshi

Rationale

Many research institute/universities all over the world are carrying out microbiological and biotechnological research. These are generating a lot of biological material/data such as DNA, RNA, vectors, plasmids, genes and promoters, etc. from their ongoing research. It is routine to keep these material, constructed after a particular research project has been finished, because they constitute a valuable reserve for research and are a way to continue capitalizing on the investment (time and funds) already made to generate knowledge or products. In addition to the sequence information, projects associated with Whole Genome Analysis generally produce biological materials i.e. cDNA clones, RFLP markers on the genetic map, cosmid clones, BAC (Bacterial Artificial Chromosome) clones, YAC (Yeast Artificial Chromosome) clones etc. These are the key of many future post-genomic researches, such as physiological or morphological characterization of a species, functional analysis of genes and comparative genomics, etc. Unavailability or loss of genetic material often makes it difficult for other scientists to reproduce and expand research program based on the past studies, like as "to reinvent the wheel". So, it is necessary to maintain an efficient

system for conservation and management of DNA materials and further supply of even the protected gene constructs within the IPR framework to the researchers. These facilities will be provided by Microbial Genomic Resource Repository (MGRR), which is a division of National Bureau of Agriculturally Important Microorganisms (NBAIM) as a stock centre to ensure availability of the protected microbiological and biotechnological resources for exploitation. Conservation of agriculturally important microorganisms came from bacteria, fungi, actinomycetes, blue green algae, including vesicular arbuscular mycorrhizae and viruses is carrying out through short term and long term preservation methods. DNA banks are established in many countries around the world having a variety of purposes. These range from small, specialized collections that have the genetic material only from individual varieties support only to a small group of researchers and bio-industries. There are a few DNA banks which conserve the genetic material of microorganisms, otherwise most of these DNA banks relate to plants, seeds, arthropods and animals. Moreover, there is no DNA bank in India itself till now for conservation and preservation of genetic material of microorganisms.

Microbial Genomic Resource Repository is not only the first microbial DNA bank in India but also in South East Asia. The MGRR DNA Bank is a non-profit organization whose activities are supported by Indian Council of Agricultural Research (ICAR), New Delhi. This centre aims to collect genetic resources, individuals and genetic information necessary for promoting researches, as well as developing novel resources and related technology and disseminating bioresources to domestic and international researches. Numerous microbial DNA, clones and vectors are deposited in the DNA Bank for long term preservation, isolated from NBAIM National Microbial Culture Collection. MGRR DNA Bank has an on-going concern with all aspects of genetic material collection activity and, in particular, with the encouragement of new initiatives and improvement of the quality standards of scientific services provided to the international user community. As DNA Bank administrators, we should consider improving the technology for quality control in supplying genetic resources. Research innovation in this field occurs a very fast pace and it is often just to keep up with it. So, all genetic material are quantified before the preservation and routinely tested for the contamination. The increasing demands on culture collections for authenticated, reliable biological material and associated information have paralleled the growth of biotechnology. Beside the preservation of microbial genetic material, MGRR is engaged in other microbiological and biotechnological research also to increase its genetic stock. Researchers can submit their microbial genetic material at MGRR DNA Bank and can also request to obtain for the genetic material in which they are interested. MGRR DNA Bank has developed its guidelines for submission and distribution of the genetic resources. MGRR DNA Bank has also set the standard for quality management and also covers biosecurity, building capacity, preservation of biological resources and data management. These guidelines have been updated to include recent developments and changes to provide basic quality management guidance for DNA Banks. To request for genetic material from MGRR DNA Bank, they need to prepare request-documents and the indicated material transfer agreement (MTA).

Mandate:

- To coordinate assemblage, conservation, quality control and validation of the microbial genomic resources to facilitate their optimal exploitation and utilization
- To act as a single window system for import and exchange of microbial genomic resources and facilitate protection of related IPR issues
- To conduct and promote basic, strategic, applied and anticipatory research for development and management of microbial genomic resources

Objectives:

- Nationwide Survey and Collection of Information about the Microbial Genetic Resources.
- Development of linkages between different research institutions, Universities, and individual researchers for obtaining microbial culture and genetic material
- Development of infrastructure facilities for the preservation and maintenance of Genetic Resource
- Technology and Protocols development for isolation and long-term preservation of the Microbial Genetic Resources
- Development of Databases or Information Bank

for Microbial Genomic Resources and linkages with other DNA Bank

- Collection of environmental Microbial Samples from different Agro climatic Regions
- Technology and protocols development for collection/transportation of microbial samples
- Exploration of non-culturable microorganisms and direct DNA isolation from environmental samples
- Documentation and Electronic Cataloguing of Microbial Genetic Resources
- Development and Implementation of Genome Projects to explore non culturable microorganisms

Significant Achievements

- Long term preservation and maintenance of genetic material (e.g. DNA, RNA, genes, clones, vectors, primers, probes, whole Genome, cDNA Libraries, etc.) related to Agriculturally Important Microorganisms is going on at the Bureau.
- Presently a total of 801 genomic DNA of which 350 Bacteria, 304 Fungi, 65 Rhizobium, 55 Actinomycetes and 27 cyanobacteria cultures has been preserved in 05 replicates with a standard concentration of genomic (50µg/µl) DNA in TE buffer at 4°C and -20°C.
- Various T-vectors, markers assisted vectors like pGFP, pUC^{18}, pUC^{19}, THα-5, T-vectors are preserved in glycerol stocks.
- Competent cells like JM109, DH5α, TOP-10F' are preserved at -80°C.
- Various universal primers for prokaryotes and eukaryotes, species specific real time primers and probes for *M. phaseolina* and *Fusarium udum*, universal sequencing primers, functional gene primers like *Nif*, Ammonia oxidizing, Mxa-methylotrophic, etc. are collected.
- 203 Various gene sequences from the In-house projects of NBAIM were collected.
- Apart from the preservation of Microbial Genetic Material, MGRR is developing databases of valuable microbial genetic information.
- 139 metagenomic clones of paper mill effluents from agriculturally irrigated lands were preserved.
- 306 letters are dispatched to the researchers of different Indian Universities and research institutes for deposition of microbial genetic material in MGRR DNA Bank.
- MGRR DNA Bank has generated a well equipped word class laboratory with all modern sophisticated equipments and instruments such as Robotic DNA Extractor, Pyrosequencer, Molecular Imager Gel Doc XR, Chemiluminiscence Gel Imaging System, DNA Fluorimeter, Electroporator, Fraction Collector, Gene Analyzer, Gradient Thermal Cycler, High Throughput Gel Electrophoresis System, Pulse Field Gel Electrophoresis, Ultra Centrifuge, Incubator Shaker, Growth Kinetics Analyzer, etc.

Conclusion

MGRR has got good achievements within short time duration by preserving a large number of microbial genetic materials. In future, this MGRR DNA Bank will play an important role in the area of microbiological and biotechnological research for the research community.

- MGRR is the first Microbial DNA Bank not only in India but also in South East Asia.
- A total of 801 genomic DNA of which: 350 Bacteria, 304 Fungi, 65 Rhizobium, 55 Actinomycetes, 27 Cyanobacteria have been preserved.
- 139 metagenomic clones of Lalkuan paper mill effluents from agriculturally irrigated lands are preserved.
- 203 gene sequences have been preserved from in-house projects of NBAIM.
- Various T-vectors, markers assisted vectors like pGFT, pUC^{18}, pUC^{19}, THα-5 are preserved in glycerol stocks.

Project: Diversity analysis of *Bacillus* and other predominant genera in extreme environments and its utilization in agriculture

Consortium Leader	:	D. K. Arora
Consortium PI	:	Rajeev Kaushik
Coordinating Scientists	:	Alok K Srivastava, Sudheer Kumar
RA	:	Anjney Sharma
SRF	:	Alok Ramkesh Rai

Rationale

Species of *Bacillus* and *Bacillus* derived genera (BBDG) are employed in industry as a source of enzymes, in agriculture as inoculants (PGPR) and biocontrol agents (BCA). They are also implicated in bioremediation and the insecticidal property of *Bacillus thuringiensis* has been exploited largely. In India, there is no baseline information available on the species richness and thus its utilization is not understood. *Bacillus* species predominate in most of the environments and are used as plant growth promoting rhizobacteria as well as potent biocontrol agents. A better understanding of BBDG diversity promises to provide an array of new products and processes. The use of BBDG as biofertilizers, biocontrol agent and bioremediators will help Indian agriculture by reducing the dependence on chemical inputs and protecting the environment. *Bacillus* based preparations would help in the development of technologies that can be utilized as bioinoculants, as PGPR that can alleviate salinity and drought stress to attain optimum yield of field and horticultural crops and bioremediation of HCH contaminated soils.

The nutritional versatility of *Bacillus* allows them to use a range of carbon sources including such things as uric acid, herbicides and nicotine. For these reasons they are extremely competitive organisms with remarkable resistance to desiccation and starvation. The optimum growth temperature of most species is 25-30°C. However, species isolated from extreme conditions have developed acclimation proteins allowing them to sustain life under extreme conditions of salinity, drought, high or low temperatures and acidity. Identification of new osmolytes and the relevant genes can be a boon to Indian agriculture, as these genes could be utilized to develop transgenic tolerant to abiotic stress.

Most of the work done in India is fragmented in nature and a comprehensive approach is needed to collect, collate the information on *Bacillus*, put them in electronic format, DNA fingerprint them, identify useful genes/constructs/vectors and develop diagnostic protocols. Hence, extensive survey of extreme environment needs to be done and isolations had to be made using appropriate media so as to bring into culture as vast diversity as possible.

Identification of *Bacillus* species is difficult as there are 205 species and more than a dozen *Bacillus* derived genera. The molecular techniques need to be developed for identification of this vast group. *Bacillus* or preparations from *Bacillus* are not commercialized for use in Agriculture as bioinoculant, biocontrol or for bioremediation. Viable technologies need to be developed based on field evaluation and validation.

Development of technologies involves characterization of strains for relevant attributes. The strains to be used for bioinoculant should be characterized for production of IAA, gibberellins, cytokinins, siderophore, ACC deaminase and solubilization of phosphorus.

Since the diversity of BBDG in the country is not known, there could be several species that may have insecticidal properties. The work in this project deals with the screening of different isolates that may lead to identification of new species or strains that have insecticidal attribute. The large database of *Bacillus* from different environment will help in selecting suitable PGPR strains and biocontrol agents.

Objectives

- Diversity analysis and identification of *Bacillus* and other predominant genera from extreme conditions of salinity, drought, acidity and mangroove
- To understand the mechanisms of adaptation in *Bacillus* and mining of relevant genes
- Study of the diversity of *Bacillus* and other predominant genera associated with plant species under extreme environments and evaluating their role as ameliorating agents for crops grown in deteriorated environments
- Selection of novel strains of *Bacillus thuriengiensis* and other *Bacillus* species with insecticidal

properties and isolation of novel cry and other insecticidal genes

Objectives assigned to the NBAIM, Mau

- Diversity analysis and identification of *Bacillus* and other predominant genera from extreme conditions of salinity from different regions of Eastern Uttar Preadesh.
- Study of the diversity of *Bacillus* and other predominant genera associated with plant species under extreme environments and evaluating their role as ameliorating agents for crops grown in deteriorated environments.

Methodology

a) Exhaustive surveys were conducted in the salinity affected regions of Mau, Azamgargh and Varanasi following grid sampling method, for the diversity analysis of *Bacillus* and other predominant genera in the saline soils of eastern UP.

b) Isolation of *Bacillus* spp. was carried out following enrichment technique. The samples were given heat treatment at 80 °C followed by dilution plating on Nutrient agar medium. Isolation of predominant genera were carried out by enrichment techniques using different media viz. Halophilic media, Jensen's N free medium, King's B (KB) medium, nutrient agar (NA), trypticase soy agar (TSA), and soil extract agar (SEA). Bacterial cultures were maintained on the respective slants and stored at 4 °C till further use.

c) The salt tolerance of the isolates was evaluated by observing the growth on NA medium amended with different concentrations of NaCl.

d) Biochemical characterization of the isolates for the purpose of evaluating plant growth promoting traits such as production of IAA, GA, Cytokinin, ACC deaminase, siderophore and mineral phosphate solubilization was carried out using standard protocols.

e) The molecular characterization of the isolates was carried out through PCR amplification of 16S and 16-23S rDNA followed by RFLP analysis using different restriction endonucleases. Species-specific molecular fingerprints will be developed for identification. Phylogenetic analyses were carried out using NTSYS – pc analysis package to group the isolates into phylogenetic clusters.

Significant Achievements:

- Rhizospheric soil sampled
- from different locations in the four districts viz., Varanasi, Mau, and Azamgarh of Uttar Pradesh (UP) have electrical conductivity varied from 3.2 to 5.0 dS m^{-1} and pH ranging from 8.0 to 10.5 of saline soil and pH (6.1-7.5), conductivity (1.0 -3.8 dS m^{-1}) .
- The cfu of the *Bacillus* in the soil samples from different region varied from *Bacillus* population ranged from 36 to 180 x 10^4 cfu/g using enrichment techniques. Total 120 isolates were picked, based on endospore presence, 51 bacilli were selected for further characterization. From chickpea rhizospheric out of 250 isolates 22 *Bacillus* and rest different predominating genera were isolated.
- The intrinsic resistance of the isolates against different salt concentration was evaluated by observing the growth on NA medium amended with various concentrations of NaCl (2,4, 6, 8, 10, 12 and 14% (w/v)). The extent of variability to NaCl tolerance was much more in bacilli obtained from Mau region than those obtained from other areas. Out of 120 isolates 85 isolates were able to tolerate NaCl stress up to 2%, 64 isolates were able to tolerate NaCl stress up to 4%, 58 isolates were able to tolerate NaCl stress up to 6%, 32 isolates were able to tolerate NaCl stress up to 8%, 26 isolates were able to tolerate NaCl stress up to 10%, 18 isolates were able to tolerate NaCl stress up to 12%, 2 isolates were able to tolerate NaCl stress up to 14% (Fig 2).

