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NBAIM
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राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो
NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT MICROORGANISMS
Understanding and conserving our national heritage of agriculturally important microorganisms

Kusumaur, Mau Nath Bhanjan - 275 101 (U.P.)

Published by
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Preface

Microorganisms are among the most ancient and adaptable organisms on the earth. They have a successful evolutionary passage across a very long period with their aptitude to adapt to diverse and often hostile environments, combined with the remarkable power of natural selection. Along with these characteristics, extremely short generation time has made these microorganisms the most resilient of life forms on this planet. As such, bacteria, cyanobacteria, actinomycetes and fungi inhabiting the soil are essential contributors in maintaining the ecological balance and agricultural wellbeing.

Looking into the potential threat to these microbes from the climate change and the possibilities of losing the microbial diversity and populations from a defined habitats in an unpredicted manner, it is very important to conserve and preserve these natural heritage for future applications. With this aim, NBAIM has successfully stored verified cultures of more than 4000 microbial accessions in different short to long-lasting preserving conditions in its culture collection "National Agriculturally Important Microbial Culture Collection (NAIMCC)". In a similar manner, the Microbial Genomic Resource Repository (MGRR) at NBAIM is conserving the genomic materials (DNA, RNA, clones, vectors etc.) of different agriculturally important microbial accessions that can be used by the researchers working with other organizations from time to time.

Because of their cosmopolitan occurrence in almost all kinds of normal, sub-normal to extreme habitats, microorganisms posed themselves as a living population with remarkable genetic, phenotypic, morphological and biochemical adaptive properties that are intrinsic to the species. These organisms, when identified and explored for their natural behavior, can act as a prototype for molecular, biochemical and physiological manipulations rendering their direct or indirect benefits to crop plants. Explorations made by the NBAIM during this year in search of microorganisms with potential characteristics from extreme habitats resulted in the identification of numerous bacteria, fungi and actinomycetes, many of them are even being reported for the first time from these habitats. The cold, heat, salt and osmo-regulatory adaptive potential of these microbes were characterized along with their *in vitro* plant growth promoting traits and certain organisms were truly potent. Further research on these organisms will uncover not only many characteristic features for their adaptability but also for their usefulness in agriculture.

It is now widely being realized that agriculturally important microorganisms have a vast potential to balance whole agroecology and transform the conventional way of performing agriculture converting into low-input-based agro-ecosystem. Their potentiality can be recognized not only in the form of their living associations with crop plants in nature or live application of bioinoculants (biofrertilizers & biopesticides) as seed or soil treatment but in the form of their potent genes, alleles and powerful metabolites that can be used in crop improvement programs. This is why, one of the most remarkable developments of the twentieth century is the discovery of the plant growth promoting microorganisms (PGPRs) that can offer a vast array of beneficial attributes to plants and inhabiting soils and thereby enhance crop productivity and soil fertility in a sustainable manner. More than 97% of our food requirements are fulfilled from the terrestrial agro-ecosystems and diverse microbial communities in varied population occur in agricultural fields to contribute for crop productivity directly or indirectly. Plants provide a valuable ecological niche for microorganisms and below ground soils along with plant roots (rhizosphere) are constantly associated with numerous microorganisms reaping benefits from microbial associations. The area of plant-

microbe interactions therefore, has remained central to the research focus of the NBAIM and several certain projects that are based on this central theme have uncovered a number of new facts on this topic. A number of fungi and bacterial isolates with promising antibiosis, enzymolytic activities and root colonizing potential were identified from the natural habitats. Rigorous and high-throughput screening of these isolates in the fields against many soil or seed plant pathogenic borne bacteria or fungi will lead to the development of next generation biopesticides with better adaptations in diverse climatic conditions.

This year has witnessed with the organization of successful National trainings on different aspects of molecular biology of microbes, metagenomics and bioinformatics. Along with the other NAIP projects successfully running the Bureau has been made the official partner of one of the prestigious ICAR's project the "Establishment of National Agricultural Bioinformatics Grid in ICAR".

I credit the constant support from the Indian Council of Agricultural Research, New Delhi. I also extend my earnest appreciation to all the scientists of the Bureau who have helped a lot in shaping this Annual Report 2010-11 in a nicely presentable manner.

Dilip K. Arora
Director

Executive Summary

Microbes are important for the Earth System, playing a very important role in maintaining the wellbeing of our global environment. Despite the obvious importance of microbes, very little is known of their diversity and its ecological function. Microorganisms are most vital life forms on the Earth and have no replacements in many areas. Be it their role in environmental succession and biogeochemical cycle, plant interactions and crop improvement, food chain, soil fertility management, agro waste recycling and bioremediation, ecosystem processing and intact relationship with higher organisms, they are the real silent workers performing their duties day and night with no reward.

National Bureau of Agriculturally Important Microorganisms (NBAIM) is functioning with the mandate to explore, identify, conserve, preserve and exploit agriculturally important microorganisms for their useful traits in agricultural well being. The Bureau aims to excel in isolation and utilization of genes for conventional and unforeseen products of high economics and value in environment and agriculture. NBAIM continues to fulfill its mandate to make Indian agriculture locally, regionally and globally competitive.

The NBAIM has well-equipped research laboratories, Central instrumentation facility, separate Fungal and Bacterial labs, Molecular Biology lab, Genomics unit with ultramodern instrumentation, Lyophilization unit, Culture collection facility, including Cyanobacterial culture unit, newly developed Microbial Genome Resource Repository (MGRR), DNA Finger Printing Lab, Bioinformatics Lab, administration block, scientists' lobby, library, Conference hall and mini-conference rooms with state-of-the-art audio-visual equipments and Agricultural Research Information Service (ARIS) cell and Prioritization, Monitoring & Evaluation (PME) Cell etc. To ensure regular water and electricity supply, tube-wells 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.

- A landmark development was the establishment of the National Agriculturally Important Microbial Culture Collection (NAIMCC) which consists of storage facility capacity for 10000 agriculturally important microorganisms. The updated second edition of "Catalogue of Strains - 2011" at NAIMCC repository is published and available having around 4000 holdings 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, information for registration of microbial cultures, IPRs issues, information about the World Federation for Culture Collections, various depositories in world and in India and their holdings, etc.
- All the microbial data available at NBAIM have been digitized and put-up in a retrievable format. Software 'MCCD' was developed at NBAIM for the digitization of AIMs. The software is on MySQL database management system. A variety of data can be accommodated in fields like information on passport data of a culture like its geographical location of isolation , name of the donor (person or Institute), name of the depositor, cultural details, the form in which it is preserved, etc. It has space for images, maintains inventory for lyophylization, generates bar codes, reminder for revival time of culture, etc.
- The first whole genome sequence of one of most agriculturally important bacterium *Mesorhizobium ciceri* Ca. 181, from India was done under leadership of NBAIM in "Application of

Microorganisms in Agriculture and Allied Sectors" (AMAAS). This is a landmark achievement in the frontier area of biological research that involves mastering of latest techniques in genome sequence assembly and annotation. The estimated genome size of *M. ciceri* Ca181 is 6.47 Mbp and it has 6742 predicted genes in 4116 transcription units. Several of these identified genes are involved in biological nitrogen fixation (34 genes) and stress tolerance (184 genes). This discovery may open up the doors for intensified research on Rhizobium strain improvement for bio-fertilizer applications. The information could also be utilized for the preparation of genetically modified improved rhizobium strains that might increase productivity and yield in pulses.

- Six mangrove ecosystems of India *viz.*, Sundarbans (West Bengal), Bhitarkanika National Park (Orissa), mangroves of Gujarat, Coringa mangroves (Andhra Pradesh), Pulicat Mangroves (Tamil Nadu) and mangroves of Goa were surveyed for the isolation, characterization and mapping of actinobacterial flora. A total of 167 morphotypes of actinomycetes were isolated using different enrichment techniques and media. These isolates were subjected to morphological, microscopic, biochemical and physiological characterization.
- The evaluation of microbial population for understanding its biogeography, community assembly and ecological processes within a particular exotic niche has been done. Exotic niches harbor population of microorganisms they represent extreme niches that have maintained some degree of pristine quality and their biotechnological potential has remained unrealized. This year 223 extremophilic microorganisms were isolated belonging to different community and genera's such as 55 and 40 halophilic fungi and bacteria from goa mangroves, 50 acidophilic isolates from tipong coal mines, 53 thermophilic isolates from sikkim hot springs and 25 psychrophilic actinomycetes from leh regions, India, respectively. They showed varied structural diversity (evident by plate photograph and Scanning electron microscope), as well as fuctional diversity such as PGP attributes (phosphates solubilizers, siderophore producers), extracellular enzyme produers (protease producers, amylase producers, cellulase producers, lipase enzyme producers).
- The isolates showed growth at 20%NaCl growth at pH 3.0 -5.0, 53 conserved in the culture collection. The results provide further evidence that, the isolation of culturable microorganisms can significantly contribute to our knowledge of species endemic to these extremophilic regions.
- 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 like plant growth promoting traits. These traits will find utilization in increasing the crop growth and biocontrol activities against plant pathogens. Further the actinomycetes will be mapped based on the structural and diversity according to the mangroves.
- NBAIM has initiated work on archaeal community structure and diversity in India. Till now a meager work has been carried on archaea from cold deserts, hot springs and saline and alkaline conditions. The project will be helpful in identifying the archaeal communities and their diversity from different ecological niche especially mineral rich ore and heavy metal contaminated industrial effluents. Their characterization will give the valuable information about the archaeal diversity and communities arising from the project will add to the existing microbial diversity studies carried out at the bureau and in turn fulfill the mandate of the Bureau.
- The Bureau has HRD component in which training programs are organized as per the mandated activities of NBAIM. This year 4 trainings have been organized in the area of metagenomics and bioinformatics The website (www.nbaim.org.in) of NBAIM was created and all the units of the NBAIM are linked with various ICAR research institutes, its design is based on the ICAR guidelines for uniformity of web contents and launched in 2010.

Infrastructure

The NBAIM has well-equipped research laboratories, Central instrumentation facility, separate Fungal and Bacterial labs, Molecular Biology lab, Genomics unit with ultramodern instrumentation, Lyophilization unit, Culture collection facility, including cyanobacterial culture unit, newly developed Microbial Genome Resource Repository (MGRR), administration block, scientists' lobby, library, Conference hall and miniconference rooms with state-of-the-art audio-visual equipments and Agricultural Research Information Service (ARIS) cell etc. To ensure regular water and electricity supply, tube-wells 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.

The administrative, finance and Director's personal section are equipped with all modern equipments like computers with internet facility, printers (Black & white as well as coloured), photocopier machines, Colour Xerox machines, lamination machine, poster printing machine, fax machine, etc.

National Agriculturally Important Microbial Culture Collection (NAIMCC)

Biodiversity Authority of India recognized the NBAIM culture collection as the National Repository. The bureau follows strict quality control and biosafety standards in the culture collection as well as in laboratories. Various types of microorganisms including filamentous fungi, bacteria, actinomycetes and yeasts are maintained under the long-term preservation. Each culture is preserved by at least two methods according to the type of microorganism. The National Agricultural Important Microbial Culture Collection (NAIMCC) has published its second addition of catalogue of microbial holdings. It has about 4000 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. In addition to that catalogue also has composition of different culture media, deposition forms, long term storage protocols and information for registration of microbial cultures. The NAIMCC has state of art facility for isolation, conservation, maintenance and storage facility for nearly 10000 microbial holdings.

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

Research facilities

The NBAIM has well-equipped research laboratories, Central instrumentation facility, separate Fungal and Bacterial labs, Molecular Biology lab, Genomics unit with ultramodern instrumentation. All the laboratories are equipped with most modern instruments required to carry-out research work in the molecular biology and microbial biotechnology. To name a few are:

- Scanning Electron Microscope (SEM)
- Confocal Laser Microscope
- Automated Lyophilization Unit
- Liquid Nitrogen Plant and Cryopreservators
- FLX454 pyroseqencing unit with hydroshear, emulsion PCR and cluster analysis
- 16 capillary ABI 3130xl automated genetic analyser
- Mini high performance computing system with bioinformatic softwares "Workstations for data annotation and analysis
- Real Time PCR
- Chemiluminiscence gel imaging system
- Robotic liquid handing system
- DNA bar-coding system
- Pulse field gel electrophoresis

- DGGE unit
- Chip based high throughput electrophoresis
- 2D gel eletrophoresis
- Metabolic growth kinetic analyzer (Bioscreen)
- Fatty acid methyl ester (FAME) analyzer
- High performance liquid chromatography (HPLC)
- Gas liquid chromatography (GLC)
- Thermocyclers
- Atomic absorption spectrophotometer
- BIOLOG microbial identification system
- RT-PCR
- Automated media preparator
- Spectrophotometer
- Ultra centrifuge
- Refrigerated incubator shaker
- Deep freezers (-80°C)
- Walk-in cold rooms
- Lyophilization
- Glass house
- MIDI identification system

Library

NBAIM library has well equipped facilities including reading and periodicals sections as well as reprography, computer and internet facilities. It has collections of fourteen scientific journals/periodicals and a number of books belonging to various subjects like bacteriology, biochemistry, bioinformatics, bioinstrumentation, biotechnology, botany, environmental sciences, integrated pest management, microbiology, molecular biology, phycology, plant pathology, virus, mycology, genetics, genomics, administration and miscellaneous literature. The library subscribes many national and international journals, has Wi-Fi internet facility though BSNL VSAT and access to various journals through Consortium of e-Resources in Agriculture (CERA).

Agricultural Research Information System (ARIS) CELL

The ARIS cell has been established in the Bureau with the latest facilities and user friendly softwares. The cell is also providing internet facilities to scientists and other staff. Microbial database of the existing culture collection is being maintained at ARIS Cell.

IPR and Bio-safety Cell at NBAIM

NBAIM has established an IPR cell for the management of intellectual knowledge and technologies generated at NBAIM, 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.

NBAIM website

NBAIM website (http://www.nbaim.org.in) contains updated information about various activities of the Bureau in different profiles *viz*., mandate, about the Bureau, culture collection, scientific plan, gene bank, library, 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. The website has links with ICAR and other research institutions, SAUs, culture collections etc.

National Genomic Resource Repository

Microbial Genomic Resource Repository (MGRR) has aims to collect and long term conservation of genomic resources like microbial DNA, clones, novel gene constructs vectors etc. The different forms of microbial genetic material e.g. DNA, RNA, cDNA, mRNA, plasmid, cosmid, primer and vector, etc. can be utilized for further research in agriculture in many ways like, for the increment of the soil fertility, crop production, crop quality and their resistance to diseases. MGRR will provide all those genetic material to the researchers/scientists, working in the field of molecular biology and microbial genomics. MGRR is maintaining the genes responsible for nitrogen fixation, nitrogen assimilation, root nodulation, bioremediation, phosphate solubilization, disease resistance, salt tolerance, stress resistance and biocontrol, etc. could be exploited to enhance crop productivity. MGRR DNA Bank has developed its guidelines for submission and distribution of the genetic resources under appropriate material transfer agreement (MTA).

Guest House

NBAIM is having a well furnished Guest House with 10 rooms including two Suites and a well managed Transit House. The charges for accommodation are according to the ICAR norms.

Major thrusts at NBAIM

Microorganisms can play key role in solving the problems related to agriculture, public health, food, environment and poverty. They are fundamentally important in ecosystems, breaking down complex animal and plant residues remains in soil and thus releasing essential nutrients for plant growth. They form beneficial mutualistic relationships with various plants, for example, nitrogen-fixing rhizobia with leguminous plants and mycorrhiza with forest trees; and they also enhance stress tolerance, provide disease resistance, aid nutrient availability and uptake, and promote biodiversity. Although major advances in genomic technologies and in situ studies of beneficial plant-microbe interactions have produced a large amount of knowledge and given insights into the mechanisms of these interactions, their application in biotechnology and agriculture has yet to be exploited. A greater understanding of how plants and soil microbes live together and benefit each other can therefore provide new strategies to improve plant productivity, while helping to protect the environment and maintain global biodiversity.

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 also perform an important function of depositing AIMs, which will facilitate the process of registration and patenting. Above all, the Bureau helps in understanding and conserving our national heritage of microorganisms not well understood and conserved so far.

Strengthening the culture collection facility

NBAIM will strengthen the microbial culture collection 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.

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.
- 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.

Characterization

- Morphological, physiological, and biochemical, characterization of AIMs.
- 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 easy access of information.
- Short and long term conservation of AIMs.

Diversity of Extremophiles

- To conserve and characterize the variable AIMs for its optimum utilization by future 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.

Diagnostics

Modern tool and technology, especially in the field of diagnostic of agriculturally important plant pathogens will prove the capabilities in the process of planning and management of plant diseases, prevention of exotic pathogens and implementation of strict quarantine.

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".

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.

Research Achievements- Institute Projects

Project 1. Characterization of beneficial rhizobacteria and its role in induced systemic resistance (ISR) and horizontal resistance in plants

PI : Alok K. Srivastava
Co-PIs : Sudheer Kumar, Prem Lal Kashyap

Rationale

Molecular phylogeny deduced from a single locus may be unreliable due to the stochastic nature of base substitutions or to rare horizontal gene transfer events. Molecular markers, such as the 16S rRNA gene, have been extensively applied to detect, identify and measure microbial diversity from environmental samples. To identify the various bacteria comprising the rhizospheric bacterial community, 16S rRNA gene sequence was initially considered to be a good indicator of so-called organismal phylogeny. In recent years, several studies have shown that the rRNA genes of rhizobia may occasionally undergo lateral transfer and genetic recombination, resulting in sequence mosaicism. These observations imply that the bacterial 16S rRNA gene phylogeny may not always accurately reflect prokaryotic phylogeny. PCR amplification of the 16S rRNA gene (rDNA), in combination with other molecular methods that generate fingerprints has been and is commonly used to analyse bacterial communities The gene coding for the beta subunit of the RNA polymerase, *rpo*B has been proposed as an alternative biomarker for microbial community studies. This gene is described as possessing the same key attributes as 16S rDNA, in that it is common to all bacteria and is a mosaic of conserved as well as variable sequence domains. Most importantly, the rpoB gene exists as a single copy in bacterial genomes. It has been demonstrated that 16S rDNA heterogeneity is a typical feature of bacteria isolated from natural environments and they state that diversity indices and correlations based on the banding patterns from 16S rDNA are not suitable for monitoring and comparing changes in microbial diversity. Consequently, it might be better to use a combination of the, *rpo*B and 16S genes to establish the phylogenetic relationships of the beneficial strains.

RPF project titled "Characterization of beneficial rhizobacteria and its role in induced systemic resistance (ISR) and horizontal resistance in plants" was started with the broad objectives for the year "Selection of potential rhizobacterial strains; Extraction of total genomic DNA and PCR amplification of 16S rDNA".

Objectives

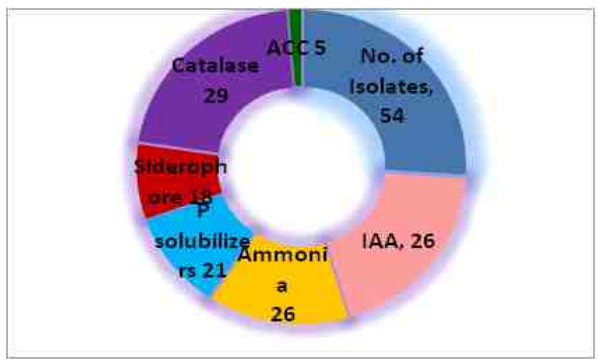
- Molecular identification of bacterial community structure in the rhizosphere of higher plants through sequence information from specific groups of genes.
- Decipher the genetic relatedness among members of the rhizospheric bacterial community through Multilocus Sequence Typing of rhizobacteria with following genes: 16S rRNA gene, *rpoB, rpoD, gyrB, atpD,* and *gacA*.
- Use of rhizobacteria to induce and record the expression of multigenic resistance, higher constitutive levels of specific isozymes of hydrolytic and/ or antioxidant enzymes.

Significant achievements

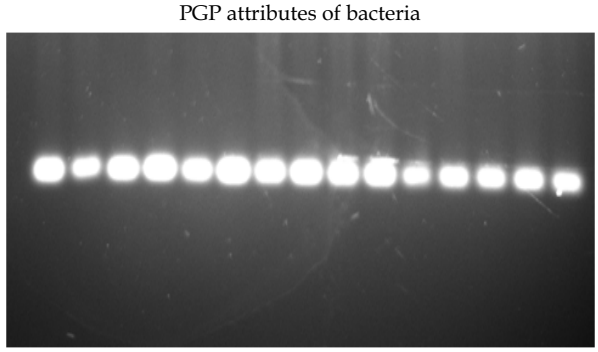
Isolation of bacteria was done from samples collected from Kanpur, Lucknow, Varanasi and Mau districts. Different morphotypes were picked up for the purification and further screening. A total of 54 different isolates have been selected and evaluated for their PGP attributes. 34 isolates were shortlisted on the basis of presence of one more than one PGP traits.

The isolates were tested for HCN production by the method of Bakker and Schipper (1987). Autoclaved broth was transferred in sterilized test tubes and

Isolates from Kanpur



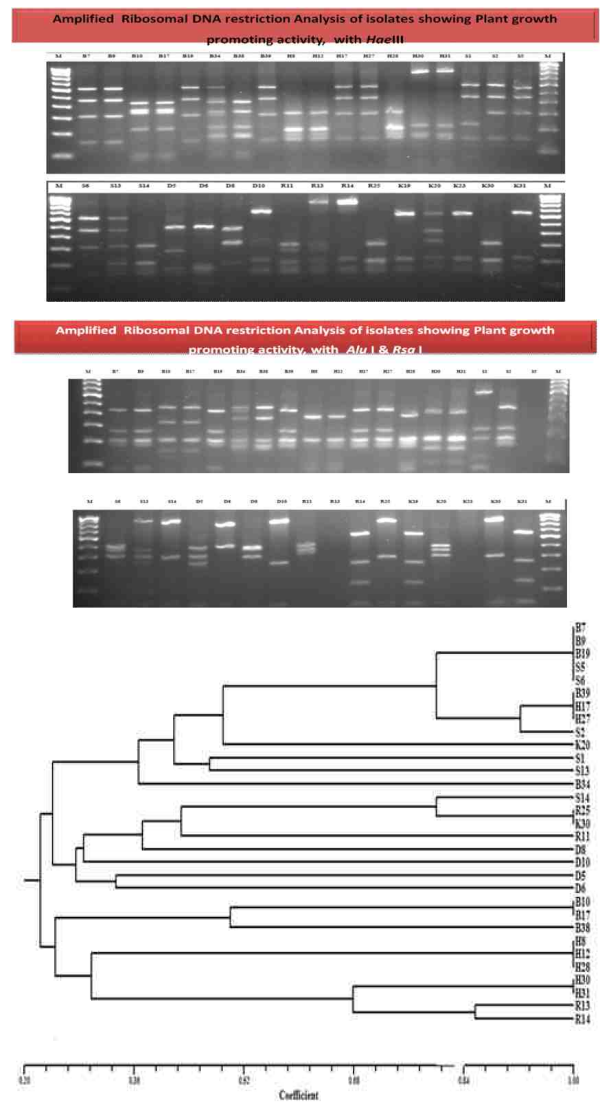
PGP attributes of bacteria



PGP traits and Genomic DNA

inoculated the isolates. Whatman no. 1 filter paper soaked in picric acid (0.05% solution in 2% sodium carbonate) was placed in the test tube. The test tubes were sealed with parafilm, incubated at 28^{0}C and recorded after 10 days. To study the production of ammonia, the isolates were grown in peptone water broth and incubated at 28^{0}C for seven days. The accumulation of ammonia was detected by addition of 1 ml of Nessler's reagent to each tube. 20 days old culture filtrates were assayed for Tetrazolium test (Snow, 1954). To a pinch of tetrazolium salt, were added 1-2 drops of 2N NaOH and 1 ml of the test sample. Instant appearance of a deep red color is indicative of hydroxamate siderophores.

Total genomic DNA extracted from all the isolates showing good quality with no smearing under UV trans-illuminator was quantified and used for PCR analysis. The yield of genomic DNA varied from 10 to 120ng/µl. The genomic DNA was diluted to get a concentration of 30-40ng in 4µl and was used as a template DNA in PCR reactions.



Dendogram showing percentage similarity using UPGMA between the isolates

A single product of 1475 bp length was obtained from the PCR amplifications. This fragment was subjected to restriction digestion by terta cutter and hexacutter restriction endonuclease to generate restriction patterns. Ribosomal-DNA sequences generated by PCR were digested separately using restriction endonuclease *AluI*, *HaeIII* and *RsaI*. It produced a good pattern which could distinguish among the bacterial groups with multiple PGP traits. The dendrogram was constructed from the matrix on the basis of presence and absence of the bands in gel. The dendrogram at 70 % similarity coefficient clearly generated polymorphic pattern and 29 isolates were clustered in 20 groups, out of which 16 isolates were selected for sequencing. Further work is under progress.

Project 2: Genotypic diversity and rhizosphere competence of potent antagonists of soil borne pathogens of vegetables

PI : Sudheer Kumar
Co-PI : Alok Kumar Srivastava

Rationale

To be effective in biocontrol of plant pathogens, microbial inoculants have to meet several important criteria, including: (i.) effective and competitive colonization of the host plant (ii.) stimulation of plant defence by induced systemic resistance (ISR) and/or systemic acquired resistance (SAR), (iii.) direct antagonistic effects on the pathogen e.g., by antibiosis or by inactivation of virulence factors of the pathogen, and iv. expression and/or production of the antagonistic traits needs to occur at the right time and place. Combining all of these traits into a single strain or mixtures of strains is likely to produce a more consistent and effective level of plant protection. In this context, efficient exploitation of these bacteria in agriculture and horticulture requires more fundamental knowledge of traits that enhance their ecological performance.

Rhizosphere competence is a dynamic process by which introduced bacterial inoculants make use of nutrients excreted by the seed and/or plant root, proliferate, efficiently colonize the root system, and survive over a considerable time period in the presence of indigenous microorganisms. Rhizosphere competence is a crucial element in beneficial plant-microbe interactions as inadequate root colonization leads to decreased biocontrol activity. Root colonization has been the subject of extensive research during the past three decades mainly due to the fact that inconsistent colonization remains one of the major limitations to the widespread use of bacterial inoculants in agriculture. A major factor contributing to the inconsistent colonization by the bacterial inoculants remains their variable ecological performance. Numerous studies have been performed in order to identify traits and factors that contribute to successful establishment, spread and survival of bacterial inoculants in the rhizosphere. These include biotic and abiotic soil factors, host genotype factors, rhizosphere-induced (rhi) genes and colonization genes. The present investigation is undertaken to understand the rhizospheric competence of potent antagonists

Objectives

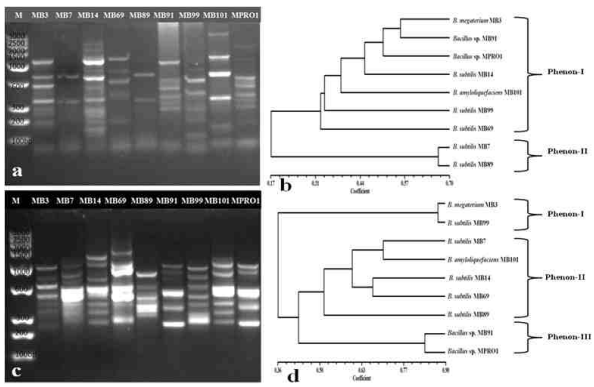
- To study the genetic diversity of potential antagonist of soil borne disease of vegetables
- To study influence of the host plant species on the population dynamics of antagonists in the rhizosphere

Significant achievements

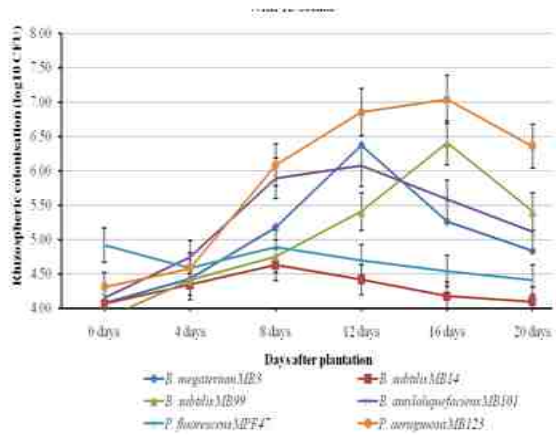
- After the preliminary screening under laboratory against different soil borne pathogens of vegetable crop and further under greenhouse condition against the Rhizoctonia root rot of tomato a total 18 bacterial strains were selected.
- The genotypic diversity among these isolates were studied on ERIC and BOX PCR analysis, a wide variability was recorded among different *Bacillus* isolates whereas narrow range of diversity was observed between *Pseudomonas* isolates.
- These isolates were further analyzed for variability, genetic relatedness and identity on the basis of 16S rDNA sequencing.
- These bacteria were also evaluated for intrinsic antibiotic resistance and further evaluated for root colonization potential with tomato roots.
- Six rhizospheric bacterial strains (*B. megaterium* MB3, *B. subtilis* MB14, *B. subtilis* MB99, *B. amyloliquefaciens* MB101, *P. fluorescens* MPF47 and *P. aeruginosa* MB123) were evaluated for root colonization and biocontrol potential.
- Out of six selected bacterial strains two bacterial strains (MB-101 and MB-123) shows better colonisation in the root associated soil with the pathogen and without pathogen. It is also concluded that rhizosphere competent antagonist B-123 was showed a competent biocontrol and plant growth promoting effects compared to all treated or non-treated controls.

Conclusion

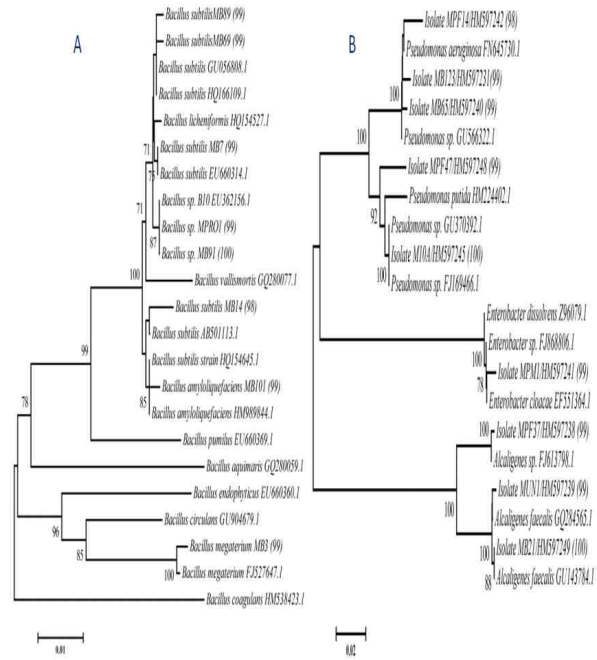
The present study also provides information on the application of bioinoculants for the management of soil borne pathogens are dependent of fitness of a



Genotypic patterns of bacterial strains obtained after ERIC (a & b) and BOX-PCR (c & d) fingerprinting.



Phylogenetic tree based on the 16S rDNA gene sequence of antagonists isolated. A = Gram positive isolates, B= Gram negative isolates



Phylogenetic tree based on the 16S rDNA gene sequence of antagonists isolated. A = Gram positive isolates, B= Gram negative isolates

stain in the rhizosphere. The strain MB 123 has better root colonizing efficiency followed by MB 99 and MB 3. However, in order to develop the best performing PGP bacterial strains for commercial applications, further selection and screening in field trials is needed.

Project 3: 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, lake, 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, mainly rice ecosystem, is proposed to be documented and possible implications of potent metabolites in agriculture and allied sectors is explored.