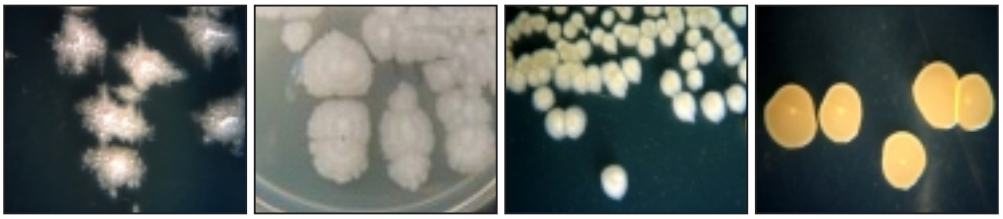


Fig 1: Pure isolates

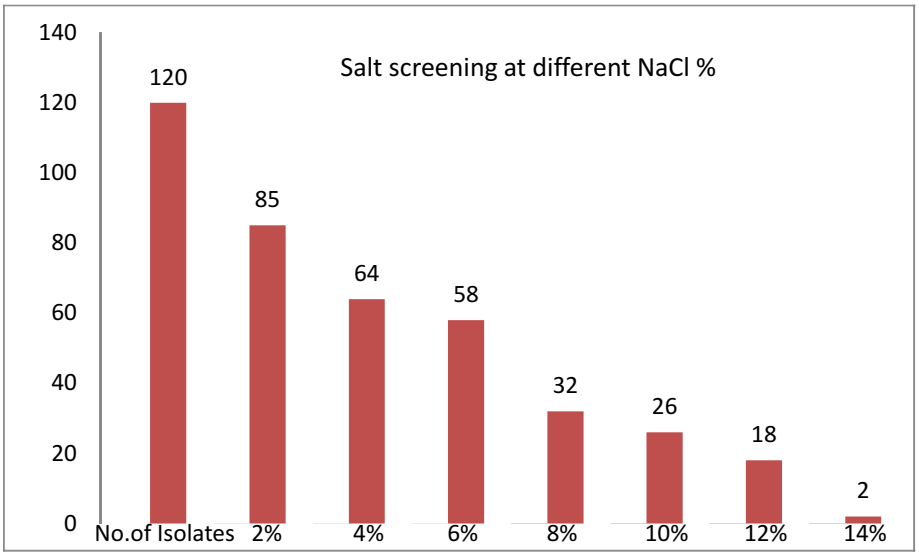


Fig 2: Salt screening of different isolates

- All the 250 isolates of chikpae rhizosphere were screened for the expression of plant growth promoting attributes. Screening results of PGP traits are depicted in Table 1. Out of 250 isolates, 185, 134, 98, 75, 205, 12 could produce IAA, ammonia, solubilise phosphate, siderophore, catalase and HCN, respectively (Fig 3).

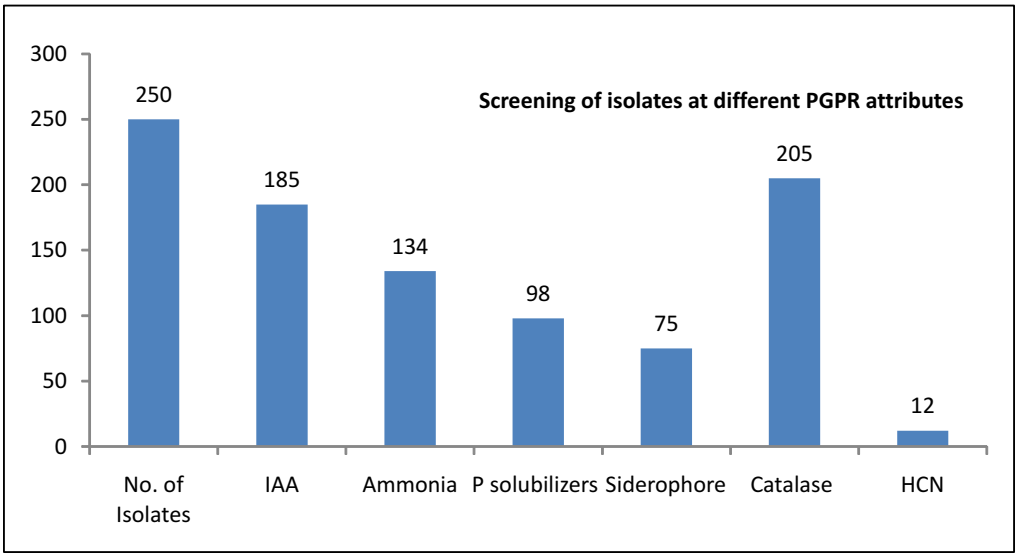


Fig 3: Screening of isolates at different PGPR attributes

- Genomic DNA of the isolates was isolated and 16 S rDNA region was amplified using universal primers pA (5'-AGAGTTTGATCCTGGCTAG-3')and pH (5'-AGGAGGTGATCCAGCCGCA-3'). Further molecular characterization is in progress.

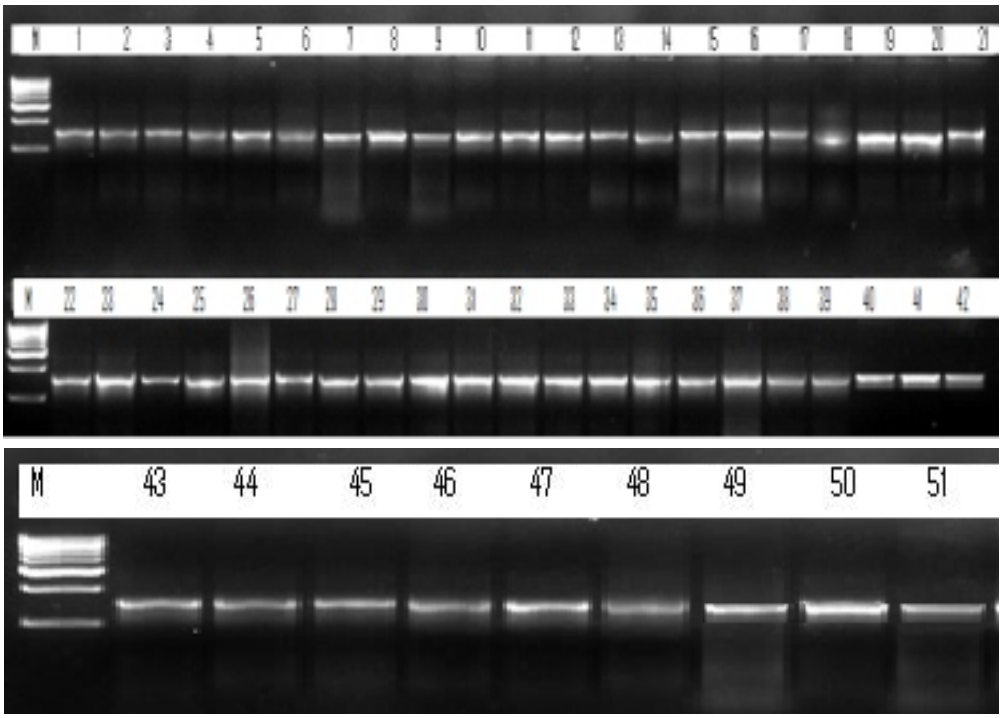


Fig 3: Agarose gel electrophoresis of PCR amplified 16s rDNA

Conclusion:

Studies made so far have resulted in the isolation of 120 and 250 distinct bacterial isolates from saline and chickpea rhizosphere soil, respectively from different region of eastern UP which tolerate NaCl concentration up to 14%. In this study we also found that the some isolates having multiple plant growth promoting attributes which may promote plant growth directly or indirectly or synergistically.

Project: Bioprospecting of gene and allele mining for abiotic stress tolerance.

PI : Rajeev Kaushik
Co-PI : Alok Srivastava, Kamlesh K Meena
SRF : Vishal Srivastava, Jaswant Yadav, Smita Rao

Rationale

Reduction in agricultural productivity to the extent of 40% due to climate changes and population explosion will have a telling effect on the national economy and livelihood security. The dwarfing genes in wheat and rice, and rust resistance genes in wheat are some of the burning examples, which stand testimony to the power of genetic technology that usher in green revolution and subsequently helped sustaining the productivity gains. With the advent of new biotechnology tools and techniques, it has been possible to access genes from diverse biological systems and deploy in target species. This has rendered the whole living world as a single gene pool. Use of crystal protein genes from the soil bacterium *Bacillus thuringiensis* in genetic engineering of crops like cotton clearly depicts how genes from evolutionarily distant organisms can bring new revolution in agricultural production. Besides Bt genes, several other genes have also been prospected, validated and are being deployed to gain commercial advantage. These efforts encourage prospecting of novel genes and new alleles of the known genes from diverse biological sources cutting across taxa and phyla and functionally validate them for future deployment to enhance and sustain agricultural productivity.

India is fortunate to have a rich bio-diversity. At present, very little baseline information is available on the diversity of extremophiles in India. Extensive survey for the characterisation and utilisation of bio-diversity is essential to meet the challenges of biotic and abiotic stresses under changing climate. In this direction, bioprospecting of extremophiles under various stress conditions would provide a database that will enrich the Indian microbial culture collection. Only fragmentary work was done on these aspects in India. There is a need for a comprehensive approach to collect, collate the information on extremophiles, digitizing the generated information and to identify useful genes/constructs/vectors. The cultures isolated from extreme environments have different mechanisms of adaptations and their characterization will lead to identification of novel genes responsible for adaptation. Further, metagenomic approach also emerged as a powerful and elegant tool to enhance the gene pool and to identify novel taxa, genes and alleles.

The work in the present project would be designed for the screening of different isolates that may lead to identification of new species/strains/genes/alleles that have ability to tolerate abiotic stresses. Multidisciplinary approach will be followed in this project to collect and preserve indigenous isolates of microbes from extreme environments.

Objectives

- Generation of genomic resource base to facilitate gene prospecting and allele mining
- Prospecting for new genes and alleles for abiotic stress tolerance (moisture stress, salinity and sodicity, soil acidity, adverse temperature and submergence/anoxia)
- Functional validation of the identified genes in model plant systems
- Use of the identified genes/allele in genetic enhancement of target species

Methodology

- The survey's were conducted at Kutch region, Gujrat and Sambhar lake for salinity, Manikaran thermal spring, HP; Chuma Thang Thermal Spring and Rajgiri Thermal Spring, Bihar for high temperature, Kutch, Gujrat; Thar Desert, Rajesthan.
- Isolation of fungi was done by using different enrichment technique on the different media (MEA, CMA, SDA, V8JA, CDA for salt stress and desiccation and YPSS media, PDA media, yeast starch media, malt extract agar for temperature stress.
- Kutch and Leh isolates were screened on different NaCl concentration (10%, 15%, 20%, 25%, 30%) amended in malt extract broth. Kutch and Leh region isolate were also screened on different concentration of PEG (5%, 10%, 15%, 20%, 25%) infused plate, while Manikaran and Rajgir isolates were screened at 40°C, 50°C, 55°C, 60°C and 65°C.
- Genomic DNA was extracted by following the Prep method. PCR amplification(Gonzalez and Mendoza et al,2008) of ITS DNA for the isolates was done by using two universal primer pA 5'-AGAGTTTGATCCTGGCTAG-3' and pH 5'-AGGAGGTGATCCAGCCGCA-3'. PCR contained 1.5 mM $MgCl_2$, 200mM dNTP, 1.5mM each primer, 3U of *Taq* polymerase (Genei, India) and 50 ng DNA template. The reaction conditions were initial denaturation at 94°C for 5 mins; denaturation at 94°C for 30 seconds for 40 cycles; annealing at 50°C

for 40 seconds; extension at 72°C, 1 min; end filling, 72°C, 10 min; holding at 4°C. The amplified product was resolved on 1.2% agarose gel.

- ITS PCR product was digested with restriction endonucleases *Alu*I, *Msp*I, *Hae*III according to the manufacturer's instructions and restricted DNA was analyzed by horizontal electrophoresis in 2.5% agarose gels. Electrophoresis was carried out at 70V for 2.5 hr with standard gels (11 x 14 cm), and documented on a gel documentation system (Alpha Imager, USA).

Significant achievements

- Total 135 isolates of fungi were obtained from Kutch region. Twenty three isolates were able to grow at 25% salt concentration (Table 1). Fungal isolates from Kutch region were also screened for desiccation tolerance and 65 isolates were able to grow at 25% PEG concentration.
- A total of 46 isolates are obtained from Water samples of Manikaran which were able to grow within a temperature range of 42° - 55 °C. From cold desert of Leh 32 different morphotypes of fungi were isolated (Table 2).
- PCR amplification of ITS region of all the isolate from various survey sites is done.

Table 1. Number of morphotypes of fungi isolated from Kutch eco region for salinity and desiccation

Isolate No.	MEA	CMA	SDA	V8JA	CDA
KR1	12	NIL	31	19	19
KR2	17	NIL	4	10	20
KR3	22	7	4	15	28
KR4	9	4	8	4	4
KR5	21	1	12	11	2
KR6	24	5	13	NIL	14
KR7	5	2	9	3	5
KR8	12	6	9	10	2
KR9	22	20	24	4	8
KR10	8	4	NIL	10	6
KR11	16	6	6	9	9

Table 2. Number of morphotypes of fungi isolated from Leh region for salinity and desiccation

Sampling Site	CMA	CDA	SDA	V8JA	MEA
Indus 01	Nil	1	2	3	4
Indus 02	Nil	1	2	1	1
Indus 03	Nil	2	3	3	Nil
Tanse 01	Nil	4	2	2	2
Tanse 02	Nil	1	3	3	1
Pangonk Tso	Nil	Nil	2	Nil	Nil

Conclusion

The proper exploration of diversity of microflora and exploration of adaptation strategy will be helpful in allele mining. Characterization of these genetic stocks and identification of useful or better variant of the genes known for conferring drought/heat tolerance will be of immense importance in improvement of gene pool. So, the study areas were chosen which is located in all the extreme part of India where only few microflora were found. Kutch Ecological region represents adapted region for high salt concentration with low water availability. Contrarily, Manikaran and Rajgir regions were chosen for the study of high temperature stress tolerance. This will fulfil the perspective of our project. Although, a diverse and wide range of germplasm still remains to be explored for abiotic stress tolerance

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Project: Georeferenced Soil Information System for Land Use Planning and Monitoring Soil and Land Quality for Agriculture

CC-PI : Alok K Srivastava
Co-PI : Kamlesh K Meena
SRF : Ashutosh Kumar

Rationale

Considering the multitude of key agricultural, environmental, economic, social and cultural functions performed by soils, it is necessary to develop a land information system for assisting land use planning systems and monitoring land quality changes. Land use and land use changes alter land quality parameter. Identification of relevant indicators and fixing baseline (reference levels) will help in forewarning the consequences of non-compatible land uses on land quality.