Objectives

- Isolation, purification and identification of cyanobacteria from different habitats including alkaliphilic and eutrophicated conditions simulating conditions of the habitats while isolating in different media
- Morphological, chemotaxonomic and molecular diversity of cyanobacteria in selected habitats
- Development of identification modules for cyanobacteria based on physiological, morphological and molecular traits 4. Exploration of metabolic diversity for value added biomolecules and pharmacologically and agrochemically important bioactive metabolites

Significant achievements

- Morphological characterization of isolates from Kanpur region led to the identification of species of *Aulosira, Cylindrospermum, Hapalosiphon, Anabaena, Nostoc, Aulosira, Cylindrospermum, Hapalosiphon, Tolypothrix, Oscillatoria* and *Scytonema*.
- HPLC profiling of extracellular and cell-free extracts from 15 cyanobacterial strains revealed high content of phenolic acids namely gallic, genteisic, ferulic and cinnamic acids. High quantity of ferulic acid in the extracts of *Plectonema boryanum* (94.52 g/g fresh cell wt) and *P. fragile* (57.22 g/g fresh cell wt) was positively correlated with the antibacterial activity against *Pseudomonas* sp., *Enterobacter* sp. and *Exiguobacterium* sp.
- Analysis 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 rice (*Oryzae sativa*) plants and rhizosphere following inoculation with cyanobacteria led to differential response on plant growth promotion and stress tolerance in saline soil.
- Cyanobacteria strains *Plectonema boryanum, Hapalosiphon intricatus, Anabaena doliolum* and *Oscillatoria acuta* grown in presence of different salt concentrations (0.5 to 2.5% NaCl conc.) in BG 11 medium showed reduced accumulation of biomass, total protein, chlorophyll and carotenoid content but accumulation of total phenol, reducing sugar and individual phenolic acids (gallic, chlorogenic and ferulic) and flavonoids (rutin and quercetin) increased many fold.

Conclusion

The results showed possible implications of different cyanobacterial strains in plant growth promotion and stress tolerance in rice plants. Besides, the identified organisms were shown to have differential capacity to tolerate high saline conditions by producing different kinds of secondary metabolites.

Project 4: 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 gram positive bacteria and are phenotypically diverse. Many species produce a wide variety of secondary metabolites, including antihelminthic compounds, antitumour agents, and the majority of known antibiotics, which have been exploited for their use in medicine and agriculture. They are tolerant to alkaline conditions and in alkaline soils, 95% population may be actinomycetes. In soil, they are involved in the decomposition and mineralization cycles by producing extracellular enzymes like cellulases, chitinases, and lignin peroxidases. 90% of the actinomycetes from soil may be *Streptomyces* and this genus alone may represent 5-20% of total microbial population. Majority of the antibiotics used for plants and animals are produced by Streptomyces. Survey and collection of motile and non motile actinomycetes from different agro-ecological zones will be useful in identifying diversity of isolates. Hence the project was formulated to

isolate, analyze the diversity and utilize the potent isolates for their use in agriculture.

Objectives

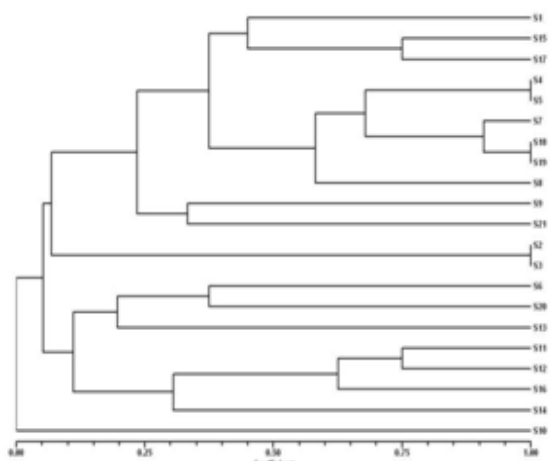
To isolate and purify motile and non-motile actinomycete isolates from mangrove ecosystem of India

- Morphological, biochemical and molecular characterization of the isolates
- To evaluate the role of some isolates for their plant growth promotion activities and nutrient management

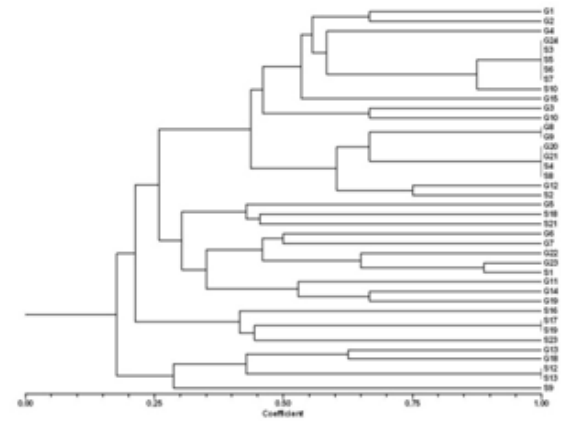
Significant achievements

A total of six mangrove ecosystems *viz.*, Sundarbans (West Bengal), Bhitarkanika National Park (Orissa), mangroves of Gujarat, Coringa mangroves (Andhra Pradesh), Pulicat Mangroves (Tamil Nadu) and mangroves of Goa were surveyed for the isolation, characterization and mapping of actinobacterial flora. A total of 167 morphotypes of actinomycetes were isolated using different enrichment techniques and

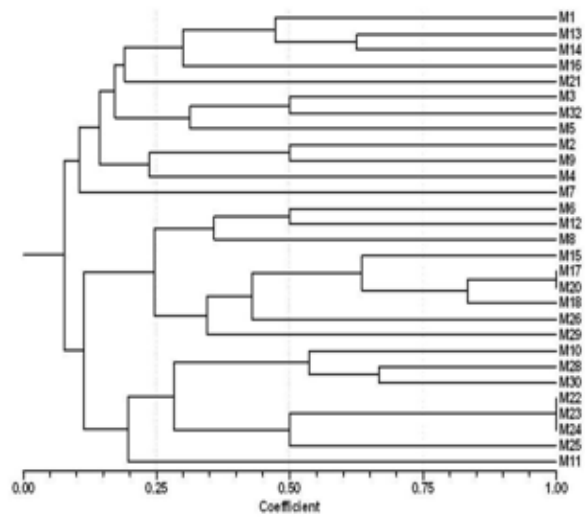
Photographs of survey and soil sampling from Mangroves of Orissa



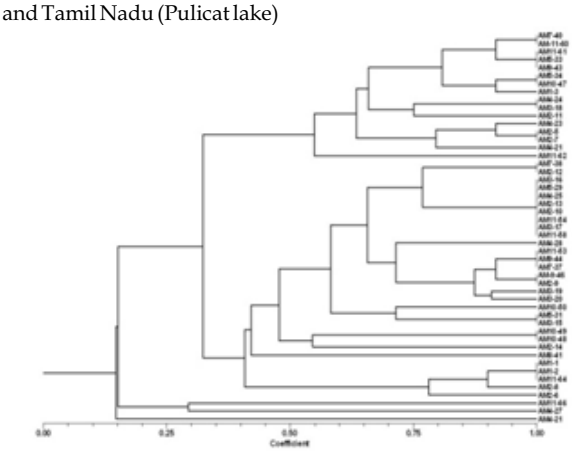
UPGMA dendrogram showing phylogenetic relationship among of the actinomycetes from Gujarat mangroves



UPGMA dendrogram showing phylogenetic relationship among the actinomycetes from Mangroves of Goa and Bitarkanika, Orissa



UPGMA dendrogram showing phylogenetic relationship among of the actinomycetes from mangroves of West Bengal and Tamil Nadu (Pulicat lake)



UPGMA dendrogram showing phylogenetic relationship among the actinomycetes from Coringa Mangroves of Andhra Pradesh

media. These isolates were subjected to morphological, microscopic, biochemical and physiological characterization.

Actinomycetes were subjected to molecular characterization using Amplified rDNA Restriction Analysis (ARDRA) using *TaqI, MspI*, and *HaeIII* restriction enzymes. Isolates could be clustered into 26 groups from Sundarban and Pulicat mangroves, 18 groups from Gujarat, 28 from coringa mangroves and 30 from Goa and Bhitarkanika mangroves. The isolates have been subjected to sequencing and identification.

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

Conclusion

Among the actinomycetes isolated from mangroves of Gujarat, West Bengal, Tamil Nadu, Andhra Pradesh, Goa and Orissa, 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 like plant growth promoting traits. These traits will find utilization in increasing the crop growth and biocontrol activities against plant pathogens. Further the actinomycetes will be mapped based on the structural and diversity according to the mangroves.

Project 5: Isolation, characterization and conservation of bacteriophages associated with some important phytopathogenic bacteria and their evaluation for use in agriculture

PI : Renu
Co-PI : Dipak T. Nagrale

Rationale

Plant diseases caused by bacteria are a major economic liability to agricultural production. Disease control has been a major challenge for many bacterial diseases. This challenge is a direct result of pathogen variability, high probability for mutation or gene transfer in the pathogen when confronted with resistance genes or bactericides, high pathogen multiplication rate during optimal conditions for disease development, and lack of adequate chemical-based approaches for control. Disease control is best achieved using an integrated management approach by combining proper cultural practices, chemicals such as bactericides or plant activators where applicable, introgression of plant resistance genes, and biological control strategies. In all cases disease control has been variable. Recently, there has been resurgence in interest in use of bacteriophages for control of bacterial plant diseases.

Bacteriophages are viruses that infect bacteria. Since their discovery in the early part of the twentieth century, they have been evaluated extensively for control of all kinds of bacterial incited diseases, including plant diseases. But with the advent of antibiotics and copper based bactericides chemical control with these compounds became the standard for controlling bacterial plant diseases.

Xanthomonas campestris pv. *Campestris* (X. campestris), the causal agent of black rot of crucifers, infects a large number of cruciferous plants, including agriculturally important crops such as cabbage, broccoli, and cauliflower. Yield can be affected in several ways: infected plants may die prematurely, heads may remain small, and quality may be reduced because of symptoms on the marketable part of the plant. Removing symptomatic leaves increases production costs. Little resistance to black rot is available in commercial lines. Long-distance spread is normally by seed and transplants, whereas local spread occurs mostly by wind-blown rain. Soft rot

may develop after black rot, further reducing quality and storage life. Management of disease is generally by proper cultural practices, usage of chemical control methods and disease free planting material and usage of resistant varieties. However, the bacteria are rapidly becoming resistant to copper sprays and copper residues are poisoning our environment. Alternative control chemicals are few, and even more toxic. A reliable and manageable biological control is needed. Bacteriophages provide highly specific control opportunities for bacterial diseases by specifically infecting and destroying the disease-causing bacteria. With little choice on environmentally safe methods for back rot control, investigating the potential for bacteriophage based biocontrol is warranted.

A number of *Xanthomonas* bacteriophages have been documented and some of them tested to show effectiveness to some extent in the control of plant diseases caused by *Xanthomonas*. As summarized in the review by Jones et al. (2006), these included bacterial spot in the genus Prunus, bacterial leaf blight on rice plant, tomato bacterial spot, bacterial blight of geraniums, bacterial blight on onion, citrus bacterial canker and citrus bacterial spot. Encouragingly, at least one of the efforts has been fruitful and a mixture of Xcv phages against tomato bacterial spot (Agriphage from OmniLytics, Incorporated) is at present commercially available. Keeping the above facts in mind, the project was formulated to collect, characterize and conserve phages of phtyopathogenic bacteria and to look out for their possible potentiality in disease management programme.

Objectives

- Isolation and characterization of pathogenic bacteria of important crop.
- Collection and isolation of bacteriophage from bacterial infected fields.
- Characterization of bacteriophages.
- Screening for evaluation of selected phages for disease control potentiality.

Significant achievement

- Survey and collection of diseased plant samples from Varanasi and nearby areas was done in the month of March, 2011. Collection of diseased plant parts from cauliflower field was done. A leaf with typical black rot symptoms (V-shaped lesions) was sampled for isolation.
- One or two leaf samples, representing one or two plants from each field, were selected for isolation of the causal agent from the typical V-shaped lesions. From each leaf sample, 0.5 to 1 cm^2 of leaf tissue was excised with a sterile scalpel from the margin of a lesion and placed in a drop of sterile 0.85% saline for 5 min. A loopful of saline suspension from a point with bacterial ooze was streaked on nutrient agar (NA) and incubated at 28°C for 48 to 72 h and observed for typical *X. campestris* pv. *campestris* colonies (pale yellow, mucoid, starch-hydrolyzing). A typical colony from each isolation plate was subcultured on NA medium and pure culture was obtained.
- Biochemical and molecular characterization of Varanasi isolate is in progress. Also further survey in and after rainy season will be done from the hotspot regions of the black rot disease of crucifers for collection of pathogenic bacteria. These bacterial strains will be used for isolation, propagation and studying host range of bacteriophages.

Typical colonies of *Xanthomonas campestris* pv *campestris* of Varanasi isolate from cauliflower

Catalase test

Project 6: Diversity analysis of plant growth promoting opiphytic and endophytic methylotrophic bacteria from different agro ecological zones of India

PI : Kamlesh Kumar Meena
Co-PI : Mahesh Yandigeri

Rationale

Methylotrophic bacteria are ubiquitous, found in water, soil and plant. Methylotrophs are 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.

Methanol is widespread, produced in nature as a result of demethylation reactions, and especially from plants. Methanol is oxidized to formaldehyde by three classes of enzymes, a quinoprotein methanol dehydrogenase (MDH) found in the *g*ram negative methylotrophs , an NAD-linked enzyme found in the *Bacillus* sp. , and a methanol:N,N'-dimethyl-4-nitrosoanaline oxidoreductase (MNO) found in other Gram-positive strains. In general, methanol oxidation is an energy-conserving step, either generating reduced cytochromes or reduced pyridine nucleotides. Aerobic methylotrophs contain specialized pathways for dissimilatory metabolism during methylotrophic growth. Rather than biochemical characterization, molecular techniques are more authanticative tool providing significant information about diversity indices and community analysis of microorganisms. Microbial molecular phylogenetic studies based on 16SrRNA gene sequence and/or conserved functional gene sequences have greatly expanded our knowledge of microbial diversity in nature. The lagoon is an estuarine one and supports an unique assemblage of marine, brackish water and freshwater species. None of the researchers have studied the methylotrophs in the lagoon covering the entire catchment area in a particular year.There is no report available containing information about the methylotrophic species occurring in Bhitakanika during a particular time and also no detail taxonomic index of each of the species available. The main purpose of this research was to isolate aerobic methanol utilizing methylotrophic strains from sediment of the mangrove and to characterize them with respect to some biochemical, physiological and molecular approach. This study unexplored dimensions of aerobic methylotrophs in this ecosystem.

Bhitakanika mangrove is one of the important hot spot for microbial diversity in India. It is a second largest mangrove of India located in Orissa. In the present study, the isolates obtained will be characterized by 16SrRNA and *mxa*F gene.

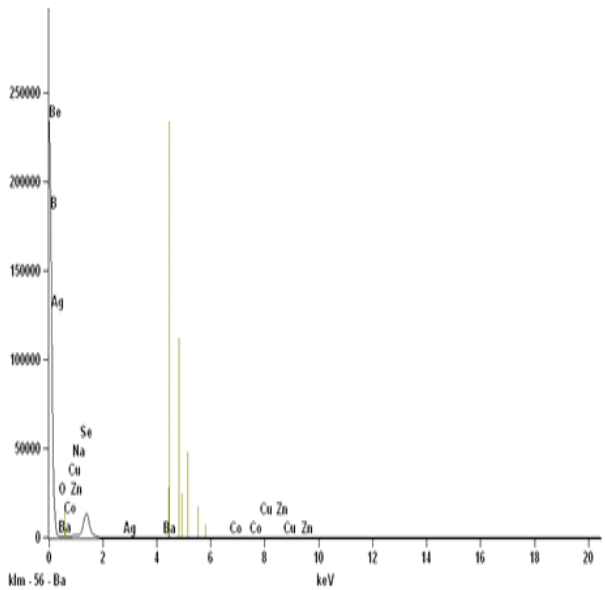
Objectives

- Isolation and enumeration of methylotrophic bacteria.
- Biochemical and molecular characterization of methylotrophs.
- Measurement of diversity indices : Species richness and Species evenness.
- Diversity analysis through metagenomic approach.