The most important link between farming practices and sustainable agriculture is the health of soils and that need regular monitoring. Soil microorganisms significantly contribute to the maintenance of the matter and energy turnover in terrestrial environment. These soil microorganisms are good indicator of soil health and contribute significantly in maintaining the fertility of soil. Each soil has a characteristic pattern of enzymes because all biochemical actions are dependent on or related to their presence. Soil enzyme assays are process level indicators and are presented as a means of determining the potential of a soil to degrade or to transform substrates. Soil enzyme activities are influenced by management practices because they are also related to microbial biomass which is sensitive to different treatments. One of the criteria used to determine microbial activity and biomass in soil is the dehydrogenase activity. The overall dehydrogenases activity of a soil depends on activities of the various dehydrogenases, which are the fundamental part of the enzyme system of all microorganism (enzyme of the respiratory metabolism, the citrate cycle and N metabolism). Dehydrogenase activity thus serve and an indicator of the microbiological redox system and may be considered a good measure of microbial oxidative activities in soil

Urease is ubiquitous cell free exoenzyme in nature that is produced by plants and microorganism. It is stabilized by the adsorption on the minerals. Either the decrease of urea concentration or the increase of the ammonium or the production of $^{14}CO_2$ are assessed in these methods. In the most cases, the rate of ammonia production is used. The quantification of urease is based on the incubation of soil sample with urea.

Land quality is conceptualized as the major link between the strategies of conservation management practices and achievement of major goals of sustainable agriculture. Assessment of land quality is invaluable in determining sustainability of land management systems. Therefore, assessment of land quality and the direction of change with time is the primary indicator of sustainable land management. Currently, complete data sets on bio-physical parameters are not available for the preparation of a soil information system under SOTER environment.

Objectives

- Determination of CFU for different microorganisms of provided sample
- Determination of soil Dehydrogenase activity

- Determination of soil Urease activity (indirect indicator of N mineralization)
- Determination of P-Solubilization Microbes and *Azotobacter*

Significant Achievements

- 223 profiles of soil have been received from different NBSS & LUP regional centers (Fig 1). 70% of total profiles have been processed to achieve the above mentioned objectives of project.
- All the profiles were analyzed for the quantitative analysis of Fungi, bacteria and actinomycetes. The soil of Rajasthan showed higher population of bacteria followed by actinomycetes and fungi. Other soil samples also exhibited similar pattern.

Table 1: Population of microorganisms in Kollu and Thar area of Rajasthan

Serial no.	*Sample code*	*Depth*	*CFU/gram soil/ml (Dilution factor 10^2)*		
			Bacteria	*Actinomycetes*	*Fungi*
Kollu (Low management)					
1.	*K-1/1*	*(0-15cm)*	*0.6 X 10^4*	*3.7 X 10^4*	*0.2 X 10^4*
2.	*K-1/2*	*(15-60cm)*	*3.0 X 10^4*	*2.5 X 10^4*	*0.2 X 10^4*
3.	*K-1/3*	*(60-120cm)*	*5.5 X 10^4*	*2.5 X 10^4*	*0.0 X 10^4*
Kollu (High management)					
4.	*K-2/1*	*(0-18cm)*	*0.5 X 10^4*	*4.0 X 10^4*	*0.6 X 10^4*
5.	*K-2/2*	*(18-36cm)*	*2.5 X 10^4*	*5.0 X 10^4*	*0.2 X 10^4*
6.	*K-2/3*	*(36-68cm)*	*6.2 X 10^4*	*2.0 X 10^4*	*0.0 X 10^4*
Thar (Low management)					
7.	*T-1/1*	*(0-38cm)*	*0.3 X 10^4*	*1.0 X 10^4*	*0.0 X 10^4*
8.	*T-1/2*	*(38-92cm)*	*0.6 X 10^4*	*2.0 X 10^4*	*0.1 X 10^4*
9.	*T-1/3*	*(92-108cm)*	*4.0 X 10^4*	*18.0 X 10^4*	*0.2 X 10^4*
10.	*T-1/4*	*(108-138cm)*	*6.0 X 10^4*	*10.0 X10^4*	*0.2 X 10^4*
11.	*T-1/5*	*(138-168cm)*	*3.5 X 10^4*	*6.0 X 10^4*	*0.0 X 10^4*
Thar(High management)					
12.	*T-2/1*	*(0-20cm)*	*1.1 X 10^4*	*0.7 X 10^4*	*0.9 X 10^4*
13.	*T-2/2*	*(20-40cm)*	*4.0 X 10^4*	*2.5 X 10^4*	*0.3 X 10^4*
14.	*T-2/3*	*(40-80cm)*	*15.0 X 10^4*	*12.0 X 10^4*	*0.1 X 10^4*
15.	*T-2/4*	*(80-120cm)*	*11.5 X 10^4*	*13.0 X 10^4*	*0.0 X 10^4*
16.	*T-2/5*	*(120-160cm)*	*9.0 X 10^4*	*15.0 X 10^4*	*0.0 X 10^4*

Table 2: Dehydrogenase and urease activity in Kollu and Thar area of Rajasthan

Serial No.	**Sample code**	**Depth**	**Activity(µg/12 hours/5gm soil)**	
Kollu (Low management)			**Dehydrogenase**	**Urease**
1.	K-1/1	(0-15cm)	0.70	8.2852 X 10^{-3}
2.	K-1/2	(15-60cm)	0.84	8.2789 X 10^{-3}
3.	K-1/3	(60-120cm)	0.80	8.6076 X 10^{-3}
Kollu (High management)				
4.	K-2/1	(0-18cm)	0.86	8.3563 X 10^{-3}
5.	K-2/2	(18-36cm)	0.72	8.3818 X 10^{-3}
6.	K-2/3	(36-68cm)	0.96	8.4446 X 10^{-3}
Thar (Low management)				
7.	T-1/1	(o-38cm)	0.84	8.2652 X 10^{-3}
8.	T-1/2	(38-92cm)	0.86	8.1088 X 10^{-3}
9.	T-1/3	(92-108cm)	1.23	7.9881 X 10^{-3}
10.	T-1/4	(108-138cm)	1.56	8.2946 X 10^{-3}
11.	T-1/5	(138-168cm)	1.30	8.1665 X 10^{-3}
Kollu(High management)				
12.	T-2/1	(0-20cm)	0.90	8.1802 X 10^{-3}
13.	T-2/2	(20-40cm)	0.82	8.2558 X 10^{-3}
14.	T-2/3	(40-80cm)	1.44	8.2663 X 10^{-3}
15.	T-2/4	(80-120cm)	1.38	8.3524 X 10^{-3}
16.	T-2/5	(120-160cm)	1.21	8.2747 X 10^{-3}

Higher level of dehydrogenase was recorded in the top soil as an indicator of active microbial biomass which reduced significantly along with the depth and least activity was found at 1 meter of depth.

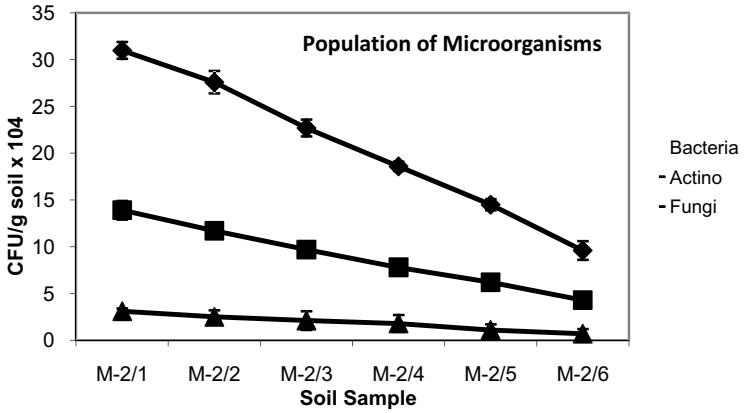

Figure 1: Population of Microorganisms in Madhpur Soil under low mamangement

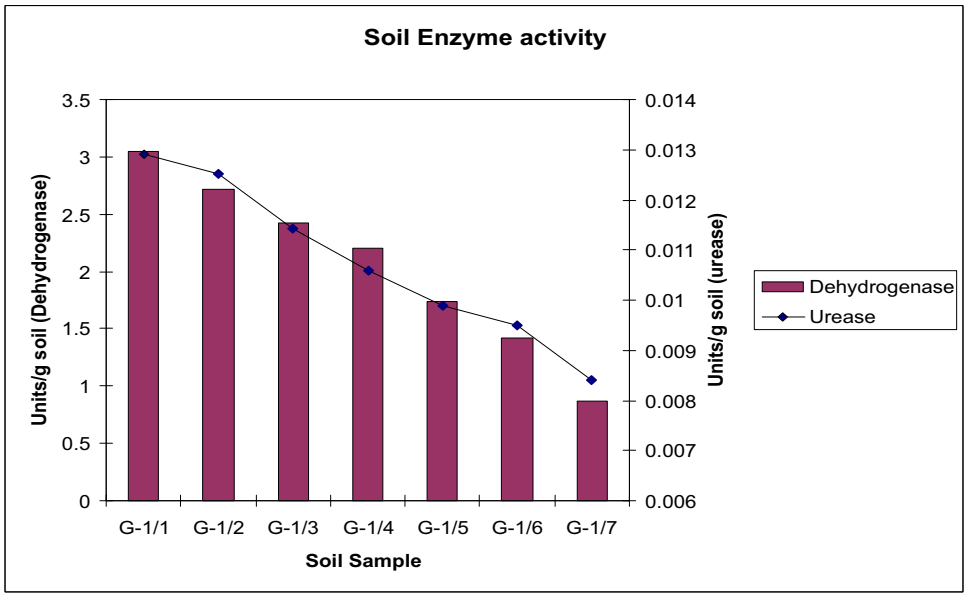

Figure 2: Microbial enzyme activity in Madhpur Soil under low mamangement

The wheat rhizosphere was evaluated for the phosphate solublizing bacteria, maximum population was recorded I Fatehpur soil followed by Ekchari soil samples. Least population was found in Haldi and Mashitawali 1 samples

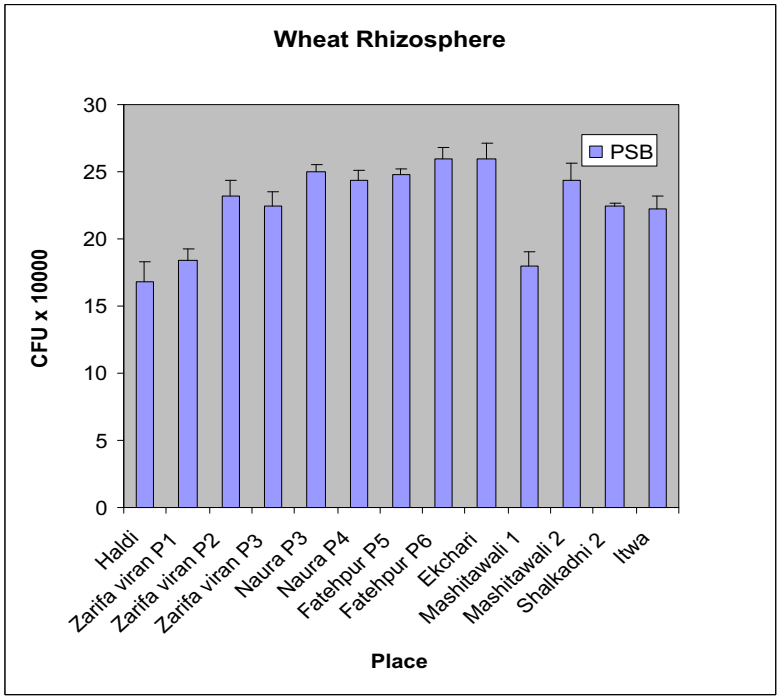

Fig. 3: The Rhizospheric soil analyzed for Phosphate Solublizing Bacteria

Conclusion:

The cfu analysis of different soil profiles provided relevant information about culturable microbial treasures. There is gradual decrease in cfu of profiles. Functional trend of urease and dehydrogenase enzyme was estimated and analyzed. It has been found that in some case urease enzyme activity is high in low management which is opposite to usual trend. The normal trend is high magnitude of urease in highly managed agricultural land. In case of soil dehydrogenase, non-significant trend is noticed i.e., value of soil dehydrogenase remain almost same throughout the profiles with minor increase or decrease. Soil enzymatic activity is responsible for important cycles, such as C, N, P, and S. Presence of *Azotobacter* and PSB microorganism in soil indirectly indicate the health of soil and crop yield.

Project: Outreach Programme on *Phytophthora, Fusarium* and *Ralstonia* diseases of horticultural and field crops.

Sub project: Conservation, characterization and documentation of different species of *Fusarium*

PI : Sudheer Kumar
Co-PI : Alok K Srivastava
SRF : Deepak Kumar Maurya, Jyoti Yadav

Rationale

Fusarium is a soil inhabiting fungi which can survive over the years in the soil in the form of resting structures (clamydospores). *Fusarium* species cause a huge range of diseases on an extraordinary range of host plants. The fungus can be soilborne, airborne, or carried in plant residue, and can be recovered from any part of a plant from the deepest root to the highest flower. These morphological features serve as the basis for formal descriptions of these taxa that meet the rules of the International Code for Botanical Nomenclature (ICBN), but are not necessarily easily applied in a diagnostic and characterization. The molecular characterization of *Fusarium* will give the clear picture of species / races and its host range present in India.

Being soil borne disease, there is limited scope of fungicidal application. The fungi caused wilt disease where the symptoms appear late at the time of flowering when the application of control measure become ineffective. The development of resistant varieties is best alternative to manage the disease under field condition. The rapid and accurate detection of pathogen before symptom expression through molecular techniques may also provide an opportunity for timely application of control measures. Moreover, understanding the variability in the pathogen is essential for resistance breeding programme. For the purpose a large number of collections of pathogenic isolates across agro-climatic regions from all over the country are needed. The molecular characterization for phenotypic and pathogenic variability is necessary for identification of race specific resistance genes in the host to develop resistant pure line and multilines.

The project is aimed to develop a repository of *Fusarium* which is lacking at present. In this network project a wide collection of *Fusarium* will be generated and a data base shall be developed regarding information like place of origin, pathogenicity, virulence, races and DNA fingerprinting. It will be user-friendly bio-informatics platform that supports the integration and use of available data on major foliar pathogens. The diagnostic techniques and kits for early and rapid detection shall also be developed.

Objectives:

- Conservation, characterization and documentation of different species of *Fusarium*
- Development of data base for Indian isolates of *Fusarium*

Significant Achievements:

- Large numbers of different species of *Fusarium* were collected in network project across the country and from different fruits, vegetables, cereals, pulses hosts. The species were collected from different agro ecological zones vary in the morphological, cultural and pathogenic characters. All these isolates have been preserved for short term as well as long term conservation in mineral oil, glycerol at -80^{0}C and lyophilized.
- The collected isolates were characterized for morphological variability on the basis of pigmentation, growth pattern, colony colour, mycelia colour, shape and size of micro conidia and macro conidia etc (Fig 1).

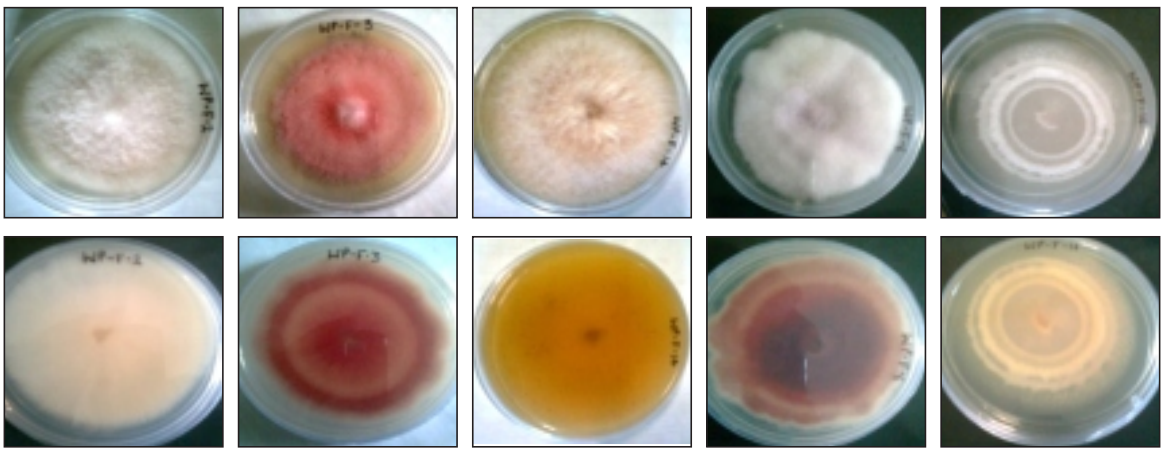

Fig 1. Variation in colony and pigmentation in different isolates of *F. oxysporum* f. sp. *lycopersici*

- The colony growth on PDA was ranged from 36.5 to 80.0 mm on 7th day. Culture of *Fusarium oxysporum* f. sp. *lycopersici* produced both micro and macro-conidia. The micro-conidia vary in size from 5.9 – 13.9 x 2.1 – 4.1 µm and macro-conidia ranges from 12.5 – 25.1 x 2.5 – 4.7 µm. Between different isolates of *Fusarium oxysporum* f. sp. *lycopersici* considerable variation were recorded in conidial size as well as growth and pigmentation. They also formed clamydospores intercalary as well as terminal.
- The PCR-based characterization of different isolates of *Fusarium* species was done by exploiting the internal transcribed spacer (ITS). Genomic DNA was isolated and amplification of ITS1 and ITS4 region of the different *Fusarium* isolates yielded a fragment of size about 550 bp (Fig 2).

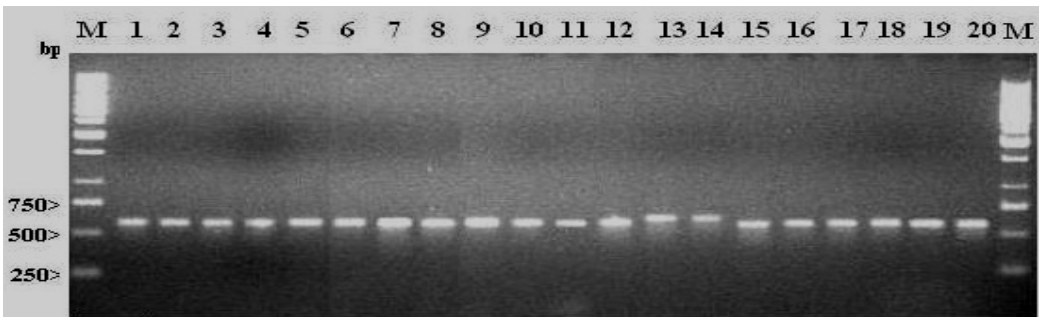

Fig. 2 ITS amplification of different Fusarium spp.

- The variability within the ITS amplified regions can be further investigated by cleaving this fragment with restriction enzyme *Alu I*. No substantial polymorphic pattern among the isolates was found by using ITS with restriction enzyme on 2.5% agarose. Restriction with other endonucleses for diversity analysis is under progress.

Conclusion

The collection and conservation of *Fusarium* with digitization of all the related information will provide a user-friendly bio-informatics platform that will facilitate the users to rapid excess of information on major foliar pathogens.

Project: Outreach Programme on Diagnosis and Management of Leaf Spot Diseases in Horticultural and Field Crops

Sub project: Conservation, characterization and documentation of different species of *Alternaria, Colletotrichum* and *Cercospora*

PI : Sudheer Kumar
Co-PI : AlokK Srivastava, Kamlesh K. Meena
SRF : Ruchi Singh, Bhavna Gangwar

Rationale

Leaf spot pathogens caused a number of economically important diseases in a wide range of hosts including cereals, legumes, vegetables, perennial crops worldwide. Leaf spot occurs in warm and humid weather conditions, hence very prominent in India. Leaf spot is a common descriptive term applied to a number of diseases affecting the foliage of crops and ornamentals. The majority of the leaf spots are caused by a variety of fungal pathogens such as *Alternaria*, *Colletotrichum* and *Cercospora* etc.

On the basis of symptoms, the identification of the causal agent of leaf spot diseases is very difficult, as the symptoms may largely vary with the host variety, crop growth stage, agronomic practices and prevailing weather conditions. Accurate species identification is critical to understand disease development or epidemiology and also to develop effective control measures.

The rapid and accurate detection of leaf spot pathogen through molecular techniques may also provide an opportunity for timely application of control measures. Being a foliar pathogen, these are greatly influenced by weather parameters and spread rapidly under favorable conditions where timely application of management practice is directly correlated with economic return.

Moreover, understanding the variability in the pathogen is essential for resistance breeding programme. For the purpose a large number of collections of pathogenic isolates across agro-climatic regions from all over the country are needed. The molecular characterization for phynotypic and pathogenic variability is necessary for identification of race specific resistance genes in the host to develop resistant pure line and multilines.

The project is aimed to develop a repository of the foliar pathogens like *Alternaria, Colletotrichum* and

Cercospora which is lacking at present. In this network project a wide collection of leaf spot pathogens will be generated and a data base shall be developed regarding information like place of origin, pathogenicity, virulence, races and DNA fingerprinting. It will be user-friendly bio-informatics platform that supports the integration and use of available data on major foliar pathogens. The diagnostic techniques and kits for early and rapid detection shall also be developed.

Objectives:

- Conservation, characterization and documentation of different species of *Alternaria, Colletotrichum* and *Cercospora*
- Development of data base for Indian isolates of *Alternaria, Colletotrichum* and *Cercospora*

Significant Achievements

- Forty three cultures of *Alternaria* species, includes *Alternaria brassicae* (5) from mustard, *Alternaria solani* (18) from tomato, and rest 20 species from different crop plant were isolated.
- Seventy one cultures of *Colletotrichum* species includes *Colletotrichum gloeosporioides* (44) from mango, *Colletotrichum falcatum* (5) from sugarcane and other *Colletotrichum* spp. from grapes were isolated. All the isolates of *Alternaria* and *Colletotrichum* have been preserved under long term conservation.
- All the isolates of *Alternaria* as well as *Colletotrichum* were characterized morphologically on the basis of colony colour, pigmentation, shape, size and arrangement of spores. Large numbers of variations were recorded within species as well as between species (Fig.1. and Fig.2).
- The colony growth on PDA was ranged from 35.8 to 36.5 of *A. solani* and 69.7 to 70.1 mm of *A. brassicaea* on 9th day.
- Conidia of *A. solani* ranged from 15.6 – 18.8 x 280.5 – 314.8 µm and *A. brassicaea* ranges from 16.4 – 20.4 x 130.8 – 172.3 µm.
- Between different isolates of *Alternaria* species, considerable variation were recorded in conidial size as well as growth and pigmentation (Fig. 3)

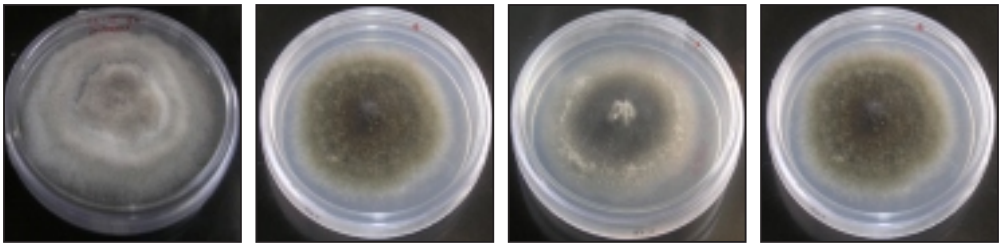

Fig 1: Variation in colony and pigmentation in different isolates of *Alternaria* species

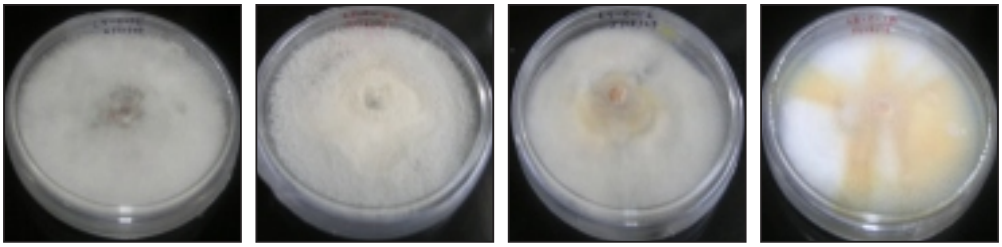

Fig 2: Variation in colony and pigmentation in different isolates of *Colletotrichum* species

Fig 3: Variability in spore size among different species of *Alternaria*

- The colony growth of *Colletotrichum* spp. on PDA was ranged from 36.5 to 63.5 mm on 9th day.
- Culture of *Colletotrichum* spp. produced conidia, vary in size from 1.9 – 4.6 x 6.3 – 13.15 µm.
- Between different isolates of *Colletotrichum* species, considerable variation were recorded in conidial size as well as growth and pigmentation (fig. 4).

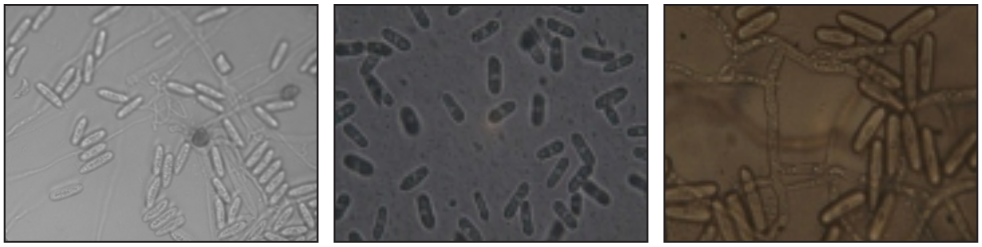

Fig 4: Variability in spore size among different species of *Colletotrichum*

- Isolation of Genomic DNA of 43 cultures of different species of *Alternaria* was completed and isolation of genomic DNA from 71 cultures of different species of *Colletotrichum* was completed.
- The amplification of all the culture of *Alternaria* and amplification of 71 cultures *Colletotrichum* species was done by exploiting the internal transcribed spacer (ITS) region (Fig 5).

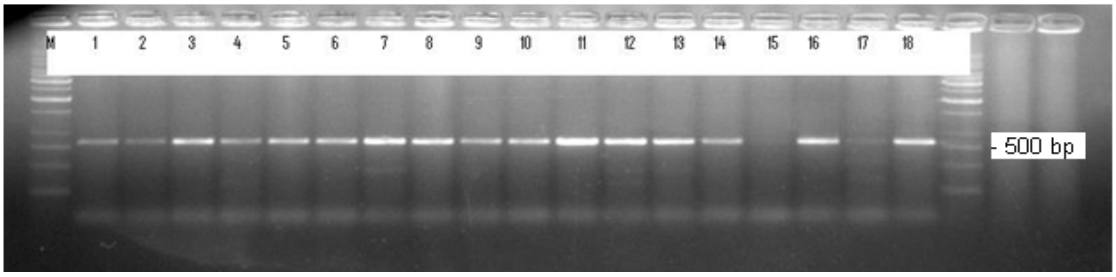

Fig 5. Amplification of ITS region of *Alternaria* spp.

- For the development of species specific primers for rapid detection of Alternaria solani, the sequences were downloaded from NCBI and aligned using Bio Edit software. Based on the results of the alignment, sequences were picked up from the hypervariable regions to design the primers. A total of 6 sets of primers specific for Alternaria solani from different conserved gene sequences of β tubulin, alt gene and ITS gene were designed using Primer3 and validated in silico (Fig 6). Work is under progress for its wet lab validation.

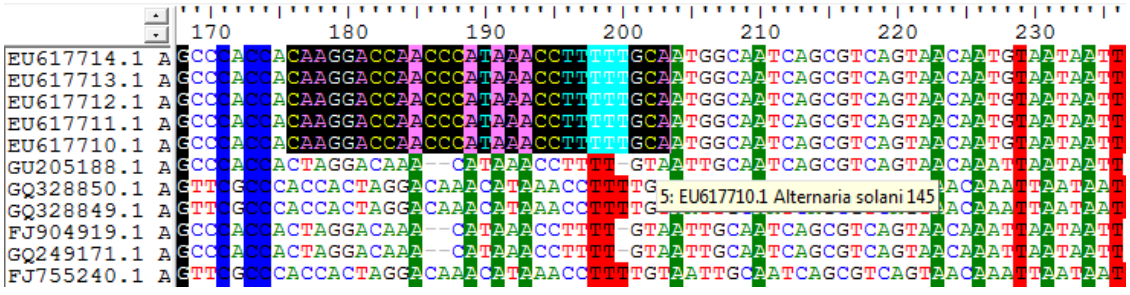

Fig 6. Alignment of alt gene sequences *Alternaria* spp.