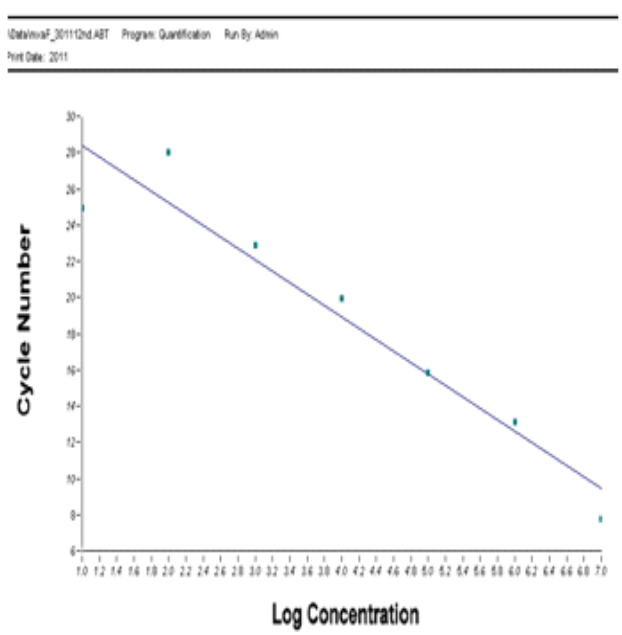
Significant achievements

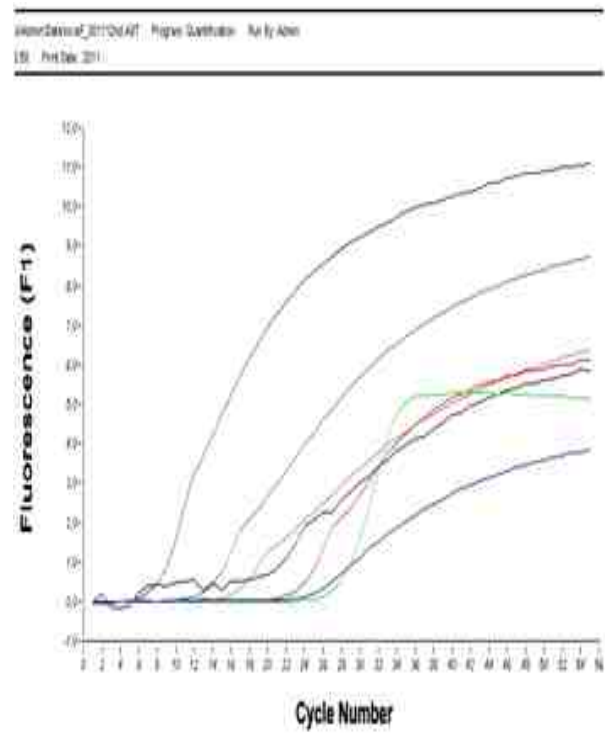
- The sediment samples were pooled from two different seasons from Bhitakanika and five composite samples were made.
- The physiochemical properties of the sediment was observed.
- The sediment samples were given with the enrichment of the different carbon sources like methanol, methylamine and methane sulfonic acid.
- From the sediment samples of season 1, 45 methanol oxidizers,23 methylamine oxidizers and no methane sulfonic acid oxidizers were seen. From season 2 isolation is in the process.
- The methanol, dehydrogenase gene was amplified and restricted to make groups and run on gene analyser.
- The sediment samples were subjected to the quanification of the the *mxa*F gene with the standards of the cloned genes.
- From season 1, the gene was quantified and in the range $4.5x10^4$-$3.2x10^5$.
- From season 2, the gene was yet to quantify from the enriched sediment samples (enrichment in the process)

Sensitivity of the Lightcycler RT-PCR was evaluated using different starting amount of DNA and standard curve. SYBR GreenI fluorescence determination at the



Sediment elemental physiochemical through EDX:





elevated temperature 83ºC resulted in a reliable and sensitive metagenomic DNA quantification assay with high linearity (Pearson correlation coefficient 0.99) over five orders of magnitude from $1x10^3$ to $1x10^7$ standard mxaF cloned DNA start molecules. The *mxa*F gene copy number calculated was in the range $4.5x10^4$-$3.2x10^5$.

Conclusion

From season 1 (June-july),the sediment samples having higher electrical conductivity and higher pH while from season2 (Feb-march) the sediments samples having relatively lower electrical conductivity and pH. These physiochemicalchanges obviously reflecting an effect on the methylotrophic community structure and therefore molecular characterization along with the quatitative estimation was compared. *mxa*F gene copy number obtained from season1 is yet to comapres with quantified copy from other season.

Project 7: Exploration, preservation and evaluation of endophytic actinomycetes from Indo-Gangetic plain

PI : Anurag chaurasia
Co-PI : D. P. Singh

Rationale

Endophytic microbes including bacteria, actinomycetes and fungi are ubiquitous in most plant species, especially in field-grown plants. There are several definitions of endophytes. Hallmann and co-workers defined any bacterium as an endophyte if it does not visibly harm the plant and it can be isolated from surface disinfected plant tissues or extracted from inside the plant. As of 1997, bacteria isolated from the internal plant tissue of healthy-looking plants were comprised of over 129 species representing over 54 genera. Although some of the endophytes are pathogenic to host plants and can locally or systemically colonize plant tissues, others latently reside in the internal tissues of non symptomatic plants without causing any adverse effects to the plants. Consequently, intimate associations between endophytes and host plants can be formed without harming the plant. Endophytes have been demonstrated to improve and promote growth of host plants as well as to reduce disease symptoms caused by plant pathogens and/or various environmental stresses. The low stress tolerance of axenic plants is commonly believed to result partly from the absence of endophytic microbes. Management of beneficial microbial communities to favour plant growth could be realized by a deeper understanding of the physiological and molecular interactions between microbes and plants. This research also may have broader economic and environmental impacts. Indo-Gangetic plains are a major crop production region of the country and have rich biodiversity, hence this project has been formulated to isolate, utilize and conserve the endophytic actinomycetes from various crops grown in this region which will be further exploited for agricultural productivity and human welfare.

Objectives:

- Isolation of endophytic actinomycetes from the crops growing in Indo-Gangetic plain
- Preservation and identification of the isolates
- Evaluation of the endophytic actinomycetes isolates for enhancing agricultural productivity and human welfare

Significant achievements

Protocol for endophytic actinomycetes isolation from the root, stem and leaves of medicinal plants like *Rauwolfia serpentina, Withania somnifera, Pimpinella anisum* and *Ocimum sanctum* using Actinomycetes isolation agar and Starch casein agar media has been standardised. Using specific media like Tap water yeast extract agar (TWYE), modified TWYE supplemented with plant extract, Inorganic salts starch agar, Glycerol asparagines agar, Humic vitamin agar, Sodium propionate agar and YIM-38 media rare endophytic actinomycetes too have been isolated. The media containing malt and yeast extract like YIM-38 give better result for rare endophytic actinomycetes isolation compare with other media. Rare actinomycetes with transparent colonies were found on TWYE agar medium. Same actinomycetes isolate shows different morphology when grown on different media. Endophytic actinomycetes from *Withania somnifera* were found to produce curd like odour on YIM-38 media. All the isolates have been preserved on the slant and in 20% glycerol. PGPR and biocontrol potential evaluation of the isolates are underway. Actinomycetes associated with cyanobacterial (Cyanomycetes) cultures have also been isolated.

O. santicum, root, HVA | *R. serpentina*, Leaf, AIA medium | *W. somnifera*, stem, SCA

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

PI : Udai Bhan Singh
Co-PI : D. P. 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. Metagenomics is a new field combining molecular biology and genetics in an attempt to identify, and characterize the genetic material from environmental samples and apply that knowledge. The genetic diversity is assessed by isolation of DNA followed by direct cloning of functional genes from the environmental sample. It is well known that less than 1% of the microbial world can be accessed using classical culturing approaches. Metagenomics attempts to overcome this bottleneck by introducing culture independent approaches. Since the metagenome technology has been introduced just a few years ago a number of significant advances have been made. Among them the partial shot gun sequencing of the Sargasso Sea, the near complete sequencing of an acid mine biofilm and the partial sequence analysis of a drinking water biofilm. These projects have led to the accumulation of more than one million novel genes and DNA sequences, which however, remain to be exploited within the next decade. Probably one of the most significant contributions which were made concerns the detection of a novel light dependent energy conservation mechanism in marine microorganisms. In addition to these achievements an increasing number of novel biocatalyst genes and genes encoding for novel antibiotics have been detected. These genes are of considerable interest to agricultural biotechnology and pharmaceutical companies. Many of these genes are currently exploited for downstream applications. Thus with respect to basic science the metagenome technology gives us new insights into the genetic makeup of microbial communities and helps us to understand how these microbial communities function. Concerning biotechnological and pharmaceutical applications the genomes of the non-cultured microbes represent a shear unlimited and very valuable resource for novel biocatalysts and genes encoding for antibiotics or other drug molecules. Metagenomics will now unlock this vast potential for biotechnological and pharmaceutical applications.

Rhizosphere ecosystem is unique in nature and harbours unique microbial diversity. Rhizosphere ecosystem is rich in organic matter and macro and micro nutrient. Rhizosphere provide a unique ecological environment for divers microbial communities like antibiotic producing, nitrogen fixing, nutrient mobilizing microorganisms. Many of the communities are involved in various activities such as antibiotic production, nutrient mobilization, bioremidiation, nutrient cycling and decomposition etc. Metagenomic approach exploited to access the whole microbial community those having 2,4-DAPG, Type I PKS and Type II PKS gene which is responsible for the production of antibiotic. The gene(s) 2, 4-DAP, Type I PKS and Type II PKS play a key role in the synthesis of a number of antibiotic such as streptomycin, tetracycline, oxy- tetracycline, chlor-tetracycline etc. which are not only agriculturally important, they have wider applicability in human and animal health. Keeping these points in new metagenomic approach has been followed in present investigation to measures the culturable as well as non- culturable microbial genomic diversity of antibiotic producing agriculturally important microorganisms.

Objectives

- Evaluation of genetic diversity of antibiotic producing AIMs in rice-wheat cropping system of Indo- Gangetic plains of Uttar Pradesh.
- Detection, prediction and diversity of antimicrobial genes (2,4-DAPG, Type-I PKS and Type-II PKS) by using metagenomic approaches.
- Screening and expression of antibiotic producing genes (2,4-DAPG, Type-I PKS and Type-II PKS)

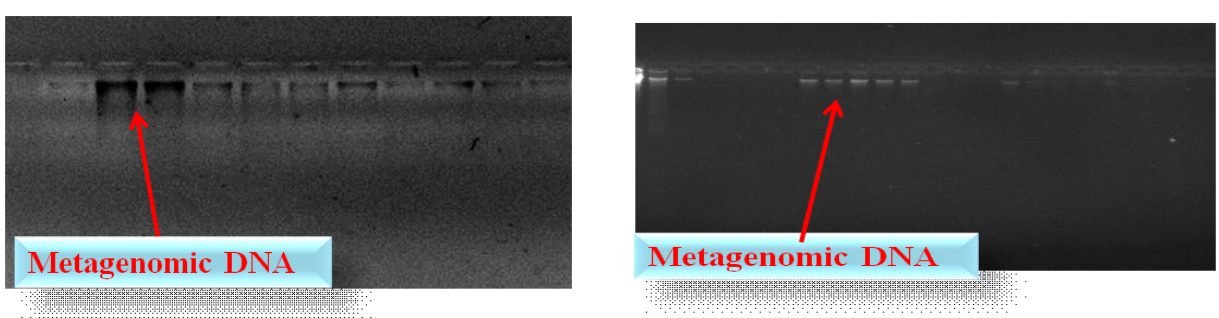
and its possible application in agriculture.

Significant Achievements

- Extensive exploration, survey and collection of soil samples were carried out from rice-wheat growing areas of Kanpur (Rural), Kanpur (Urban) and adjoining areas of Allahabad of Uttar Pradesh during 2010- 11. Survey and collection of rhizospheric and non-rhizospheric soil sample were carried out from wheat rhizosphere of more than 25 villages of 10 blocks. 36 sample of rhizospheric soil with plant roots have been collected for the further isolation of antibiotic producing agriculturally important microorganism (AIMs). Testing of physic chemical properties of soil samples to know the ecological aspect of the soil and micro flora- are going on. A total of 124 different morphotypes belonging to fungi, bacteria and actinomycetes were isolated using different media. A total of 26 isolates of fungi, 46 isolates of bacteria and 52 isolates of actinomycetes were isolated (Fig. 2). For the characterization of antibiotic producing AIMs a dual plate technique was used for preliminary screening. These isolates were studied by using morphological parameters like

Map depicting the wheat growing areas chosen for the survey and diversity analysis of antibiotic producing AIMs

Representative morphotypes of actinomycetes isolated from wheat rhizosphere from Kanpur soil sample.



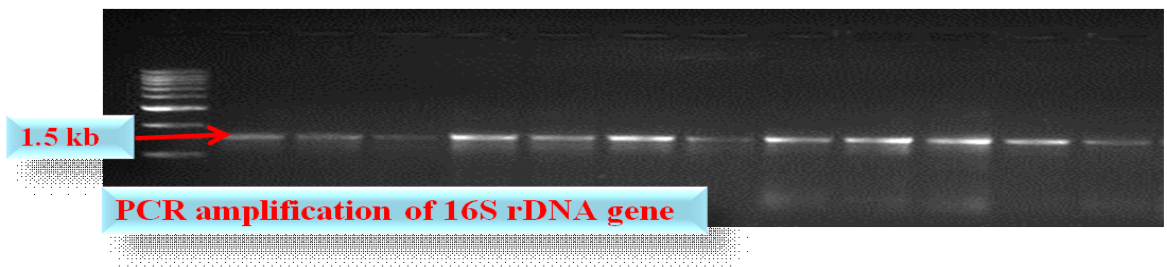
Environmental whole-genome of wheat rhizosphere of Kanpur and Allahabad samples

colour of pigment, growth pattern, colour of colony etc. a number of actinomycetes were found to be as secondary metabolite producer. All the isolates were screened for the antimicrobial potential. 37 isolate showing antifungal and antibacterial activities against *Rhizoctonia, Fusarium, Ganoderma, Macrophomina, Sclerotium* and *Sclerotinia*.

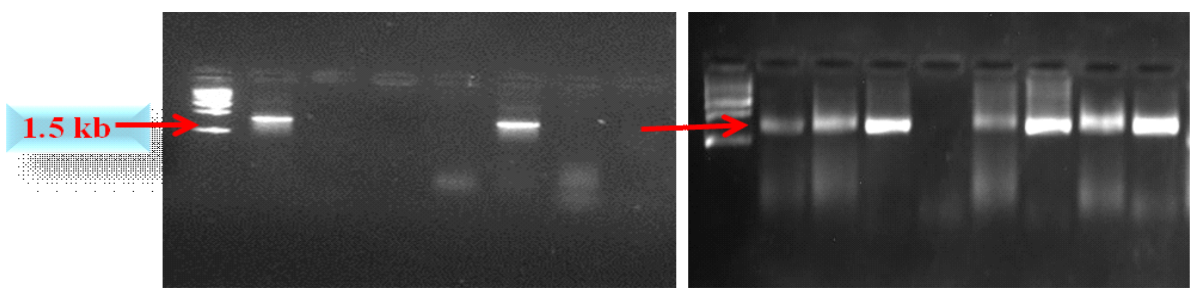
- To see the variation at species level for the different selected morphotypes, PCR amplification of 16S rDNA followed by ARDRA analysis using three different restriction endonucleases was carried out.
- Environmental whole-genome amplification was carried out to access biodiversity of bacterial and actinomycetes community present in the wheat rhizosphere, PCR amplification of 16S rDNA followed by ARDRA analysis using three different restriction endonucleases was carried out.