Conclusion

The collection and conservation of leaf spot pathogens with digitization of all the related information will provide a user-friendly bio-informatics platform that will facilitate the users to rapid excess of information on major foliar pathogens. The spices specific primer will help for detection of *A. solani*

Significant Achievements- AMAAS

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- During 2009-10, 80 grids in Central Western Ghats were covered for sampling and a total of 487 samples (80 composite soils, 243 from roots of 105 plant species, 76 leaf, 13 leaf litter, 21 decaying wood, 23 mushroom and 31 termite mound samples) were collected. A total of 988 isolates including 298 Azotobacter, 86 Azospirillum, 260 Beijerinckia, 212 phospahte solubilizers, 17 lignin degraders, 84 fluorescent pseudomonads and 31 pink pigmented facultative methylotrophs were purified and preserved.
- From Trivandrum, Idukki & Palakkad districts, 51 samples were collected that resulted in 50 N fixers, 41 P-solubilizers, 19 P. fluorescens, 11 cellulose degraders, 8 lignin degraders and 13 Trichoderma.
- A total of twenty nitrogen fixing cyanobacteria have been identified as Anabaena constricta Nostoc piscinale, Nostoc species LCR17, Nostoc paludosum, Nostoc spongiaeforme, Nostoc species LCR21, Anabaena fertilisssima LCR23, Nostoc species LCR12, Anabaena toruolsa, Nostoc ellipsosporum, Nostoc carneum, Nostoc linckia, Nostoc punctiforme, Nostoc rivulare, Nostoc sp. LCR1NK, Nostoc sp. LCR2NK, Nostoc sp. LCR3NK, Nostoc sp. LCR4NK, Nostoc sp. LCR1NK and Nostoc sp. LCR2NK.
- Out of 733 bacterial isolates isolated from ten soil samples of Arunpur, Chilika, Haripur, Humma, Indrakhi (Orissa), Kalipatnam, K. P. Palem, Gondhi, Shankaraguptam and Undi (Andhra Pradesh), 186 isolates were IAA producers, 41 isolates solubilized P and 70 isolates utilizing ACC as a sole source of nitrogen.
- A total of 158 wild mushrooms were collected from the forests of Uttarakhand, Arunachal Pradesh, Himachal Pradesh and Andaman & Nicobar Island that included Phellodon tomentosus, Laetiporus sulphureus, Thelephora and Dictyophora.
- Thirty fluorescent Pseudomonas were isolated from Jabalpur, Sehore, Raisen, Hoshangabad and Guna districts of M.P and there plant growth properties like production of protease, lipase, chitinase cellulase and pectinase were studied.
- A total of 146 soil samples were collected from different arid and semi arid zones of Haryana. Out of which 60 soil samples were analyzed for pH, EC, organic C, ammonical N, nitrate N and total N. Soil pH ranged from 6.34 to 7.55 while the soil EC was in the range of 0.09 to 5.54 dSm^{-1}. The organic C and total N were in the range of 0.05 to 0.61% and 0.01-0.05%, respectively. The ammonical and nitrate N varied from 5-10ug/g soil and 10-50ug/gsoil, respectively.
- A total of 5 explorations in 11 districts of Bihar resulted in 721 sample from which Rhizobium, Azotobacter, PSB, Azospirillum and Fungus (Fusarium spp, Pythium apharidermeta, Aspergillus spp, Mucor, Alternaria alternate, Helminthosporium) in the rhizosphere of 10 different crops of 11 mentioned district viz. Khagaria, Begusarai, Saharsa, Supaul, East Champaran, Shivhar, Darbhanga, Sitamarhi, Madhubani, Samastipur, Kishanganj.
- 175 bacterial and 40 Streptomyces spp. from soil of Tawang, Arunachal Pradesh and thirty bacterial strains were isolated from rhino-dung of Kaziranga National Park, Assam. Out of 30 strains, 4 bacterial strains were identified as Providencia sp. RD1, Pseudomonas sp. RD2, Pseudomonas sp. RD3 and Achromobacter sp. RD4.
- Based on biochemical characterizations and scanning electron microscopy, 21 isolates of

Streptomyces and 9 salt tolerant bacteria were identified from rhizosphere of Camellia sinensis, Citrus reticulata and Cryptomeria japonicum in Darjeeling districts of North Bengal.

- Forty six (46) cyanobacterial strains belonging to 9 genera have been isolated from different ecological habitats of Manipur, Arunachal Pradesh, Assam, and Mizoram states of NE region. The strains are Phormidium (25), Oscillatoria (02), Lyngbya (04), Plectonema (01), Nostoc (05), Anabaena (04), Aulosira (01), Microcheate (01) and Calothrix (03).
- 360 fungal isolates from all eight districts of Mizoram i.e. Aizawl, Champhai, Saiha, Lunglai, Mamit, Kolasib, Serchip and Lowngtalai were isolated.
- Potential antagonistic and PGP isolates were identified as Bacillus subtilis (6), B. pumilus (5), B. megaterium (3), B. cereus (3), B. lichiniformis (1), Staphylococcus spp (3), Enterobacter cloacae (2), Pseudomonas spp (2) and Alcaligenes faecalis (1) based on carbohydrate utilization pattern using Microbial Identification System (BIOLOG).
- 200 samples collected from different districts of Orissa included different functional types like cellulase, xylanase, lipase, geletinase, protease, phosphate solublizing activity, pectinase, chitinase and amylase producers. 9 ammonia oxidizing bacteria were also isolated.
- 160 new diazotrophic bacteria were isolated from wheat cropping system of Punjab. Ninety isolates were found to be positive for Nif H using two different Nif H primers (Nif H 1 and Nif H 2).
- Twenty-two bacterial strains have been isolated from five marine fishes (Siganus spp., Carangoides spp., Leiognathus spp., Caranx spp., and Sillago spp.) collected from Mandapam, Tamil Nadu. Bacillus and Pseudomonas spp. were the predominant skin isolates. While, gills harbored Acinetobacter, Arthrobacter, Bacillus, Enterobacteriaceae, Flavobacterium and Pseudomonas spp. The visceral isolates included Arthrobacter, Bacillus, Enterobacteriaceae and Pseudomonas spp.
- 520 isolates, 65 were identified as organisms belonging to the genus Bacillus with 24 belonging to B. subtilis, 26 belonging to B. cereus, 10 belonging to B.pumilis, five belonging to B. coagulans, 29 isolates were identified as Micrococcus sps with 10 isolates belonging to M. luteus, 15 belonging to M. varians and four belonging to M. lylae, seven isolates were identified as Pseudomonas aeruginosa.
- A total of 525 samples like milk, curd, buttermilk were collected from different possible sources viz., cow, buffalo, goat, sheep, camel from various dairy units and individual farmer's and screening for bacteriocin production by the LAB isolates against food spoilage/pathogenic organisms like Staphylococcus aureus and Micrococcus luteus resulted in 30 out of 275 isolates screened were showing antibacterial activity.
- A complete database of the most promising bacteria (90 isolates of PGPR and rhizobia) for growth promotion of soybean, chickpea and wheat in vertisols was prepared. 11 highly effective PGPR improved nodule numbers and mass in field by 225 and 285% and grain yields by 54.8%. 4 effective strains improved nodule numbers and mass by 156 and 214% and grain yields by 30.9%.
- Two consortia were developed and tested for integrated nutrient supplementation through biofertlizers and plant growth promotion in sorghum (Pseudomonas–P17 + Azospirillum–Azs4 + Glomus fasciculatum) and pigeonpea (Pseudomonas–P17 + Bradyrhizobium–IC 4060 + Glomus fasciculatum).
- Seven carrier materials i.e., Purified talc, Charcoal, Deodar sawdust, Cidarwood sawdust, Charcoal and soil mixture, Raw talc (kadiya) and Alginate beads were evaluated for the development of formulation.
- Selected rhizobacterial strains were evaluated in ginger for their biocontrol and growth promotion in green house. For biocontrol screening against Ralstonia solanacearum, GRB 35 and GRB 36 showed highest disease suppression followed by GRB 70 and GRB 91.
- Evaluation of 22 PGPR isolates from coconut and 21 from cocoa were completed for growth promotion in coconut and cocoa seedlings. Pseudomonas putida KnSF208, Bacillus coagulans RSB14, Paenibacillus alvei KiEB 25 were found to be the best plant growth promoters of coconut. Pseudomonas putida biovar A KDSF

23, Pseudomonas sp. KDSF 7 and Bacillus licheniformis KGEB 16 were found to be the best plant growth promoters of cocoa.

- Thirty three efficient heterotrophic nitrifiers were isolated from CRRI, Canning, Talchua, Khola, Gupti and Ersama rice field sopils. Three of them viz. CRRI- 12 and CRRI- 14 and Gupti G-10 have been identified by 16s rDNA sequencing as Bacillus sp., Lysinibacillus sp. and Bacillus sp., respectively.
- Twelve actinomycetes were identified as having plant growth promotion and biocontrol traits (against M. phaseolina), of which 7 actinomycetes were demonstrated for their potential in green house.
- Two hundred and ninety three bacteria were isolated from the 45 samples of Vineyards of the main commercial cultivars viz. Thompson Seedless, Tas-A-Ganesh and Sonaka from the research farms. Out of these 136 were endophytes; 87 from grape shoots, 18 from leaf lamina, 13 from petioles and 18 from grape roots.
- HPLC analysis confirm PAH degradation potential of an actinomycete, Streptomyces rochei PAH-13 and a fungal strain, Phanerochaete chrysosporium VV-18 at three different concentrations (10 ppm, 50 ppm and 100 ppm).
- Agricultural land situated around hexachlorocyclohexane (HCH) dumpsites was surveyed for HCH residues and 25 strains were isolated by enrichment method, which were analyzed for HCH-degradation by gas chromatography. Only 7 strains were found to have HCH-degrading capabilities.
- Compost was prepared under LMC in 20 days time with the help of thermophilic fungi, S. thermophilum (X-21), H. insolens (I-33) and H. insolens (I-3) and their consortium. It was observed that thermophiles inoculated piles harboured least numbers of competitors and productive compost could be prepared with the help of S. thermophilum (14.88 kg/ 100 kg compost) as compared to control which gave a yield of 12.14 kg of mushrooms.
- Saccharomyces cerevisiae strain has been developed through regular recycling on a galactose medium. It produced about 30% more ethanol from kinnow waste through SSF, compared to the conventional S. cerevisiae strain
- Statistical optimization of simultaneous saccharification and fermentation (SSF) process using cellulase at 5FPU/g, pectinase at 60 IU/g, galactose adapted S. cerevisiae cells and temperature of 37 ^{o}C resulted in ethanol concentration and volumetric productivity of 43g/l and 2.86g/l/h, respectively, from kinnow waste which shows potential for scale-up studies.
- Nineteen β-HCH degrading bacteria (Is1-19) were isolated from the pesticide treated soils and checked for degradation of the pesticide. In MS medium, the organisms were able to degrade about 50-97% β-HCH. The decreasing order of degradation of β-HCH by 4 most potent isolates was Is12 (98%), Is5 (89%), Is3 (88%), and Is18 (83%) after third application @10 µg/ml. The retention time of the degradation product was 1.73 min.
- Ammonia oxidizing bacteria were enriched and enumerated from water samples collected from fish/prawn rearing unit. Six new isolates of cellulose-degrading bacteria were obtained from water samples were collected from Surat. One of the isolates (Bacillus megaterium) gave very high cellulase as well as xylanase activities, whereas another (Bacillus subtilis) gave high xylanase activity.
- Three fungal cultures i.e. Thrichoderma longibrachiatum, T. fasciculatum, Phanerochaete chrysosporium were tested for removal of Pb, Cd and Ni from liquid medium containing 20 ppm concentration of each heavy metal at different pH i.e. 3.5, 4.0, 4.5, and 5.0. In case of Pb, all the three fungi removed maximum Pb at pH 5.0. The highest Pb removal was observed by P. chrysosporium (94.56%) followed by T. fasciculatum (90.6%) and T. longibrachiatum (67.66%).
- Four out of seven ammonia oxidizing archaeabacterial isolates from brackishwater samples were found to harbor amoA gene. Two out of 23 -proteobacterial isolates from brackishwater ecosystems were positive for amoA gene. Twelve different media were evaluated for enrichment and isolation of Beggiatoa spp. from brackishwater ecosystems.
- Isolation of 81 lactic acid bacteria from 94 dairy and non-dairy samples was carried out for the

phenotypic and molecular characterization of Lactococcus lactis.

- Among 20 microbial isolates isolated from different starch rich substrate a Fusarium sp. exhibited the best amylase activity (0.332±.081U/ml)at 5 % (w/v) mango kernel. CMCase, FPase and β-Glucosidase were produced using mahua pomace as substrate by Trichoderma sp. through submerged fermentation at substrate concentration of 10% (w/v) for former two enzymes production and 5% (w/v) for the later β-Glucosidase.

Abiotic

- Out of 14 Azotobacter isolates, 7 isolates were tentatively identified as A. chroococcum, 4 as A. paspali, 2 as A. vinelandii and one as A. nigricans. Similarly out of 18 salt tolerant Azospirillum isolates, 7 were identified as A. halopraeferens, 6 as A. brasilense and 5 as A. irakense.
- GroES (hsp 10) gene amplified in Pseudomonas sp. strains. Induction of heat shock proteins (hsp60) under heat stress in Pseudomonas aeruginosa P6, confirmed by Western blotting. Trehalose production in Pseudomonas strains under heat stress observed by TLC. Trehalose synthase activity under heat stress, confirmed by HPLC.
- Out of twelve, eight cold tolerant bacterial strains (NPRs3, NPRp15, NARs9, PPERs23, PCRs4, PGRs1, PGRs4 and PGERs17) showed freezing resistance (88.8%–94.4%) and (31.4%-74.1%) after 96hrs at -10 and -40°C respectively. Maximum freezing resistance were observed in strains PGRs4 (74.1%) and PGERs17 (73.5%) at -40°C.

Genomics

- A total of 24 nifH gene sequences are in public domain through NCBI database.
- Metagenomic nifH library highlighted the prevalence of nifH genes in Uttarakhand Himalayan region.
- Small insert shotgun Genomic DNA insert plasmid library preparation is started. Glycerol mounts of 1152 (96 x 12 plates) clones are prepared.
- Plasmid DNA of 1152 (96 x 12 plates) clones was isolated and inserts size analyzed. 1008 Clones were found to contain insert of 1-5 Kb.
- 702 clones have been sequenced from 10 plates (5 forward and 5 reverse) spanning 4,43,407 nucleotides (443.4 Kb).
- The fresh isolate of Mesorhizobium cicerae Ca181 authenticated by NBAIM Mau was sequenced using the Roche 454 Pyrosequencing technology.
- Tn5 induced mutant library of Pseudomonas putida S11 comprising 3900 mutants was generated.
- On screening the library for sensitivity to 8-Hydroxyquinoline (8HQ), six 8HQ sensitive mutants and four tolerant mutants were obtained.
- High molecular weight genomic DNA was isolated from Mesorhizobium ciceri Ca181. Shearing of genomic DNA into 40kb fragments was done by passing it through a 200 µl small bore pipette 50-60 times. Sheared DNA was end repaired to generate blunt ended and 5'-phosphorylated DNA. Size selection for 25-40 kb fragments of end repaired DNA was done.
- PCR based screening of genomic library of Anabaena laxa (RPAN8) revealed positive results with primers directed towards fungicidal enzymes, compounds and cyanotoxins; Fungicidal activity was shown by 30 clones.
- Functional analyses and Bioinformatic tools guided characterization revealed the presence of two novel genes (end 1 and end 2) encoding endoglucanase associated with Peptidase M20 family and glycoside hydrolase family 5 (GH5), respectively, which also showed fungicidal activity.

New Initiatives at NBAIM

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Microbial Genome Resource Repository (MGRR)

Significance of MGRR

Many research institute/universities all over the world are carrying out microbiological and biotechnological research, which results to generate lot of genomic resources like cDNA libraries, gene constructs, cloned gene sequences, promoter regions, transgenes etc. These are valuable resources that need to be exploited through functional and comparative genomic approach for gene discovery and transgenic product development. Hence collection and maintenance of these genomic resources at a central place would play a vital role in bioscience research and education by enormous reduction in the cost of research. Besides, conservation of genomic DNA or part thereof the threatened and endangered plant, animal, fish and microbial species would ensure availability of the hereditary material of these endangered species for exploitation at a later stage. Further collection and supply of even the protected gene constructs within the IPR framework to the researchers in India is necessary to ensure availability of these protected biotechnological resources for exploitation. There is an urgent need to establish a strong centre to act as a hub of the network with essential facilities, expertise and mechanisms connecting the similar biotechnological activities of various national bureau and research institutes.

Considering the need of conservation and distribution of genetic resources, ICAR has established a National Genomic Resource Centers under which "Microbial Genome Resource Repository" (MGRR) was added as a new theme area under the Network project "AMAAS" in the XI plan. MGRR is a facility for the long term storage of the genetic material of agriculturally important microorganisms, maintained in selected individuals or clones and plasmids accompanying the data.

This specified area is concerned with the maintenance of DNA integrity for which the requirements in terms of storage time and fidelity are significantly different. Both storage time and fidelity must be carefully considered and defined when discussing strategies for "preservation." Bio-repositories are most concerned with maintaining the ability to obtain DNA sequence information from stored specimens. Current sequencing technology depends on the polymerase chain reaction (PCR) to make copies of stored DNA that are ultimately used for sequence identification. As a result of this process, accurate sequence information can be obtained even from samples in which the DNA has been reduced to fragments. Although the desire for sequence identification does allow substantially greater levels of degradation to be tolerated, bio-repositories typically strive to preserve samples "permanently" so that future scientists can utilize specimens for studies at some undefined point in time. DNA samples are stored at -80°C or in liquid nitrogen (-196°C), but there is a significant expense associated with maintaining these conditions in hundreds of millions of samples. In contrast, dehydrated samples could be stored at room temperature, thereby greatly reducing cost and increasing convenience. Considering the lack of studies concerning the long-term storage stability of DNA, there is an obvious need for systematic storage studies on both purified DNA and intact cells. Regardless of storage conditions, it is important to question whether storage for indefinite periods (e.g., millennia) is truly necessary, even if it is attainable under certain storage conditions.

In addition to the sequence information, MGRR is also aimed to produce biological materials such as cDNA clones, RFLP markers on the genetic map,

cosmid clones, BAC (Bacterial Artificial Chromosome) clones, YAC (Yeast Artificial Chromosome) clones etc. These are the key of many future post-genomic researches, such as physiological or morphological characterization of a species, functional analysis of genes and comparative genomics, etc. Unavailability or loss of genetic material often makes it difficult for other scientists to reproduce and expand research program based on the past studies, like as "to reinvent the wheel". Therefore, it is necessary to maintain an efficient system for conservation and management of genetic materials and further supply the protected genes/constructs within the IPR framework to the researchers. These facilities will be provided by "NBAIM Microbial Genomic Resource Repository" in future to ensure availability of these protected biotechnological resources for exploitation.

Mandate

1. To coordinate assemblage, conservation, quality control and validation of the microbial genomic resources to facilitate their optimal exploitation and utilization
2. To act as a single window system for import and exchange of microbial genomic resources and facilitate protection of related IPR issues.
3. To conduct and promote basic, strategic, applied and anticipatory research for development and management of microbial genomic resources.

Objectives

1. Nationwide survey and collection of information about the genetic resources/DNA
2. Development of linkages between different research institutions, Universities, and individual researchers for obtaining microbial culture and genetic material.
3. Development of infrastructure facilities for the preservation and maintenance of Genetic Resource.
4. Technology and Protocols development for isolation and long-term preservation of the Microbial Genetic Resources.
5. Development of Databases or Information Bank for Microbial Genomic Resources and linkages with other DNA Bank.
6. Collection of environmental Microbial Samples from different Agro climatic Regions.
7. Technology and protocols development for collection/transportation microbial samples.
8. Exploration of non-culturable microorganisms and direct DNA isolation from environmental samples.
9. Documentation and Electronic Cataloging of Genetic Resources.
10. Development and Implementation of Genome Projects to explore non-culturable microorganisms.

Major Thrust of MGRR DNA Bank

- The major thrust of DNA Bank activities will focus on the collection and distribution of DNA materials from microorganisms and materials derived from researchers in molecular genetics, and associated genomics information.
- A DNA Bank Repository will facilitate storage of DNA and the accompanying data, until the Foundation authorizes the release of a portion of the DNA for an approved and funded research investigation.

The Centre will maintain genetic materials like:

1. Whole Genome
2. PAC/BAC/YAC clone vectors, competent Cells from sequencing projects.
3. A collection of vectors/gene constructs contributed by researchers.
4. Promoter DNA-fragments fused to the reporter genes.
5. RFLP probes specific for different microbes.
6. cDNA/ EST Libraries.
7. Expression plasmids including binary vectors.
8. Cloned DNA

Existing Facilities at MGRR

MGRR has generated a well equipped laboratory with all modern equipments and instruments such as:

1. Robotic DNA Extractor
2. Pyrosequencer
3. Molecular Imager Gel Doc XR System
4. Chemiluminescence Gel Imaging System
5. Confocal Microscope
6. DNA Fluorimeter System
7. Electroporator
8. Fraction Collector System
9. Gene Analyzer
10. Gradient Thermal Cycler

11. High Throughput Electrophoresis
12. Pulse Field Gel Electrophoresis System
13. Ultra Centrifuge
14. General Incubator
15. Incubator Shaker
16. Hybridization oven
17. Growth Kinetics Analyzer
18. Media Preparator
19. Micronics Starterpack with TPE caps, etc.


Laboratory facilities


Robotic DNA extractor


People at work


Storage

National Agriculture Innovation Project (NAIP)

1. Diversity analysis of *Bacillus* and other predominant genera in extreme environment and its utilization in agriculture

NBAIM is a **consortium leader** of this project and the objectives of the project are:

- Diversity analysis and identification of *Bacillus* and other predominant genera from extreme conditions of salinity, drought, acidity and mangrove.
- To understand the mechanisms of adaptation in *Bacillus* and mining of relevant genes.
- Study of the diversity of *Bacillus* and other predominant genera associated with plant species under extreme environments and evaluating their role as ameliorating agents for crops grown in deteriorated environments.
- Selection of novel strains of *Bacillus thuriengiensis* and other *Bacillus* species with insecticidal properties and isolation of novel cry and other insecticidal genes.

Consortium Partners

1. National Research Centre for Plant Biotechnology (NRCPB), IARI, New Delhi
2. Indian Agricultural Research Institute (IARI), New Delhi
3. National Research Centre for Groundnut (NRCG), Junagadh
4. Central Plantation Crop Research Institute (CPCRI), Kasargod

2. Bioprospecting of microbial genes and allele mining for abiotic stress tolerance

NBAIM is a consortium partner in this NAIP Mega Project and its objectives of the project are:

- Prospecting novel genes, promoters and alleles for economically important traits using indigenous bioresources with emphasis on less studied species
- Functional validation of the new genes in model systems and different genetic backgrounds
- Transfer of the validated genes and alleles to recipient species cutting across biological barriers.
- Generation of genomic resource base to facilitate gene prospecting and allele mining
- Prospecting for new genes and alleles for abiotic stress tolerance (moisture stress, salinity and sodicity, soil acidity, adverse temperature and submergence/anoxia)

3. Georeferenced soil information system for land use planning and monitoring soil and land quality for agriculture

NBAIM is a consortium partner in this NAIP Project and its objectives of the project are:

Objectives

1. Microbiological analysis of soil for fungi, bacteria and actinomycetes
2. Estimation of soil dehydrogenase as an indicator

of active biomass.

3. Estimation of soil urease as an indicator of microbial activity

4. Conservation, characterization and documentation of different species of *Alternaria*, *Colletotrichum* and *Cercospora*

Objectives

1. Conservation, characterization and documentation of different species of *Alternaria, Colletotrichum* and *Cercospora*
2. Development of database for Indian isolates of *Alternaria, Colletotrichum* and *Cercospora*

5. Conservation, characterization and documentation of different species of *Fusarium*"

Objectives

1. Conservation, characterization and documentation of different species of *Fusarium*
2. Development of database for Indian isolates of *Fusarium*

Human Resource Development

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Activities being carried out to strengthen human resource

- Organizing National level training programs on "Molecular Characterization" of AIMs at NBAIM for researchers, research students and scientists
- Developing Memorandum of Understanding (MOU) between NBAIM and Banaras Hindu University, Varanasi, Barkatullah University, Bhopal, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar; Sardar Patel Agricultural University, Meerut and Bundelkhand University, Jhansi for academic exchange among research students pursuing Ph.D. programs in order to share the facilities and expertise available in each other's institution.

Specialized Trainings organized

The Bureau is gaining nation-wide recognition for organizing specialized training programs on novel and innovative methods of molecular identification and characterization of AIMs including fungi, bacteria, actinomycetes and cyanobacteria. The aim of these trainings always remains skill development pertaining to microbial taxonomy and modern systematics based on molecular techniques among the researchers, students and faculties from Institutions, Industries and Universities.

During 2009-10, following specialized trainings have been organized.

Trainings Organized:

- The evolutionary diversification of Cyanobacteria: Biochemical, molecular and Phylogenetic approaches from July, 14 to 19, 2009

- Summer School on "Recent advances in Molecular Identification and Characterization of Agriculturally Important Microorganisms" from September 1, 2009 to September 21, 2009 at NBAIM, Mau with the following theme area:

1. Molecular biological tools to study microbial diversity
2. Molecular characterization of AIMs
   - FBacteria
   - FFungi
   - FActinomycetes
3. Biolog Automated Microbial Identification System
4. Development of molecular probe for detection of microbes
5. DNA sequencing and Microbial gene bank.
6. Bioinformatics: Tools and Applications

- National Training Programme in the area of "Molecular Approaches for Identification and Characterization of Actinomycetes" from 01st December 2009 to 10th December 2009 at NBAIM, Mau consisting following theme: (i) chemical taxonomy of actinomycetes; (ii) BIOLOG a carbon source utilization method for microbial identification; (iii) Ribosomal RNA based microbial identification for actinomycetes; (iv) application of Real Time PCR for Molecular detection of microbes; (v) Sequencing Chemistry; (vi) Use of bioinformatics tools in molecular identification.

Meetings and Visits

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Meetings/Conferences/Training attended by the Scientists and Staff of NBAIM

- CIC NAIP meeting at NASC, New Delhi in August 2009.
- Attended the Annual Review Meeting of the AMAAS project at NASC Complex, New Delhi on 25.08.09.
- Delivered lecture before the Hon'ble Agriculture Minister at New Delhi on 09.09.09.
- The CABI Global Summit – Food Security in a Climate of Change held on 19-21 October 2009 at CABI, UK.
- The half yearly review meeting of the AMAAS project at Kasargod on 17.11.2009.
- The Institute Management Committee of the Directorate of Mushroom Research, Solan on 07.12.09.
- The Institute Research Committee meeting on 19.12.2009
- The half yearly review meeting of AMAAS project held on 09.01.2010 at NBAIM, Mau
- The Director's meeting on 15-16th January 2010 at NASC, New Delhi
- The half yearly review meeting of the AMAAS project held on 02.02.2010 at NBAIM, Mau.
- The "Workshop on Sensitization on Networking and Web Hosting" held on February 2-3, 2010 at the National Academy of Agricultural Research Management, Hyderabad.
- Attended Institute Management Committee of NBAIM, Mau on 03.02.2010.
- The Director's Conference on 15-16th February 2010 at NASC, New Delhi.
- Attended the Vice Chancellors' Conference on 17-18 February 2010 at NASC, New Delhi.
- The "National Conference on Quality Seeds and Planting Material – Health Management in Horticultural Crops" from March 11 to March 14 2010 at New Delhi.
- The Research Advisory Committee meeting of the NBAIM on 18.03.2010 at Mau.

ADD THE TRAINING COURSES TAKEN BY SCIENTISTS AND STAFF....

Meetings Organized

- The evolutionary diversification of Cyanobacteria: Biochemical, molecular and Phylogenetic approaches from July, 14 to 19, 2009
- Summer School on "Recent advances in Molecular Identification and Characterization of Agriculturally Important Microorganisms" from September 1, 2009 to September 21, 2009
- National Training Programme in the area of "Molecular Approaches for Identification and Characterization of Actinomycetes" from 01st December 2009 to 10th December 2009
- Organized the Annual Review Meeting of the AMAAS project at NASC Complex, New Delhi on 25.08.09.
- Organized the half yearly review meeting of the AMAAS project at Kasargod on 17.11.2009.
- Organized the half yearly review meeting of AMAAS project held on 09.01.2010 at NBAIM, Mau
- Organized the half yearly review meeting of the AMAAS project held on 02.02.2010 at NBAIM, Mau.
- Organized Institute Management Committee of NBAIM, Mau on 03.02.2010.
- Organized the Research Advisory Committee meeting of the NBAIM on 18.03.2010 at Mau.

Visits Abroad

- Prof Dilip K. Arora attended the UK CABI Global Summit on **"Food Security in a Climate of Change"** from 19-22 October 2009.
- Foreign deputation to Dr. Rajeev Kaushik, Senior Scientist, in the area of Gene Mining in Bacillus at Humboldt University, Berlin, Germany from 16th November 2009 to 13th February 2010.

Awards/Honours

- Associateship of National Academy of Agricultural Sciences, New Delhi is conferred to Dr. Dhananjaya Pratap Singh, Senior Scientist of this Bureau.