Functional approach

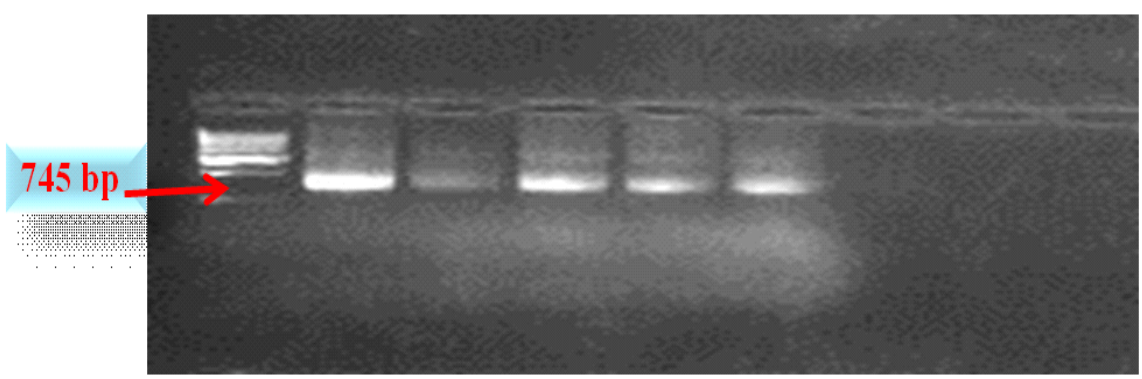
- The gene(s) 2, 4-DAPG, Type I PKS and Type II PKS are the key genes which is responsible for the synthesis of a number of antibiotic such as streptomycin, tetracycline, oxytetracycline, chlor-tetracycline urdamycin A, urdamycin B, urdamycin F, kinamycin D, medermycin, actinorhodin etc. which is agriculturally important and have wider applicability in human and animal health.
- The gene employed in this study 2, 4-DAPG, Type I PKS and Type II PKS were used to antibiotic



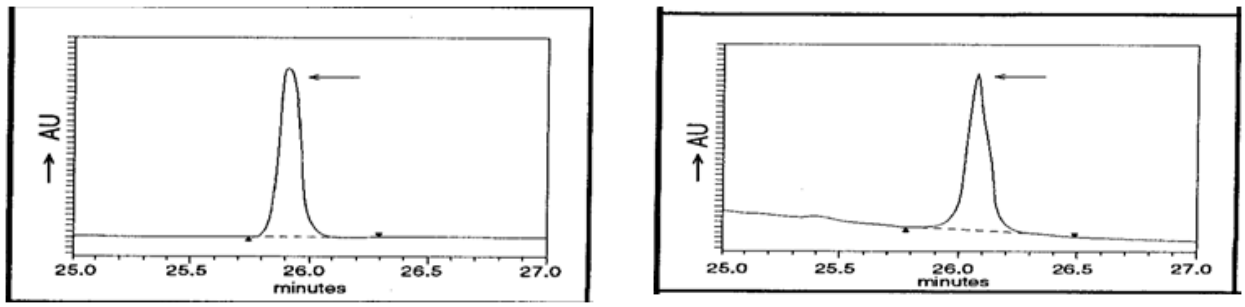
Environmental whole-genome amplification of 16S rDNA gene in Kanpur soil sample



Specific amplification of Type II PKS gene in wheat rhizosphere metagenome in Kanpur and Allahabad soil sample



Specific amplification of 2,4-DAPG gene in wheat rhizosphere metagenome in Kanpur soil sample.



High pressure liquid chromatography (HPLC) chromatograms of 2,4-diacetylphloroglucinol (Phl) (Pure compound as standard and in wheat rhizosphere: a case study taken as for reference in our study)

producing microbial community analysis in wheat rhizosphere. The presence of 2, 4-DAPG, Type I PKS and Type II PKS gene in the isolate and soil metagenome detected by the partial amplification of the gene using specific primers i.e. 20-mer primers for antibiotics 2,4-diacetylphloroglucinol (Phl)-Phl2a(F) GAGGACGTCGAAGACCACCA, Phl2b(R) ACCGCAGCATCGTGTATGAG, oligonucleotides primers for type I polyketide synthase F' TSAAGTCSAACATCGGBCA, R' CGCAGGTTSCSGTACCAGTA, oligonucleotides primers for type II polyketide synthase U1 F' GCCGGAATTCATGATCCCGGTCGCGGTCA, U1 R' GCCAATGCATAAGCTTCACCG CCCGGCACG CACCGC.

- HPLC based procedure of isolation and characterization of bioactive principles (in secondary metabolites) from culture filtrates and in wheat rhizosphere were standardized. HPLC based determination of 2,4 -DAPG, streptomycin and tetracycline from culture filtrates and rhizospheric soil is in process. Relationship between rhizospheric population dynamics and nature and amount of antibiotic produced was correlated based on HPLC data.

Project 9: Exploration of pathogenecity gene(s) of *Magnaporthe grisea* responsible for rice blast epidemic in hot spot regions of India

PI : Prem Lal Kashyap
Co-PI : Sudheer Kumar

Rationale

Rice blast caused by *Magnaporthe grisea* Barr (*Pyricularia grisea* anamorph Cav.) is one of the most destructive diseases of rice. The fungus is distributed world-wide and causes losses of up to 100% of the yield depending on cultivar susceptibility, environmental conditions and management system. Almost in each year, the fungus destroys rice enough to feed an estimated 60 million people. The pathogen infects most sections of the plant, but infections of the node or the panicle are the most damaging phases of the disease. Since the pathogen is highly variable, breeding for durable resistance to blast become a major challenge. Emergence of new pathotypes has been reported in different regions, causing the breakdown of cultivars developed with single major resistant genes. However, population evolution and virulence diversity of *M. grisea* in the field was still unexplored. Moreover, the detailed investigations on the exploration of diversity of pathogenicity genes of *M. grisea* have been fuelled by the necessity to develop cost-effective, eco-friendly and durable and novel strategies for the control *M. grisea*, in spite of ongoing strong efforts to develop and introduce new fungicides and resistant plant varieties. An essential cue in this ongoing battle is the identification and search for diseases controlling targets via the identification of pathogenicity determinants, encoded by virulence genes. Relatively little information exists on these aspects as fragmentary and preliminary efforts were made globally by various researchers to explore the significance of virulence genes in epidemiology and management of rice blast disease. In india, so far, no such type of study based on the diversity of virulence gene(s) on large scale was done. It is believed that the information

Table: In *silico* designing of primers for the amplification of gene controlling virulence in *M. Grisea*

Gene/ Gene product	Forward Primer	Reverse primer	Predicted amplicon size (bp)
Protein kinase *(CHM1)*gene	ATTGGCACCGCAGGCTATG	GCAGCTCGACTAGTGGTAGTG	450
Cytochrome b *(LpMDO1-1)*	AATCCTCTTGGTGTTTCAG	ATAAATGGATCTTCAACGTG	385
Neutral Trehalase (NTH1)	GCGGAAAGTTGAGAGCC	ATCTCATCGATCTCGTGAC	463
Trehalase precursor *(TRE1)*	GACCCATACAATCAACGC	ACATTAGCTAGGTTTACGG	362
MPLC1	CCACTTTGACACCGAGACAG	GGATGTGGTAGAGGAAGTCATC	359
MAC1	GCCGATAGAGCAACATACAC	GCGTTTGTGCTGCGTTG	363

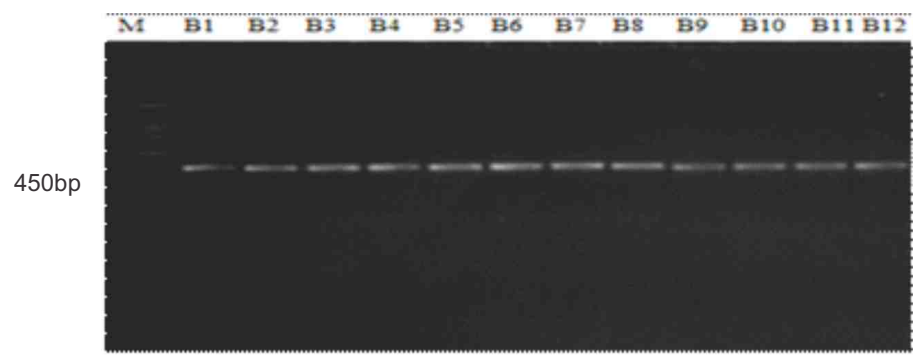
generated from this research project will provide answer why rice blast epidemic occurs at a particular location at particular time-points and what gene(s) of *M. grisea* act as a master switch for the occurrence of rice blast epidemic. The identification and further exploration of these genes will help in proper identification of novel target sites to restrict the menace of the disease. The information generated through this project act as a model to devise eco-friendly, cost effective and integrated approach for the effective management of *M. grisea* under field conditions.

Objectives:

- Isolation, identification and characterization of *M. grisea* isolates prevalent in hot spot region of India region
- Detection, prediction and diversity analysis of pathogenicity gene(s) by using molecular tools.
- Expression profiling of pathogenicity gene(s) to identify virulence pattern of *M. grisea*.

Salient achievements:

- Thirty two isolates of *Magnaporthe grisea* were isolated from infected rice leaves, spikes and panicles collected from rice blast hot spot regions of India (Hyderabad, Srinagar, Cuttack, Bangalore and Almora etc.) and maintained on the oat meal agar medium supplemented with kinetin (0.01%). These isolates were further identified and characterized using morphological, microscopical and pathogenecity assay. It was found that the isolates collected from various locations were varied in terms of conidia size.
- Different pathogenecity gene(s) controlling virulence of *M. grisea* were identified from NCBI database and primers for each gene were



Lane M: 1Kb ladder; Lane B1, B2-B5, B6-B9, B10-B11 and B12 depicts the amplification of 450 bp amplicon size product of the *Magnaporthe grisea* ***CHM1*** **gene in various** isolates collected from Hyderabad, Srinagar, Cuttack, Bangalore and Almora respectively

designed and validated in silico.

- The experiment was conducted to the diversity of CHM1 gene controlling viruelnce in twelve different isolates of *M. grisea* screened on the basis of pathogeneicity and microscopy. Total genomic DNA of all isolates was isolated and amplified using CHM1 specific forward (ATTGGCACCGCAGGCTATG) and reverse (GCAGCTCGACTAGTGGTAGT) primers to detect the presence of protein kinase gene (CHM1) gene. The thermal profile used for the amplification of target gene was: 94^{0}C for 5 min followed by 35 cycles of denaturation at 94?C for1 min, annealing at 50^{0}C for 1min and final Extension at 72^{0}C for 10 min. All the twelve isolates were able to amplify the genomic DNA and resulted in the 450 amplicon size product.

Conclusion

From the present study it is conclude that the there is a large variability in the isolates of *M. grisea* collected from different locations of India. The protein kinase gene (*CHM1*) is universally available in all the twelve isolates.

Project 10: Diversity analysis and microbial management of salt stress in solanaceous crops in UP

PI : Sanjay Kumar Goswami
Co-P I : P.L. Kashyap

Rationale

Soil salinity is a major constraint to food production because it limits crop yield and restrict use of land previously cultivated. The United Nations environment programme estimate that approximately 20% of agricultural land and 50% crop land in the world is salt stressed. In India about 10 million ha of cultivated land is salt affected. 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. Saline habitats are frequently inhabited by an abundance of microbial communities adapted to these ecosystems. Among the microorganisms, the bacteria play a major role as important and dominant inhabitants of saline and hypersaline environments. The bacteria that live in saline environments may be assigned to two categories: archaebacteria and eubacteria. Archaebacteria are extremely halophilic microorganisms which grow optionally at salt saturation (up to 30% NaCl).

Objectives

- Isolation of microorganisms from rhizotic zones of solanaceous crops (potato, tomato, brinjal and chilli) grown under salt stress.
- Selection of salt tolerant micro-organisms
- Diversity analysis and identification
- Biochemical and molecular characterization of selected micro-organisms.
- Evaluation of selected micro-organisms in the rhizosphere of solanaceous crops (Green house studies) and plant-microbe interaction studies in the rhizosphere.

Significant achievements

- Survey of solanaceous crops (potato, tomato, chilli and brinjal) in Mau, Balia, Gajipur, Banaras and Faizabad areas was done for the collection of rhizospheric salt affected soil samples.
- 80 soil samples were collected
- 35 micro-organisms were isolated from the soil samples of Mau and Faizabad.
- 26 microorganisms were isolated from the soil samples of Gajipur, Balia and Varanasi
- A3, A4, A13, A17, A21, A22 , A22, A25 are some salt resistant isolates
- Screening for more salt tolerant microorganisms is under progress
- Morphological and Biochemical characterization is in progress

Project 11: Isolation, characterization of bacterial communities and their metabolites in rhizospheric rice ecosystem

PI : Lalan Sharma
Co PI : Sanjay Goswami

Rationale

Rhizosphere is considered the soil volume surrounding the root-tissue and the term was firstly coined by Hiltner in 1904. It is well established that microbial life only occupies a minor volume of soil being localized in hot spots such as the rhizosphere soil, where micro flora has a continuous access to a flow of low and high molecular weight organic substrates derived from roots. This flow, together with specific physical, chemical and biological factors, can markedly affect microbial activity and community structure of the rhizosphere soil. Both beneficial and detrimental interactions occur between microorganisms of rhizosphere soil and plants. The composition and activity of microbial communities of rhizosphere soil, which are affected by rhizodeposition, a term that includes all substances released from roots to soil. Type of compounds released by roots, and systems used to study the rhizosphere effect will be studied. Rhizodeposition includes both low and high-molecular weight compounds including monomers such as glucose and amino acids, polymers such as polysaccharides and proteins, root debris and root border cells, root cap cells separated from the root apex during root growth. The organic substances released from roots to the rhizosphere soil support higher microbial biomass and microbial activity in the rhizosphere than in the bulk soil. Not all compounds released from roots are organic because roots can also release proton, oxygen and water. Root products can be classified according to their perceived function in excretions (CO_2, bicarbonate ions, H^+, electrons, ethylene, etc.) and secretions (mucilage, H^+, electrons, enzymes, siderophores, etc.) with the former being thought to facilitate internal metabolism and the latter external processes such as nutrients uptake. The root products can also be classified according to their chemical properties (composition, solubility, stability, volatility, molecular weight, etc.) and site of origin. Secretions can be classified according to their biological activity (chemical signals, phytoalexins, phytohormones, ectoenzymes, allelochemicals, etc.). Low-molecular-weight exudates can diffuse to a longer distance than high-molecular weight compounds but they are more readily assimilated by soil microorganisms. It is also well established that low molecular weight exudates are immediately available to microorganisms inhabiting rhizosphere soil and rhizoplane whereas high-molecular weight compounds are generally hydrolysed by hydrolases in smaller compounds which can be taken up by microbial cells.

Objectives

- Survey and collection rhizospheric soil samples from Indo-gangetic plain of Uttar Pradesh
- Characterization of HCN/siderophore producing bacterial isolates and develop consortia of beneficial isolates for their nutrient utilization
- To determine the secondary metabolites produced by the bacterial isolates in broth culture medium by Mass spectrometry
- To determine the compounds profile in root tissue and present in rhizosphere soil by using Mass spectrometry
- To study plant-microbe interaction by using Gnotobiotic system

Significant achievements:

- Survey and collection of rhizospheric soil samples from four different regions (Gorakhpur, Lucknow, Kanpur and Varanasi) of Indogangetic plain.
- Isolation of rhizobacterial populations were made by the different inoculation techniques (soil plate and serial dilution) on various culture media (Nutient agar, Jenson agar, Pikovshaya agar, Bark's medium, NFB medium, Malate medium and YEMA medium). A total of 83 rhizobacterial population have been isolated from rhizospheric soil samples that were visually characterized for their different morphotypes.
- Rhizospheric soil is determined for their pH (ranges 7.0-8.4), EC-value (ranges 1.3-1.9 dsfm) and organic carbon in soil (ranges medium to medium high).

- A total of 26 isolates have been tested for the HCN production, siderophore production and phosphorus solubilization, only the GR-1, GR-3, GR-6, GR-23 and LUCK-24 isolates showed significant results to concern.

Conclusion

Soil samples were recorded for their pH, EC- value and organic carbon in soil, slight variation was observed. A number rhizobacterial population were isolated by the different inoculation techniques on various culture media. The isolates were also characterized for their HCN production, siderophore production and phosphorus solubilization. The work is under progress.

Project 12: Diversity analysis of archaea from different ecological niches and their characterization

PI : Dipak T. Nagrale
CO-PI : Renu

Rationale:

Archaea typically thrive in extreme environmental condition. Domain archaea show an increased resistance to extreme conditions like cold desert, hot springs, hypersalinity and sulphate rich niche underlying their importance in studies of other possible habitable regions.Methanogenic archaea are a vital part of sewage treatment, since they are part of the community of microorganisms that carry out anaerobic digestion and produce biogas. In mineral processing, acidophilic archaea display promise for the extraction of metals from ores, including gold, cobalt and copper. Archaea host a new class of potentially useful antibiotics. Archaea can provide novel insights into possible early life formation since they are known for their longevity and their ability to survive for several million years.