Distinguished visitors

- Dr. Mangala Rai, the then Secretary (DARE) and Director General, ICAR, New Delhi; Dr. Swapan K. Datta, Deputy Director General (CS), ICAR, Krishi Bhavan, New Delhi, Dr. H. P. Singh, Deputy Director General (H), ICAR, New Delhi;; Dr. A. K. Singh, Deputy Director General (NRM), ICAR, New Delhi; Sh. Ajay Kumar, Director, DARE, Krishi Bhavan, New Delhi; Dr. A. N. Rai, Vice Chancellor, Mizrom University, Mizoram; Sh. Madhusudan Raizada, Commissioner, Azamgarh Mandal, Azamgarh; Sh. K. K. Tripathi, DIG, Azamgarh, Azamgarh; Sh. A. K. Upadhyay, Registrar, BHU, Varanasi; Dr. S. P. Mathur, Deputy Registrar, BHU, Varanasi; Dr. W. S. Lakra, Director, NBFGR, Lucknow; Dr. R. S. Dwivedi, Ex-Head, BHU, Varanasi; Dr. Sanjay Swarup, Professor, National University of Singapore, Singapore; Dr. N. K. Tyagi, Member, Agricultural Scientist Recruitment Board, New Delhi; Dr. C. M. Singh, Former Director Extension, NDUAT, Kumarganj, Faizabad; Dr. A. N. Mukhopadhyay, Former Vice Chancellor, AAU, Assam; Dr. K. V. B. R. Tilak, Emeritus Scientist, Osmania University, Hyderabad; Dr. S. Shivaji, Scientist –F (Dy. Dir), CCMB, Hyderabad; Dr. R. P. Tewari, Former Director, DMR Solan; Dr. D. L. N. Rao, PC(Biofertilizer), IISS, Bhopal.

Publications

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Publications

Research papers

1. Mahesh S. Yandigeri, Arvind K. Yadav, Kamlesh Kumar Meena and Sunil Pabbi (2010) Effect of mineral phosphates on growth and nitrogen fixation of diazotrophic cyanobacteria *Anabaena variabilis* and *Westiellopsis prolifica*, Antonie van Leeuwenhoek, 97:297–306. (NAAS Jrn ID: A149, rating: 8.5)
2. Mahesh S. Yandigeri, Kamlesh K. Meena, Sunil Pabbi and Anil Kumar Saxena (2010), Effect of phosphate solubilization on biological nitrogen fixation by diazotrophic cyanobacteria, Indian Journal of Microbiology (Accepted)(NAAS Jrn ID: I061, rating: 4.0)
3. Kamlesh K. Meena, Sukumar Mesapogu, Manish Kumar, Mahesh S. Yandigeri, Geeta Singh and Anil K. Saxena (2010), Co-inoculation of the endophytic fungus *Piriformospora indica* with the phosphate-solubilising bacterium *Pseudomonas striata* affects population dynamics and plant growth in chickpea, Biology and Fertility of Soils, 46:169–174. (NAAS Jrn ID: B058, rating: 8.3)
4. Patil, H.J., Srivastava, A.K., Kumar, S., Chaudhari, B.L., Arora, D.K., 2010. Selective isolation, evaluation and characterization of antagonistic actinomycetes against Rhizoctonia solani. World J. Microbiol. Biotechnol. DOI: 10.1007/s11274-010-0400-0 (In press)
5. Yadav A. K., Srivastava A. K., Yandigeri M. S., Modi D. R., Arora D.K. (2010). Characterization of indigenous copper resistant Streptomycetes from Chickpea (*Cicer arietinum* L.) fields, Annals of Microbiology (Accepted).
6. Arvind K. Yadav, Mahesh S. Yandigeri and Dilip K. Arora (2010) Effect of co-inoculation of antagonistic *Streptomyces macrosporeus* strain N19 in chickpea (*Cicer arietinum* L.) against charcoal root rot caused by *Macrophomina phaseolina*. (World Journal of Microbiology and Biotechnology) Comm. (NAAS rating: 7.5).
7. Kamlesh Kumar Meena, Mahesh S. Yandigeri, Geeta Singh and Anil Saxena (2010) Co-inoculation of endophytic fungus *Piriformospora indica* with phosphate solubilizing bacteria *Pseudomonas striata* affects soil phosphatase activity and plant growth attributes in different chickpea (*Cicer arietinum* L.) genotypes, (Mycorrhiza) Comm. (NAAS rating: 8.5).
8. Subhash Yadav, Rajeev Kaushik, A. K. Saxena and D. K. Arora. Influence of Long term irrigation of paper and pulp mill effluent of the Diversity of Bacillaceae. (Biology & Fertility of Soils) Comm.
9. A. K. Yadav, R. Kumar, R. Saikia, T. C. Bora and D. K. Arora. Novel Copper resistant and antimicrobial Streptomyces isolated from Bay of Bengal, India. (Journal of Medical mycology) Comm.
10. A. K. Yadav, S. Vardhan, Rakesh Kumar, Nityanand Malviya, D. R. Modi, A. K. Srivastava, A. K. Saxena and D. K. Arora. Thermostable α-amylase: Purification, production and optimization from streptomycetes spp. (Proceeding of National Academy of Sciences, India) Comm.
11. Arvind Kumar Yadav, Shachi Vardhan, Rakesh Kumar, Ratul Saikia, Dinesh Raj Modi, Alok Kumar Srivastava, and Dilip K. Arora. Production and optimization of protease from Thermoalkalophilic streptomycetes. (Enzyme and Microbial Technology) Comm.

12. Dhananjay Pratap Singh, Anamika Srivastava and Shvianshu Upadhyay. A rapid, sensitive and high yielding standarlized protocol for genomic DNA isolation from filamentous cyanobacteria. (Phycologia) Comm.
13. K. K. Meena, Manish Kumar and D. K. Arora. Genetic Diversity of Aerobic Cultivable Methylotrophic community from Brakishwater chilka lake. (Microbial Ecology) Comm.
14. Binu Mani Tripathi, Rajeev Kaushik, Ram Nageena Singh, A. K. Saxena and D. K. Arora. Genetic and functional diversity of Streptomyces in pulp and paper mill treated crop fields. (Applied Soil Ecology) Comm.
15. Sudhir K. Upadhyay, Shweta Tiwari, Brijendra K. Kashyap, Rameshwar Tiwari, Rajeev Kaushik, D. P. Singh, A. K. Saxena and D. K. Arora. Utilization of salt tolerant PGPR for improving the productivity of wheat crop under saline conditions. (Folia Microbiologica) Comm.
16. D. P. Singh, Shivanshu Upadhyay, Arvind Kumar, Anamika Srivastava and D. K. Arora. Cyanobacteria mediated induced metabolic responses in rice correlates with plant health. (International Symposum on Phycological Research 2010) Comm.
17. Arvind K. Yadav, Mahesh S. Yandigeri and Dilip K. Arora. Effect of coinoculation of antagonistic Streptomyces in chickpea (Cicer) against charcoal root rot caused by Macrophonica phaseolina (tassi) grid. (Biological control) Comm.
18. Shachi Vardhan, Rajeev Kaushik, Anil Kumar Saxena and Dilip K. Arora. ARDRA and identification of Bacillus upto sp. level. (Antonie Van Leewenhock) Comm.
19. Manoj K. Solanki, Nidhi Singh, Alok K. Srivastava, Sudheer Kumar and Dilip K. Arora. Chitin mediated enhancement of bicontrol afficacy of Trichoderma against tomato root rot. (Communicated)
20. Harmesh Sahay, Rajeev Kaushik, Surinder Singh, A. K. Saxena and Dilip K. Arora. Culturable diversity of moderately halphilic bacteria. (Journal of Microbiology and Biotechnology) Comm.
21. Upadhayay S. K., Tiwari S., Kashyap B.K., MishraB.K., Tiwari R., Kaushik R., Saxena A.K., Arora D.K. 2008. Utilization of salt tolerant PGPR for improving the productivity of wheat crop under saline conditions. (Folia Microbiologica) Comm.

Books:

- Mahesh S. Yandigeri, Dilip K. Arora and Arvind K. Yadav (2010) 'Synoptical Keys for Identification of Streptomyces Genera' published by National Bureau of Agriculturally Important Microorganisms (NBAIM), Kushmaur, Mau Nath Bhanjan (U.P.), India (ISBN: 978-81-909892-0-6).

Book chapters:

- Mahesh Yandigeri, Sukumar Mesapogu, Arvind Yadav and D. K. Arora (2010) Microbial Culture Banks: Custodian of Real Natural Wealth. In: Souvenir of National Conference on Biodiversity, Development and Poverty Alleviation on the occasion of International Day for Biological Diversity (22nd May 2010), H.B. Singh, R.J. Srivastava, D.P.Singh, B.K. Sarma and R.K. Dubey (Eds.), Uttar Pradesh State Biodiversity Board, Lucknow, pp. 34-39.

Abstracts:

1. Yadav A., Malviya N., Yandigeri M. S., Roy M., Singh D., Srivastava A.K. and Arora D. K. Genotypic diversity of actinobacteria from Indo-gangetic plains of India: In 50th Annual Conference of AMI, 15-18 December, 2009 at NCL, Pune.
2. Yadav A., Malviya N., Yandigeri M. S., Singh D., Roy M., Srivastava A.K. and Arora D. K. Actinobacterial diversity from Sambhar salt lake: In 50th Annual Conference of AMI, 15-18 December, 2009 at NCL, Pune.
3. Field evaluation of Bacterial consortia to alleviate salt stress for growth and yield of wheat. (First Asian PGPR Congress 2009, Hyderabad)
4. Molecular Diversity of Halotolerant bacteria from Chilka Lake, India. (50th AMI Conference 2009, Pune)

Training manuals

- The evolutionary diversification of Cyanobacteria: Biochemical, molecular and Phylogenetic approaches
- Summer School on "Recent advances in Molecular Identification and Characterization of Agriculturally Important Microorganisms"
- National Training Programme in the area of

"Molecular Approaches for Identification and Characterization of Actinomycetes"

DNA sequences deposited at GenBank:

- Yandigeri, M.S., Malviya, N., Yadav, A.K. and Arora, D.K. (2010). Diversity of actinomycetes from Indogangetic plains of India. GenBank Accession No. GU817410 (*Streptomyces macrosporeus* N19), GU817411 (*Streptomyces albogriseolus* N40), GU817412 (*Streptomyces griseorubens* N45), GU817413 (*Streptomyces albogriseolus* N53), GU817414 (*Streptomyces viridodiastaticus* N71).

Linkages and Affiliations

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Local Institutes

The NBAIM has effective linkages with different ICAR research institutions and SAUs situated in U.P. Since the Bureau is actively participating into the Networking with NRC's on microbial biodiversity along with other Institutes having expertise. These linkages also provide library facility, characterization and fingerprinting of selected AIMs, including logistic support in R&D activities.

National Institute, Agricultural Universities and organizations

The NBAIM has strong linkages with national institutes, agricultural and conventional universities, other Government and Non-Government organizations. We are strengthening the "Indian Microbial Genetic Resources Management System" by making up the National base for collection and deposition of AIMs meant for long term storage and evaluation. The Bureau is presently linking with different small and private organizations along with individual collector of AIMs and persuading them to deposit their collection at NBAIM.

International Institutes

- NBAIM is an affiliated member of World Federation of Culture Collection (WFCC).
- The Bureau has linkages with International microbial resource centres covered under the umbrella of WFCC and OCDE. NBAIM has imported cultures from CABI, Bioscience, U.K., ATCC, USA, HUT, Hiroshima University, Japan, Fungal Genetic Stock Centre, USA, Agricultural Research Service Culture Collection, USA., Bacillus Genetic Stock Centre, USA
- Under the World Bank aided National Agricultural Technology Project, ICAR approved projects under Bioscience

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Research Advisory Committee (RAC)

Chairman

Dr. A. N. Mukhopadhyay
Former Vice Chancellor
Assam Agricultural University, Assam

Members

Dr. T. P. Rajendran
ADG (PP), ICAR
New Delhi

Dr. A.N. Rai
Vice Chancellor
Mizoram University
Mizoram

Dr. S. M. P. Khurana
Director
Amity Institute of Biotechnology
Amity University, Noida

Dr. K.V.B.R. Tilak
Emeritus Professor
Department of Botany
Usmania University
Hyderabad

Dr. O.P. Rupela
Consultant, FAO
Hyderbad

Prof. D.K. Arora
Director, NBAIM

Member Secretary

Dr. Alok K. Srivastava
Senior Scientist, NBAIM

Recommendations

1. Acute shortage of scientists and technical staff should be considered very seriously by the ICAR and man power should be posted here at the earliest.
2. Cryopreservation facility need to be developed at the Bureau and an expert consultant(s) for the same be appointed at the discretion of the Director, NBAIM for short durations.
3. More scientists of the Scientists from the NBAIM should get trained in metagenomics, community analysis, and other specialized areas of agriculturally important microorganisms at abroad and ICAR should be requested to manage the same.
4. A separate building and infrastructure for the MGRR may be developed to strengthen the facility.
5. The culture collection may be equipped with DNA finger printing and EGSS facilities.
6. Storage of the patentable cultures may be encouraged by putting it as "safe deposit" and by ensuring the depositer that the same should not be distributed without the prior permission of the depositer.
7. NBAIM should get accreditation by the recognized govt. body like DAT and other International accreditation agencies. It should be strengthened with the biosafety concerns and P3 labs. This will raise the reputation of the Bureau in National and International arena.
8. Director should be authorized to appoint consultant for short term (2 -4 weeks) and the services may be extended thereafter.
9. NBAIM should invite expert people from the Nation to improve its inhouse capacity. Also it should organize National seminars/conferences for the exchange of ideas.
10. RAC felt that the cultures stored by the NBAIM should be placed as "Replica" at some other places, may be in Delhi and the Council should generate facilities for the same.
11. SRFs of the Bureau should be encouraged to registered themselves for the Ph. D. programs at Universities.