Objectives:

- Diversity analysis of archaea from different ecological niches using culturable and metagenomic approaches.
- Community analysis of archaea from different ecological niche.
- Development of molecular diagnostic tools of some agriculturally important archaea.

Significant achievements

- Samples from coal and iron mines were collection and processed.
- Work on the isolation of potent microbial species and metagenome is in progress.

Conclusion:

From the earlier reports it is clear that a meager work has been accomplished on archaeal community structure and diversity in India and abroad. Also a meager work has been carried on archaea from cold deserts, hot springs and saline and alkaline conditions. The project will be helpful in identifying the archaeal communities and their diversity from different ecological niche especially mineral rich ore and heavy metal contaminated industrial effluents. Their characterization will give the valuable information about the archaea(s).Valuable information about archaeal diversity and communities arising from the project will add to the existing microbial diversity studies carried out at the bureau and in turn fulfill the mandate of the Bureau.

Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)

Research Achievements

Microbial Diversity and Identification

Project: Diversity analysis of *Bacillus* and *Bacillus*-derived genera in the Indo-Gangetic plains of India

PI : Dilip .K. Arora

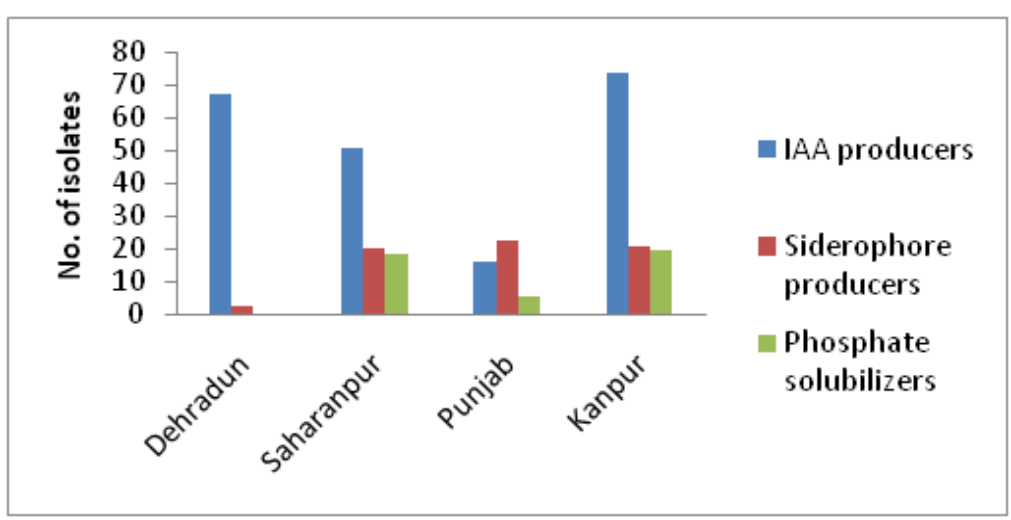
Rationale

The IGP is the 'bread basket' for much of South Asia. Rice and wheat, the major cereal crops of this region are grown in rotation on almost 12 Mha of land. Together they are the principal source of food and livelihood security for several hundred millions of people in this densely populated region (Paroda et al., 1994). During the last four decades, the wheat production no doubt increased 6 times and that of rice 2.5 times, but the present scenario suggests declining trend in rice as well as wheat productivity. To compensate the effect of declination farmers have started applying higher doses of N than the recommended ones. Such indiscriminate use of N has further worsen the nutrient imbalance in soil systems, besides increasing the pest incidence, cost of production and shift in the microbial community. Shift in microbial community may lead to declination in productivity of soils. The objective of the present investigation was to understand the microbiological reasons for the decline in the agricultural productivity and species richness of *Bacillus* in this region.

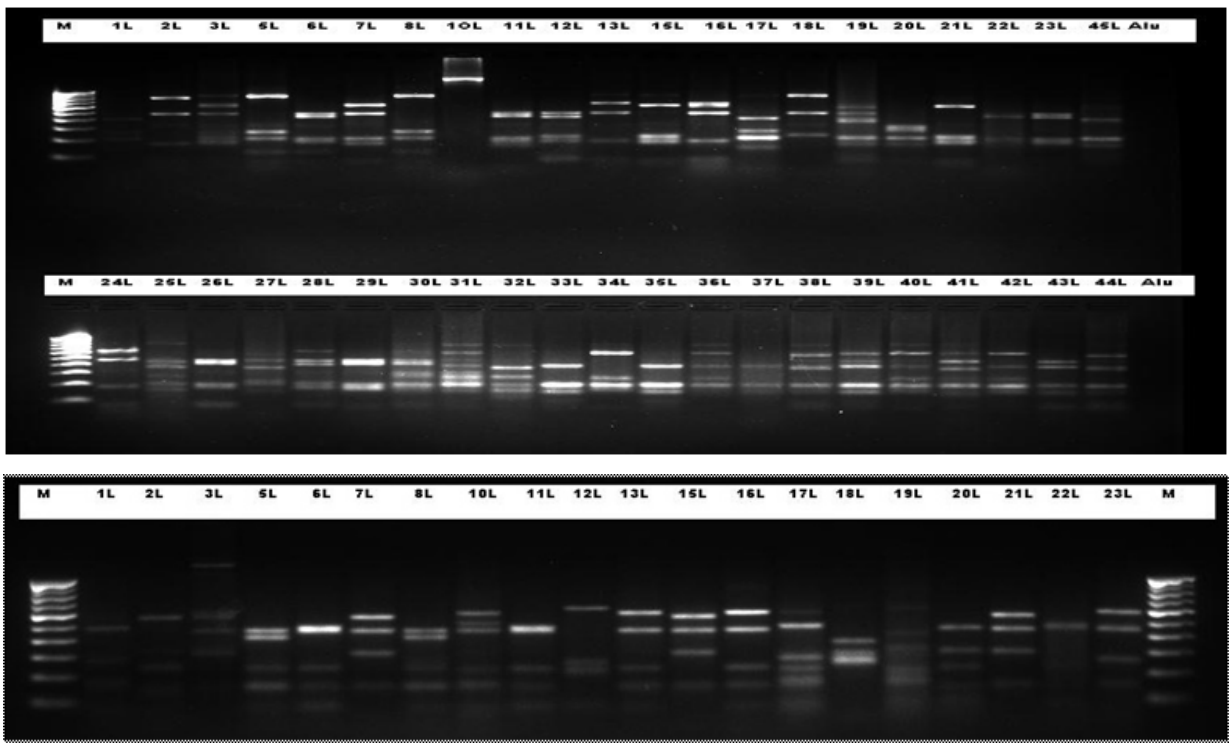
Objectives

- 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.

Significant achievements



Percentage of PGP attributes of IGP regions



RFLP analysis of 16S rDNA with *Alu*I and *Hae* III restriction enzymes respectively

- A total of 245 isolates were isolated from the soils of Trans (Punjab) and Central IGP (U.P) regions. These isolates were screened for various PGP traits i.e., Indole Acetic Acid production (IAA), Phosphate solubilization and Siderophore production.
- 20.5% of the isolates from Central IGP region were found to produce siderophores, whereas from Trans IGP region, a total of 22.5% isolates showed siderophores production.
- IAA production without any precursors was observed for both Central and Trans IGP regions. 74% and 52% of the isolates from Kanpur and Saharanpur regions (Central IGP) showed IAA production. Higher quantity of IAA ranging from 1200µg/ mg to 600 µg/mg of protein was recorded; whereas, from Punjab region (Trans IGP) only 16% isolates were IAA producers.
- 8% and 20% phosphate solubilizing *bacillus* from Saharanpur and Kanpur belt (Central IGP) respectively were recorded. Whereas, Punjab region showed only 6% of the isolates as phosphate solubilizer.
- Molecular characterization of Central and Trans IGP region isolates by using PRA analysis of 16S rRNA with three restriction enzymes showed great diversity among the isolates in IGP regions. On the basis of the hypothesis given earlier (previous annual report), we predict that the large number of the isolates are *Bacillus*-derived genera.
- Some of the isolates from the major cluster have been sequenced and were identified as *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) .

Conclusion

A great diversity among the isolates was recorded in IGP but the isolates having insignificant PGP activity dominates which confirms the rationale. This dominance is more in Trans IGP region than the Central IGP region where the use of chemical fertilizer was more.

Project: Development of diagnostic kit for identification of soil microbes (*Bacillus* and *Pseudomonas)*

PI : Dilip K. Arora

Rationale

The genus *Bacillus* and *Pseudomonas* includes the Gram-positive spore forming and gram negative aerobic bacteria, respectively. They are phenotypically heterogeneous, 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 very wide range of important medical, agricultural, pharmaceutical and other industrial products. They have a long and distinguished history in the realms of biotechnology. Both the genus includes very versatile members and the most effective biocontrol agents for various insects pest. They are also good sources of numerous antibiotics, flavor enhancer such as purine nucleosides, surfactants and various other products. They can actively metabolize decomposing plant and animal matter and various inorganic nutrients. These are important Plant Growth Promoting Rhizobacteria as they are good IAA producers and Phosphate and Siderophore producers and thus find wide application in agriculture.

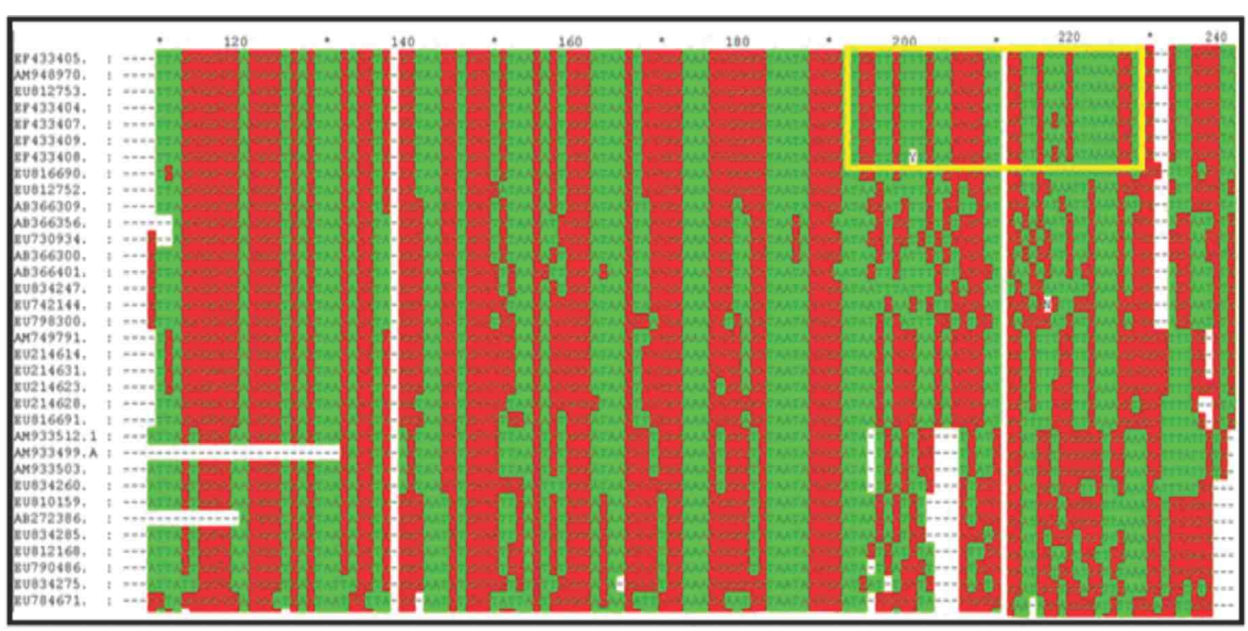
There are more than 200 species of *Bacillus* and it is difficult to identify the different species on morphological, cultural and biochemical methods. Diagnostics based on molecular techniques could be employed to distinguish *Bacillus* and Bacillus derived genera. For rapid identification of Pseudomonas gacA markers which is a response regulator involved in post-transcriptional regulation of gene expression have been selected for designing specific primers.

Objectives

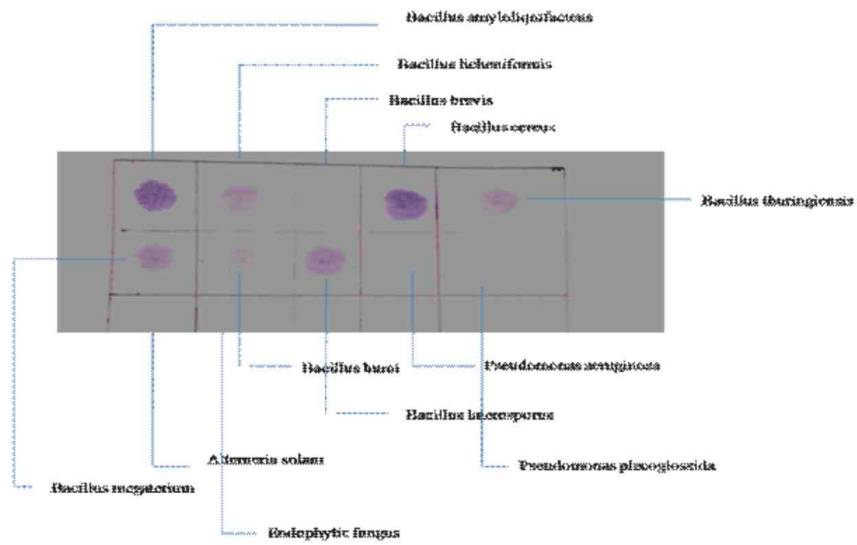
- To isolate and characterize the soil microbes (*Bacillus* and *Pseudomonas*)
- To develop rapid diagnostic kits for identification of soil microbes. (*Bacillus* and *Pseudomonas*)

Significant achievements

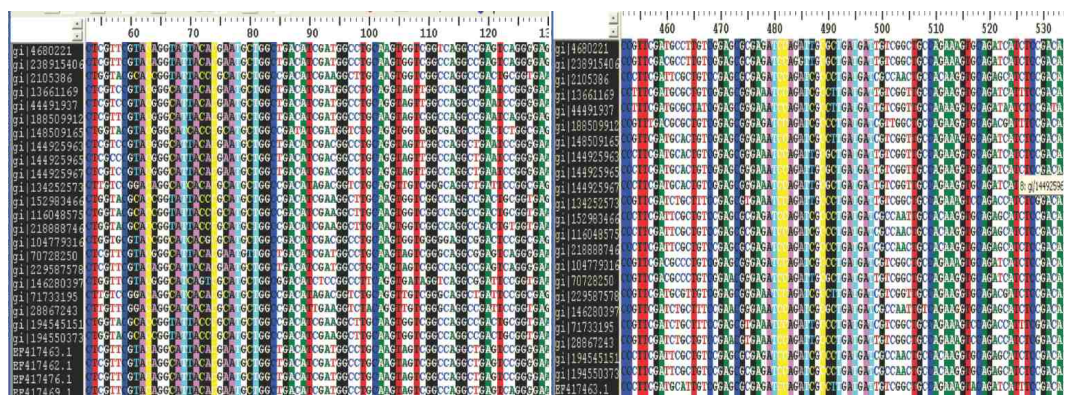
- In the last annual report, it was reported that amplification of 220bp region of 16S rDNA with nested primer pair followed by sequencing could help in the identification of *Bacillus* species and *Bacillus* derived genera.
- This small hypervariable region contains all the information for delineation of the species.
- To prove the hypothesis further, complete 16S rDNA and 220 bp fragment were amplified from 20 different species of *Bacillus*. All the sequences were BLAST searched and identical identity of



Alignment of 16S rDNA sequences of *Bacillus*, *Bacillus* derived genera and *Bacillus* related genera



Dot Blot hybridization of designed probe for the identification of Bacillus (at 64.4^{0}C, probe showed hybridization signal for Bacillus 16S rDNA samples and negative results with Pseudomonas and fungal samples)



Regions targetted for development of PCR primer based on GacA(Global activator of antibiotic and cyanide production) gene

the species was obtained from either sequencing complete or partial sequencing.

- Another approach was also used to design the probe for identification of genus *Bacillus*. An oligonucleotide probe for identification of genus *Bacillus* was designed from the internal conserved regions of 16S rDNA following alignment of 16s rDNA sequences of *Bacillus*, *Bacillus* derived genera and *Bacillus* related genera. The probe is non-radioactively labeled and is validated for its sensitivity and specificity.
- Degenerate primers for the *gacA* gene of *Pseudomonas* genus was developed based on the available gene sequences.
- DGGE profiling of phylogenetic gene (16S rRNA) of central and eastern Indo gangetic soil flora was done.
- The target regions for PCR primer have been validated both in silico as well as in wet lab with some other genus *Xanthomonas* group, *Vibrionaceae, Alteromonadaceae, Enterobacteriaceae*.

Conclusion

An oligonucleotide 50 mer probe were designed for the identification of genus *Bacillus* from the internal conserved regions of 16S rDNA following alignment of 16s rDNA sequences of *Bacillus*, *Bacillus* derived genera and *Bacillus* related genera. The probe is non-radioactively labeled and is validated with annealing temperature of 64.4^{0}C, the probe showed hybridization signal for *Bacillus* 16S rDNA samples and negative results with Pseudomonas and fungal samples.

Project: Diversity analysis of microbes in extreme conditions

PI : Dilip K. Arora
CoPI : Alok Kumar Srivastava, Sudhir Kumar and Mahesh Yandigeri

Rationale

Microbial diversity encompasses a spectrum of microscopic organisms including bacteria, actinomycetes, fungi, algae and protozoa. An estimated 50% of all living protoplasm on earth is microbes. There may be 1.5 million species of fungi yet only 5% are described; as many as one million species of bacterium exist but only about 5000 have been described in the last century. A gram of typical soil contains about billion bacteria, but only 1% can be cultured in the laboratory. Fewer than 5% of all microbial species have been discovered and named- even less is known about the diversity within these species. Microorganisms represent the richest gamut of molecular and chemical diversity in nature, as they comprise the most diverse forms of life. Over the period of time they abound in all kinds of habitats *viz*., with extreme of pH, salinity, water stress, temperature etc. interest in the exploration of microbial diversity has been spurred by the fact that microbes are essential for life since they perform numerous functions essential for the biosphere that includes nutrient cycling and environment detoxification. In India it is even more relevant due to our enormous wealth of available biodiversity. Therefor, continued research is needed to describe and protect the unexplored resources for the preservation of natural ecosystems and future benefits of mankind.

Objectives

- Survey and collection of soil and water samples from extreme climates
- Isolation of microorganisms employing different media and screening for high or low temperature, salt tolerance, acidic or alkaline pH.
- To look for the production of enzymes protease, amylase, xylanases and cellulases.
- Molecular characterization through PCR amplification of ribosomal genes.
- DNA sequencing and identification of novel extremophilic microorganisms.

Significant achievement

- Samples were collected from different extremophilic niches such as acidophilic (Tipong coal mines), Halophilic (Goa mangroves), Psychrophilic and Thermophilic hot spring of Leh and Sikkim, India.
- Physiochemical parameters including pH of water and sediment samples was analysed as, 7.5, 2.5, 6.5 and 8.6 of Goa mangrove, Tipong coal mines (Assam, high sellinium: low nickel metal concentration), Sikkim thermal spring and Leh psychrophilic regions were recorded respectively.

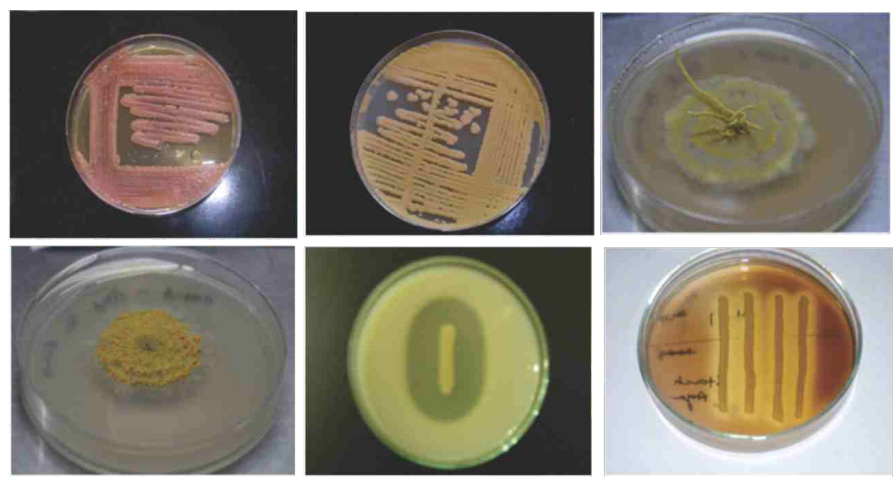
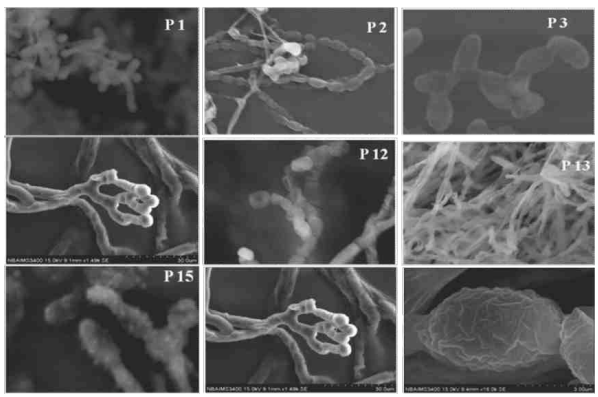
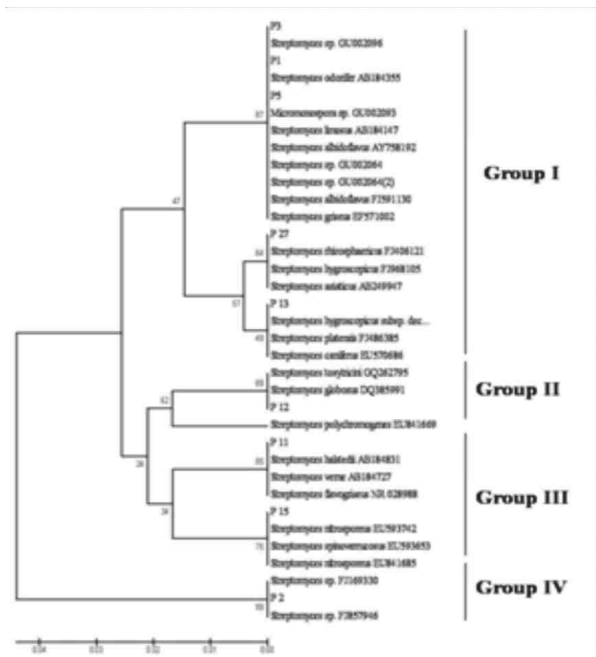


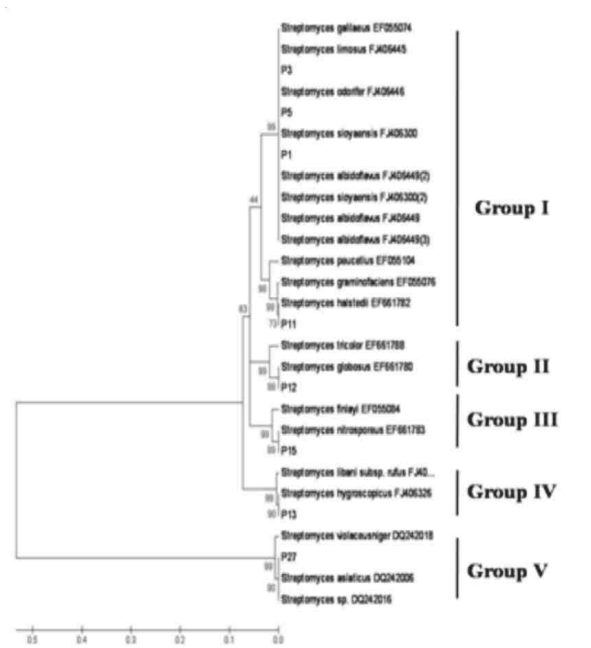
Plate photograph and biochemical characterization of extremophilic isolate, from extreme regions of India.



Scanning electron micrograph of psychrophilic actinomycetes and halophilic isolates obtained from Leh (psychrophilic niche)

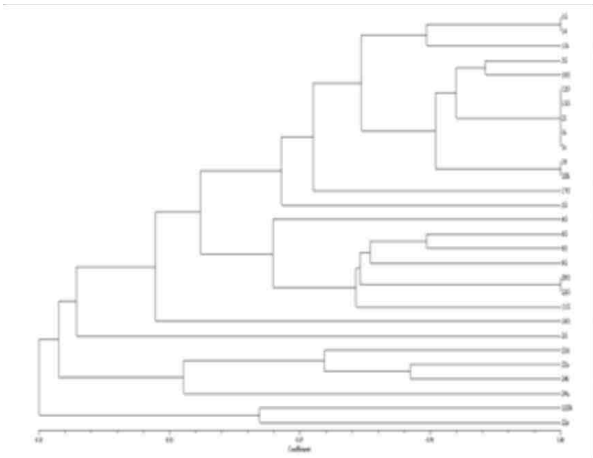


Phylogenetic relationships of psychrophilic actinobacterial species based on complete nucleotide sequences (1.5 kb) of the 16S rDNA. The tree was generated by the neighbor-joining method. Percentages at nodes represent levels of bootstrap support from 1000 resampled datasets. Bar 0.001% indicates estimated sequence divergence.

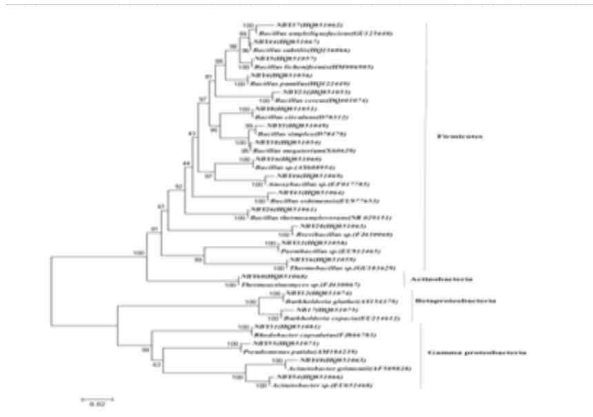


Phylogenetic relationships of psychrophilic actinobacterial species based on partial nucleotide sequences (540 bp) of the DNA directed RNA polymerase beta subunit (rpo β) gene. The tree was generated by the neighbor-joining method. Percentages at nodes represent levels of bootstrap support from 1000 resampled datasets. Bar 0.001% indicates estimated sequence divergence.

- Enumeration of microbial loads includes Goa mangrove (fungi 3-5×10^5; bacteria 20-32×10^4), Sikkim hot spring (2×10^3) and Leh psychrophilic region (8×10^2 cfu/g soil/mL), using different media as well as various enrichment techniques.
- Collectively a total of 223 microorganisms were isolated from these extremophilic regions includes (95 isolates from Goa mangrove, 50 isolates from Tipong coal mines, 53 isolates from sikkim hot springs, and 25 actinomycetes from leh psychrophilic regions) showing different phenotype as evident by morphological characterization by using Scanning electron microscopy.
- Goa mangrove harbours halophilic microorganisms and have ablilty to grow 20% NaCl (6 fungal and 4 bacterial isolates), showed luxurient growth at high salt concentration.
- 3 isolates from tipong coal mine were grows at pH 3.0. Sikkim hot spring isolates showed growth at 45°C and of them 11 isolates showed growth at 65°C temperature, while leh region actinomycetes isolates showed luxuriant growth at 10 °C respectively.
- Bacterial isolates from Goa mangrove ecosystem were further characterized for its PGP attributes and showed 5 and 6 isolates produces phosphate solubilization and siderophore production, while extracellular enzymes production from Goa and Sikkim thermal spring showed that 9, 10, 3, 10 isolates produces protease, amylase,



Phylogenetic analysis of halophilic microbes showing diversity among the Goa isolates.



Neighbor-joining tree showing the phylogenetic relationships between culturable bacterial 16S rRNA gene sequences from **Yumthang and Yumesamdong hot spring** and closely related sequences from the GenBank database

cellulase and lipase enzymes, respectively.

- Molecular characterization of halophilic fungus and bacterial isolates includes amplification of 16S rDNA (bacteria) and ITS region (fungi), followed by ARDRA analysis of halophilic, thermophilic bacteria as well as psychrophilic actinomycetes isolates by using *AluI*, MspIII, *HaeIII* (*HhaI, HaeII*I and *TaqI* for actinomycetes) restriction endonuclease showed 16 and 22 clusters for halophilic and thermophilic bacteria. Molecular characterization of actinomycetes was also carried out by amplification and sequencing of DNA directed RNA polymerase beta subunit (*rpo*β) gene.
- Idnetification of thermophilic bacteria showed the bacterial groups belongs to *β- Proteobacteria, -Proteobacteria, Firmicutes* and *Actinobacteria*, while psychrophilic actinomycetes were taxonomically identified as *Streptomyces odorifer* (P3), *Streptomyces limosus* (P5), *Streptomyces* sp. (P2), *Streptomyces albidoflavus* (P1), *Streptomyces globosus* (P12), *Streptomyces asiaticus* (P27) & *Streptomyces nitrosporeus* (P15), *Streptomyces hygroscopicus* (P13), respectively, with an accession numbers for 16S rRNA as HQ1387472-HQ1387479 and for DNA directed RNA polymerase beta subunit gene (*rpo* β) genes HQ1387480- HQ1387487 respectively.

Conclusion

The evaluation of microbial population is required for understanding its biogeography, community assembly and ecological processes within a particular exotic niche. Exotic niches harbor population of microorganisms they represent extreme niches that have maintained some degree of pristine quality and their biotechnological potential has remained unrealized. Total 223 extremophilic microorganisms belongs to different community as well as genera's such as 55 and 40 halophilic fungi and bacteria from goa mangroves, 50 acidophilic isolates from tipong coal mines, 53 thermophilic isolates from Sikkim hot springs and 25 psychrophilic actinomycetes from Leh regions, India were isolated respectively. They showed varied structural diversity (evident by plate photograph and Scanning electron microscope), as well as fuctional diversity such as PGP attributes (5 phosphates solubilizers, 6 siderophore producers), extracellular enzyme produers (9 protease producers, 10 amylase producers, 3 cellulase producers, 10 lipase enzyme producers).

The findings showed that 7 isolates showed growth at 20%NaCl, followed by 50 isolates grow at pH 3.0 -5.0, 53 isolates from thermal springs grow at 45°C as well as 25 psychrophilic actinomycetes isolates showed luxuriant growth at 10°C were isolated from Leh psychrophilic regions' respectivley. The results provide further evidence that, the isolation of culturable microorganisms can significantly contribute to our knowledge of species endemic to these extremophilic regions.

Project: Diversity of actinomycetes from Indogangetic plains

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

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. In India, Indogangetic 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. 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 Indogangetic plains.
- Functional characterization of isolated strains using BIOLOG microbial identification system and conventional biochemical methods.