Institute Management Committee (IMC)

Chairman

Prof. Dilip K. Arora
Director, NBAIM

Members

Dr. R. P. Tewari
Former Director
DMR, Solan

Dr. D. L. N. Rao
Project Co-ordinator
IISS, Bhopal

Dr. S. K. Sharma
Director, NBPGR
New Delhi

Dr. C. Manoharachary
Emeritus Professor
Department of Botany
Usmania University
Hyderabad

Member Secretary
Dr. Alok Srivastava
Senior Scientist, NBAIM

Recommendations

1. The IMC recommended that the Bureau should renovate the hostel in order to provide necessary facilities to SRF and RAs as given below:
2. In every room reading table, chair and bed with mattress may be provided.
3. The windows of rooms may be fixed with alluminium section and slide glass panels.
4. The expenditure for room furnishing and window fixing should be meet from furniture fixture budget of NBAIM plan
5. The cutting and policing of room floors should be done. Small wash basin with running water tap may fixed inside the room. Necessary provision may be done under minor repair.
6. IMC approved the purchase of tissuelyser
7. The budget allocated under works (NBAIM plan) and AMAAS was discussed in detail
8. It was felt and recommend that Rs. 200 lakhs sanctioned for construction of Auditorium may be returned to ICAR as the work may not be started in current financial year as CPWD could not provided even preliminary estimate despite of repetitive efforts by NBAIM.
9. The budget allocated for MGRR lab and extension of new lab may be transferred to CPWD after getting the approval from Director Works ICAR
10. IMC appreciated the efforts for revenue receipt.
11. IMC was satisfied with replies of the para related to use of equipments and other issued related to staff welfare.
12. IMC recommended the posting of Scientists to the NBAIM. IMC further suggested that the posts of Senior Scientist etc. will be advertised with specialization in the related fields and the matter should be taken up at priority level through the Director (Plan), ICAR.

NBAIM Personnel

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Scientist	Staff
Dilip K. Arora Director Rajeev Kaushik Senior Scientist Alok Kumar Srivastava Senior Scientist Sudheer Kumar Senior Scientist Dhananjaya P. Singh Senior Scientist Anurag Chaurasia Scientist Mahesh Yandigeri Scientist Kamlesh Kumar Meena Scientist Udai Bhan Singh Scientist	S. N. Yadav Shyamji Shukla Ashok Kumar Manish Kumar Jain Sudesh Kumar Satish Pal Pratap Singh Mahesh Yadav Pilloo Meena Anchal Kumar Srivastava Amit Kumar Rai S. S. Reddy Rajkumar Meena Alok K. Upadhyay Manish Kumar Roy Ashutosh Rai Amar Nath Singh Patel Manoj Kumar Bali Ram Chetan Singh Ram Gopal Rekha Gupta Chandra Kishore Anil Kumar Rana Ram Avadh Singh Asheesh Kumar Ajay Kumar Vishwakarma

कार्यकारी सारांश

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प्रशासकीय संक्षेपण

कृषि उपयोगी सूक्ष्मजीवों के अनुप्रयोग, प्रबन्धन, अनुरक्षण, पृथक्करण और संरक्षण के क्षेत्र में अपनी जिम्मेदारियों को निभाते हुए राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो देश के महत्वपूर्ण संस्थानों में से एक है। यह ब्यूरो सूक्ष्मजैविक विविधता की स्थापना, जीव वैज्ञानिक नियंत्रण, जीनोमिक्स (जीनमिति), सूक्ष्म संग्रहण, रख-रखाव और सूक्ष्मजैविक जीन पूल बनाने के बहुआयामी कार्यों को पूरा करने में लगा हुआ है। सूक्ष्मजीवों की आण्विक पहचान और उनके गुण-चिह्नांकन के लिए शिक्षा और प्रशिक्षण द्वारा अनुसंधायकों, वैज्ञानिकों, छात्रों और उद्यमियों में तकनीकी और वैज्ञानिक दक्षता का विकास करना ब्यूरो के मुख्य उद्देश्यों में से है और इस दिशा में उल्लेखनीय प्रगति हो रही है।

देश में सूक्ष्मजैविक सम्पदाओं की राष्ट्रीय धरोहर के अनुरक्षण और रख-रखाव के राष्ट्रीय उद्देश्यों के लिए वर्ष 2001 में ब्यूरो की स्थापना की गयी और अपनी स्थापना के साथ ही पूरी वचनबद्धता के साथ ब्यूरो ने कार्य करना प्रारम्भ कर दिया। चूँकि कृषि उपयोगी सूक्ष्मजीवों का अनुरक्षण किसी भी देश के लिए एक चुनौतीपूर्ण कार्य रहा है अतः सूक्ष्म जैविक विविधता की आगामी चुनौतियों से निपटने, आधारभूत और प्रायोगिक अनुसंधान कार्यों और सूक्ष्म संग्रह केन्द्र के सफल संचालन हेतु अनुसंधानकों, छात्रों और सेवारत वैज्ञानिकों में तकनीकी विशेषज्ञता और उच्च तकनीकी ज्ञान का आधार तैयार करने के लिए ब्यूरो का कार्य-अधिपत्र बनाया गया है।

गत वर्षों में राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो द्वारा सूक्ष्मजीवियों को एकत्र करने, जीन संसाधन भण्डार तैयार करने और सूक्ष्मजैव तकनीकी क्षेत्र में एक सक्रिय और उत्तरदायी संस्था के रूप में समुचित प्रयास किये गये हैं। अपने समर्पित वैज्ञानिक और तकनीकी कर्मचारियों के सहयोग, सुसज्जित भवन, अनुसंधान और विकास कार्यों एवं अत्याधुनिक उपकरण सुविधाओं के साथ हम अपने महान राष्ट्रीय उद्देश्यों की पूर्ति में लगे हुए हैं। वर्ष 2009-10 की शानदार और बहुआयामी उपलब्धियॉ निम्न हैं-

- इस वर्ष ब्यूरो के सूक्ष्म संग्रह केन्द्र में 3500 सूक्ष्मजैविक अवाप्तियों का लक्ष्य प्राप्त किया गया। इस सूक्ष्म संग्रह में बैक्टीरिया, फंजाई, एक्टीनोमाईसीट्स एवं नील हरित शैवाल की अनेक प्रजातियों के सूक्ष्मजीव शामिल हैं। यह सूक्ष्म संग्रह इकाई विविध सूक्ष्मजीवों के गुण-चिह्नांकन, अनुरक्षण और रख-रखाव करने, उनके अवाप्ति क्रमांक देने, लाइफोलाइजेशन सूची तैयार करने, उनके पुनरूज्जीवन और इलैक्ट्रानिक सर्विलॉस के लिए, (कस्टम मेड डाटा मैनेजमेंट साफ्टवेयर में) इलैक्ट्रानिक फार्मेट आदि कार्यों के लिए पूर्ण रूप से सुसज्जित है।
- माइक्रोबियल जीनोम रिसोर्स रिपोजिटरी (एम0 जी0 आर0 आर0) द्वारा वर्ष के दौरान कई महत्वपूर्ण उपलब्धियॉ प्राप्त की गयी हैं।
- 350 बैक्टीरिया, 304 फंजाई, 65 राइजोबियम, 55 एक्टीनोमाईसीट्स और 27 सायनोबैक्टीरिया के जिनोमिक डी0एन0ए0 मिलाकर कुल 801 जीनोमिक डी0एन0ए0 संग्रहीत किये गये हैं। इसके अलावा लालकुँआ पेपर मिल जल प्रवाह से सिंचित कृषि भूमि से प्राप्त 139 मैटाजीनोमिक क्लौन को भी संरक्षित किया गया है। राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो में चल रही आन्तरिक परियोजनाओं से 203 जीन अनुक्रमों को भी संरक्षित कर लिया गया है। विविध टी-वैक्टर्स और मार्कर्स-एसिस्टेड वेक्टर्स को ग्लिसरॉल में संरक्षित किया गया है।
- वैज्ञानिकों और तकनीकी कर्मचारियों की कार्यकुशल टीम के साथ ब्यूरो भारतीय कृषि अनुसंधान परिषद, सी0 एस0 आई0 आर0, डिपार्टमेंट ऑफ बायोटैक्टनौलॉजी, डिपार्टमेंट ऑफ साइंस एण्ड टैक्नौलॉजी, राज्य और केन्द्रीय विश्वविद्यालयों, कृषि संस्थानों और अन्तर्राष्ट्रीय सूक्ष्मजैव संसाधन केन्द्रों समेत कई राष्ट्रीय संस्थाओं से अपने संबंध मजबूत कर रहा है। बैक्टीरिया, फंजाई, एक्टीनोमाईसीट्स, मिथाइलोट्रौप्स, सायनोबैक्टीरिया की आण्विक पहचान और गुण-चिहनंकन पर अपने ज्ञान को बढ़ाने के लिए देश-विदेश से वैज्ञानिक, प्रवक्ता और शोध छात्र बार-बार राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो

में आते हैं। वर्ष 2009-10 के दौरान तीन राष्ट्रीय प्रशिक्षण कार्यशालाएँ और भारतीय कृषि अनुसंधान परिषद द्वारा प्रायोजित एक ''समर स्कूल'' का आयोजन किया गया जिसमें कुल 150 प्रतिभागियों ने भाग लिया।

- राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो भारत सरकार द्वारा प्रायोजित एक राष्ट्रीय परियोजना ''नेशनल एग्रीकल्चर इनोवेशन प्रौजेक्ट'' (एन0 ए0 आई0 पी0) के एक मैगा प्रोजेक्ट ''बायोप्रोस्पेक्टिंग आफ माइक्रोबियल जीन्स एण्ड एलील माइनिंग फार एबायोटिक स्ट्रेस टॉलरेंस'' पर कार्य कर रहा है। एक अन्य एन0ए0आई0पी0 वित्त पोषित परियोजना ''डायवर्सिटी एनालिसिस आफ बैसिलस एण्ड अदर प्रीडोमिनेन्ट जेनेरा इन एक्सट्रीम एनवायरनमेंट एण्ड इट्स यूटिलाइजेशन इन एग्रीकल्चर'' में ब्यूरो एक सहयोगी संस्था के रूप में अगुआई कर रहा है।
- राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो द्वारा ''एप्लीकेशंस आफ माइक्रोआर्गनिज्मस इन एग्रीकल्चर एण्ड एलाइड सेक्टर्स'' ;।ड।ैद्ध नामक नेटवर्क परियोजना एक प्रमुख केन्द्र के रूप में सफलतापूर्वक चलायी जा रही है और पूरे देश में फैले इसके 61 केन्द्रों पर इसका प्रबन्धन ओर समन्वयन (सह-संचालन) किया जा रहा है। इस परियोजना में 81 पी0आई0, 139 को-पी0आई0 और 139 अनुसंधान अध्येता और अनुसंधान सहायक लगे हुए हैं।
- अमास नेटवर्क परियोजना के अन्तर्गत हुए प्रशंसनीय अनुसंधान और विकास कार्यों को प्रलेखित किया गया है। अत्याधुनिक 454 पायरोसीक्वेंसर का प्रयोग करके महत्वपूर्ण बैक्टीरिया मेसोराइजोबियम साईसेरी जीवाणु के पूरे जीन अनुक्रम को प्रलेखित किया जा चुका है।
- 2009-10 के दौरान तीव्र सम्भावना वाले देश के विभिन्न भागों में 20 से अधिक खोजी अभियान (सर्वे) किये गये। जिन स्थानों का सर्वे किया गया उनमें उत्तर प्रदेश, हिमाचल प्रदेश, राजस्थान, बिहार, उड़ीसा, पश्चिम बंगाल, अरूणाचल प्रदेश, आसाम, सिक्किम, मेघालय, केरल जैसे राज्यों के विभिन्न परिवेशों की मिट्टी, वनस्पति, ताजे पानी, गर्म जल स्रोतों के नमूनों, समुद्री और कच्छ जलवायु परिवेशों तथा कृषि अपशिष्ट मृदा आदि के नमूने लिए गये ताकि सूक्ष्मजीव और उनके उपोत्पादों का पृथक्करण, पहचान और गुण-चिहनंकन किया जा सके।
- कुछ महत्वपूर्ण सूक्ष्मजीव जैसे बैसिलस, स्युडोमोनास, फुजेरियम, मैक्रोफोमिना, सिरेशिया आदि के आण्विक अनुप्रतिमुद्रण (फिंगरप्रिंटिंग) का कार्य पूर्ण किया गया। महत्वपूर्ण सूक्ष्मजीवाणुओं के जीन अनुक्रम लेकर जीन बंकि, एन0सी0बी0आई0 में रखे गये और उन्हें अवाप्ति क्रमॉक (असेसन नम्बर) प्रदान किये गये।
- मिथाइलोट्रॉप्स, एक्टीनोमाईसीट्स एवं सायनोबैक्टीरिया समेत विभिन्न वर्गों के जीवाणुओं के जैव एवं रासायनिक पूर्वेक्षण किये गये और जिन पौधों में इन जीवाणुओं के टीके दिये गये वे ''हाई परफोरमेंस लिक्विड क्रोमेटोग्राफ'' ;भ्च्स्ब्द्ध के माध्यम से फाइटोहारमोनस, एमीनोएसिड्स, कार्बोहाईड्रेट्स एवं एल्कालोइड्स आदि के पृथक्करण के लिए प्रयोगाधीन हैं।
- वर्तमान में, फसलोत्पादन में अति महत्व के सूक्ष्मजीवों की एक विशाल विविधता राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो के पास संरक्षित है-
- जैव नियंत्रण उत्प्रेरकः ट्राइकोडर्मा प्रजातियॉ, पीसाइलोमाईसीज थर्मोस्कस, पीसाइलोमाईसीज लीलासिनस, बिवेरिया प्रजातियॉ, ग्लायोक्लैडियम विरेंस एवं वर्टिसीलियम प्रजातियॉ.
- जैव कीटनाशकः बिवेरिया प्रजातियॉ, मेटारीजियम प्रजातियॉ, पीसाइलोमाईसीज प्रजातियॉ, वर्टिसीलियम एवं नोमुरिया प्रजातियॉ.
- वृद्धिवर्धकः स्युडोमोनास फ्लुरिसेंस, स्युडोमोनास सिरिंगि, राइजोबियम प्रजातियॉ एवं ब्राडिराइजोबियम प्रजातियॉ.
- किण्वक, प्रति-जैविक, जीव-विष उत्पादकः फुजेरियम पेलीडोरोसियम, फुजेरियम औक्सीस्पोरम, पेन्सीलियम साइट्रोनम, पेन्सीलियम फ्रीक्वेन्टेंस एवं एजपरजीलस आदि।
- कीटरोग मूलकः बिवेरिया, मेटारीजियम, वर्टिसीलियाम एवं नोमूरया प्रजातियॉ.
- अण्ड परजीवी कवकः पीसाइलोमाईसीज लीलासिनस, वर्टिसीलियम, कैलेमाईडोस्पोरियम, माइकोपैरासिटिक फॅजाई एवं ग्लियोक्लैडियम प्रजातियॉ.
- बैक्टीरिया प्रसंस्करण कृमिनाशी और कीटनाशी पदार्थः बैसिलस आर्कुलांस, बैसिलस ब्रेविस, पेनीबैसीलस अल्वेई एवं ब्रेविबैसिलस लैटरोस्पोरस.
- जैव उर्वरकः नोस्टोक, एनाबिना, राइजोबियम, ऐजोस्पिरिलम और एजोटोबेक्टर आदि।