Significant achievements

- A total of 238 colonies of *Streptomycetes* were isolated from different regions of IGP, among which 145 isolates showing wide variation in the colony morphology were chosen for further studies. The population count of Streptomycetes fluctuated from 14 X 10^2 to 32 X 10^2 g $soil^{-1}$), showed to be fluctuated.
- Genus *Streptomyces* was tentatively identified by the morphological characterization using aerial mycelial colour, substrate mycelial colour, pigments, and spore chain arrangements such as rectiflexibles (RF), retinaculaparti (RA), or straight chain (Scanning Electron Microscopy.
- PGP activity of all the isolates revealed that a total of 57.2% were ammonia producers, 8% siderophore producers and 34.4% of phosphate solubilizers. All Streptomycetes isolates were assayed for salinity tolerance (2, 4, 6, and 8% NaCl), antimicrobial assay (>15 mm inhibition

Table 1. Geographical location and enumeration of Streptomycetes isolates from Indo-gangetic Plains, India

Coordinates	Location	Soil Type	pH	Total number of colonies/ *Streptomyces* count (× 10^2)
30.33°N 78.06°E	Northern Indogangetic plain	Alluvial soils	7.5-7.8	35/23
29°23°N 79°27°E	Northern Indogangetic plain	Loamy to sandy loam	6-6.5	29/14
29.96°N 78.16°E	Northern Indogangetic plain	Loamy to sandy loam	7-7.5	31/14
29.59°N 79.65°E	Northern Indogangetic plain	Loamy to sandy loam	6-6.5	32/19
26.50°N 80.50°E	Upper Indogangetic plain	Saline-alkaline alluvial	7.8-8.5	47/32
27.57°N 80.68°E	Upper Indogangetic plain	Saline, alluvial	7.5-7.8	36/23
27.58°N 81.60°E	Upper Indogangetic plain	Saline, alluvial	7.3-7.7	38/22

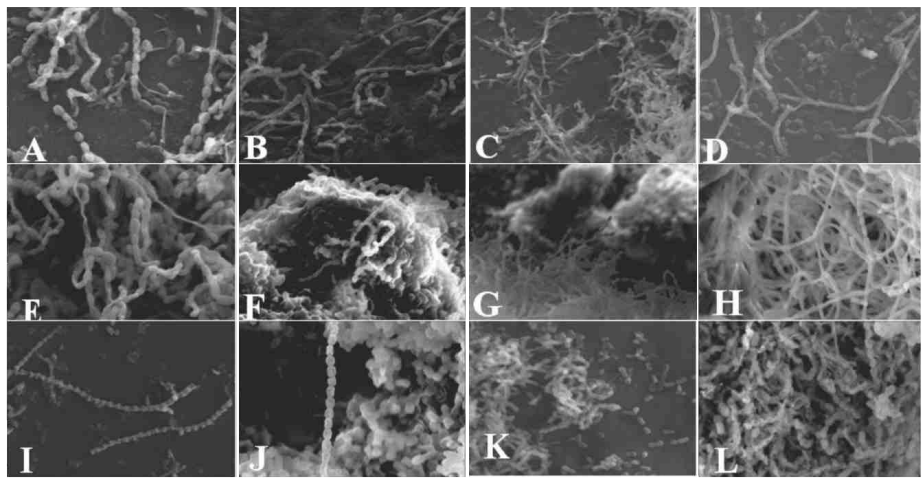


Figure 1 Scanning Electron Microscopy (SEM), of actinobacterial isolates isolated from IGP, India, showing variations in spore chain morphology as: (A) C4 (*S. fumigatiscleroticus*), (B) C46 (*S. fradiae*), (C) D14 (*S. bacillaris*), (D) D21 (*S. flavidofuscus*), (E) N61 (*S. avidinii*), (F) D13 (*Streptomyces* sp.), (G) N37 (*S. vinaceus*), (H) N29 (*S. carpaticus*), (I) N64 (*S. albus*), (J) D43 (*S. rubrolavendulae*), (K) N40 (*S. albogriseolus*), and (L) N71 (*S. Viridodiastaticus*).

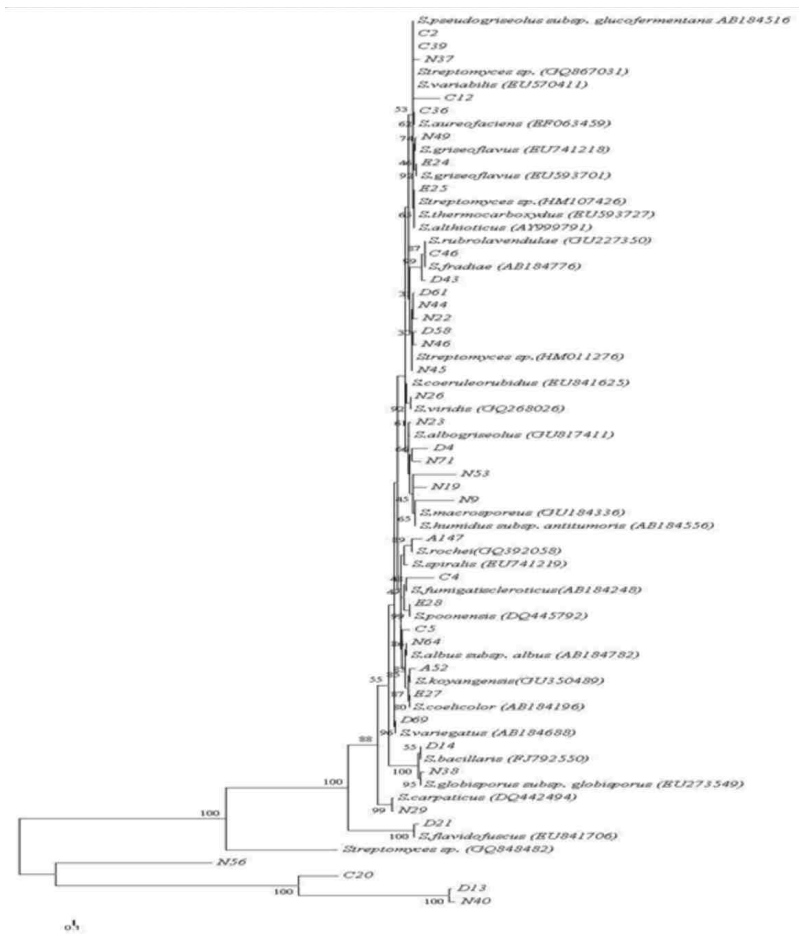


Figure 2. NJ phylogenetic tree of full 16S rRNA sequences from selected isolates. The sequence data for several closely related actinobacterial type cultures were recovered from genebank and included in the tree. The accession numbers for the sequences are as follows: AB184404, EU570411, EU294135, GQ268026, GU350489, EU841624, FJ481066, AB184139, EF063459, GU817411, AY999791, AB184248, FJ486350, DQ445792, EU741219, AB184688, GU383166, AB184556, AB184776, FJ792550 and EU273549. The boot strap values from 5,000 pseudoreplications are shown at each of the branch points on the tree. Bar indicates % similarity.

zone) against *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium ciceri* and *Bacillus subtilis*, and cyanide production.

- Molecular characterization of *Streptomyces* from different regions of IGP was carried out based on 16S rRNA gene amplification and its RFLP pattern with a set of three restriction enzymes *Hha*I, *Hae*III and *Taq*I. A total of 40 isolates were chosen as representatives based on RFLP clustering at >70% similarity level and were sequenced by Sanger's di-deoxy nucleotide sequencing method and identification were based on percentage similarity (>97% compared with public database sequences, NCBI), by BLAST homology. Further phylogenetic analysis of 40 representatives was carried out for their similarity to known actinobacteria aligned together with the sequences (closest representatives), available in public databases (Genbank, NCBI), of actinobacteria.
- Among the representative isolates N53 although showed 96% sequence similarity to *S. albogriseolus* and it may be a new species of *Streptomycetes*, but morphological as well biochemical characterization of N53 isolate was compared with type strain *S. albogriseolus* (DSM 40003), did not reveal significant differences.

Conclusion

In conclusion, these results provided further evidence that species diversity of actinobacteria is higher in Indo-Gangetic Plains of India. Also, these had promising potential for plant growth promoting and various other attributes. The culturable *Streptomycetes*, isolated from IGP regions in the India were clustered into three groups. The isolation of culturable actinobacteria has contributed to our knowledge of diversity and population structure of actinoacteria from India's most fertile regions and further increased the information of actinobacteria available for the plant growth promoting attributes. Further isolate N53 from this study seemed to be new species and needs to be validated by DNA-DNA hybridization, (%) GC content as well as FAME analysis for its identification up to species level.

Nutrient Management, PGPR and Biocontrol

Project: Exploration, collection and characterization of some agriculturally important biocontrol agents suitable for disease management

PI : Dilip K. Arora
Co PI : Alok Kumar Srivastava, Sudheer Kumar

Rationale

Chickpea (*Cicer arietinum L.*) is the world's third most important pulse crop after bean and pea, with India accounting for approximately 75% of the world chickpea production. Chickpea productivity, however, remained virtually stagnant from past few decades because of its susceptibility to diseases such as wilt (*Fusarium oxysporum* f. sp. *ciceri,* FOC), black root rot (*F. solani, FS*) and charcoal rot (*Macrophomina phaseolina, MP)*. The synergistic interaction of these pathogens causes more than 80% reduction in yield, if proper and timely measures are not taken to control menace of these pathogens. Moreover, farmers heavily rely on the application of fungicides and pesticides to manage these seed and soilborne pathogens. Because of concerns regarding both human health and environmental protection, viable alternatives to these chemicals are being sought. It has been recognized from a long time that a large number of naturally occurring rhizospheric bacteria are antagonistic towards plant pathogens and as a result may offer a viable substitute for abandoning the use of these chemicals.

The interest in the use of biological approaches to replace hazardous pesticides in fertilizing soils or improve plant resistance against phytopathogens is steadily gaining worldwide acceptance. In this regard, the use of plant growth promoting rhizobacteria (PGPR) has a potential role in developing sustainable agricultural systems for crop production and protection. Plant roots influence soilborne microbial communities via several

mechanisms, including excretion of specific organic compounds, competition for nutrients, and providing a solid surface for attachment. *Bacillus* spp. has many characteristics as an excellent biocontrol agent, including the production of structurally diverse antibiotics, formation of viable spores, promotion of plant growth, and an ubiquitous presence in soil. Some of the well documented characteristics of *Bacillus* spp. related to soil fertility and plant nutrition optimization are the production of bacterial phytohormones and/or the solubilization of mineral phosphates. Additionally, strains of *Bacillus* have paramount advantages over other bacteria involved in biocontrol in being mostly soil inhabitants, capable of sporulation, easy to cultivate, long shelf life and showing increased yields of various crops.

Objective

- 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
- Field evaluation of potent biocontrol agents

Significant achievements

A total 480 bacterial strains were obtained from the rhizosphere of different crops from Indogangetic plain regions (Mau, Varanasi, Lucknow and Kanpur) of India. Out of them, only fourteen bacterial strains (B-CK11, B-ML26, B-PV53, B-CL94, B-PL125, B-MV146, B-WM77, B-MM208, B-WV249, B-CV280, B-CM331, B-CL392, B-PK413 and B-PM444) showed significant inhibitory effect on mycelial growth (>5mm diameter) against all the three plant pathogenic fungi. Similarly, culture filtrates of *Bacillus* spp. inhibited radial colony growth of *FOC race 1*, *FS* and *MP* at varying degrees. Strain B-CM331, B-TM444, and B-WM177 were the most efficient antagonists and showed 38.33, 37.03 and 36.80 mm inhibition zone against *FOC race 1*, respectively.

Phenotypic characterization of strains

Phenotypic characterisation of fourteen bacterial

In vitro screening of antagonistic population of *Bacillus* strains against chickpea pathogens

Sampling site	Crop	C.F.U (g^{-1} soil)	Strains(s) obtained	Number of isolates antagonistic to different pathogens (% antagonists)		
				***FOC* race 1**	***FS***	***MP***
Mau	Chickpea	22.4×10^6	50	14 (28.0)	13 (26)	15 (30.0)
	Mustard	12.8×10^6	35	8 (22.86)	10 (28.57)	12 (34.29)
	Potato	16.3×10^6	30	6 (20.0)	9 (30.0)	10 (33.33)
	Wheat	9.8×10^6	25	5 (20.0)	8 (32.0)	7 (28.0)
Varanasi	Chickpea	20.4×10^6	45	11 (24.44)	12 (26.67)	13 (28.89)
	Mustard	13.6×10^6	30	6 (20.0)	8 (26.67)	10 (33.33)
	Potato	15.2×10^6	35	5 (14.29)	12 (34.29)	12 (34.29)
	Wheat	8.7×10^6	30	5 (16.67)	9 (30.0)	12 (40.0)
Lucknow	Chickpea	18.4×10^6	30	9 (30.0)	7 (23.33)	8 (26.67)
	Mustard	10.9×10^6	25	4 (16.0)	7 (28.0)	9 (36.0)
	Potato	18.8×10^6	25	4 (16.0)	8 (32.0)	9 (36.0)
	Wheat	8.9×10^6	20	5 (25.0)	7 (35.0)	5 (25.0)
Kanpur	Chickpea	17.5×10^6	35	12 (34.29)	8 (22.86)	7 (20.0)
	Mustard	11.1×10^6	25	5 (20.0)	7 (28.0)	7 (28.0)
	Potato	9.9×10^6	20	5 (25.0)	6 (30.0)	5 (25.0)
	Wheat	7.6×10^6	20	4 (20.0)	7 (35.0)	6 (30.0)

Table 2: Effect of culture filtrates of the *Bacillus* spp. on the radial growth of *FOC* race 1, *FS and MP*

Strain(s)	Inhibition zone (mm)		
	***FOC* race1**	***FS***	***MP***
B-CK11	30.53 ± 2.08^{cde}	25.30 ± 2.66^{def}	31.17 ± 1.40^{cde}
B-ML26	32.10 ±1.35^{cd}	31.50 ±1.91^{abc}	32.03 ±1.75^{cd}
B-PV53	28.13 ±3.45^{def}	23.43 ±0.91^{f}	26.93 ±1.29^{fgh}
B-CL94	34.43 ±1.98^{abc}	22.77 ±1.50^{f}	30.37 ±2.99^{c-f}
B-PL125	33.83 ±2.16^{bc}	33.00 ±2.03^{ab}	29.60 ±0.92^{def}
B-MV146	31.03 ±2.15^{cde}	22.80 ±1.15^{f}	27.10 ±1.41^{fgh}
B-WM177	36.80 ±1.45^{ab}	29.37 ±1.50^{bc}	33.20 ±1.40^{c}
B-MM208	27.47 ±2.35^{efg}	28.80 ±2.65^{cd}	28.07 ±1.92^{efg}
B-WV249	25.13 ±2.20^{fgh}	27.97 ±1.63^{cde}	25.17 ±1.84^{gh}
B-CV280	23.23 ±1.17^{h}	23.47 ±2.20^{f}	24.53 ±2.90^{gh}
B-CM331	37.03 ±1.15^{ab}	30.37 ±1.63^{abc}	37.50 ±2.14^{b}
B-WL392	24.07 ±2.48^{gh}	24.53 ±2.75^{ef}	23.60 ±2.61^{h}
B-PK413	18.17 ±2.93^{i}	22.90 ±1.77^{f}	25.40 ±1.05^{gh}
B-PM444	38.33 ±2.80^{a}	33.60 ±2.72^{a}	40.97 ±2.16^{a}

Mean ±SE value (n=3) in the same column followed by the same letter(s) are not significantly different (P= 0.05) according to Duncan's Multiple Range Test. For each experiment, values represent the means of three independent experiments. Each experiment was conducted in three replicates.

antagonists showed that most of the strains were rod shaped, motile and endospore forming (Table 3). B-WM177, B-CM11 and B-PM444 strain were coco-bacilli in shape while B-PK413 strain showed ellipsoidal morphology. These morphological results confirmed the close relationship of all the bacterial antagonists with *Bacillus* spp.

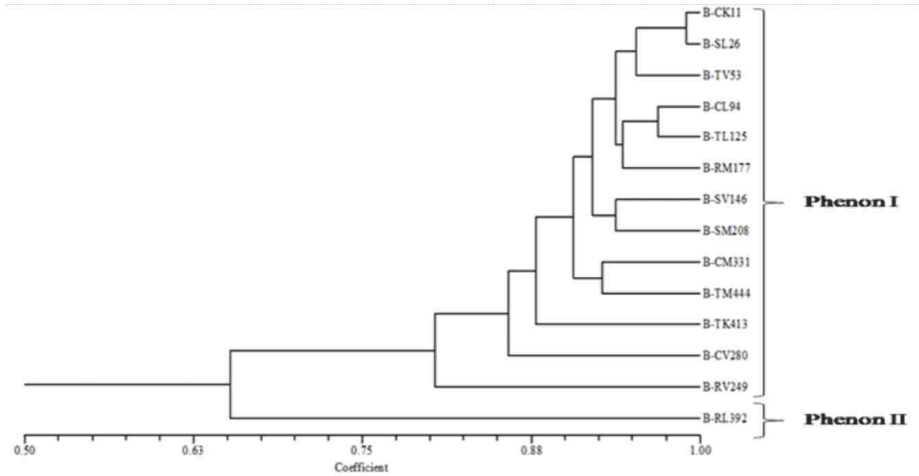
Phenogram of antagonistic strains of *Bacillus* based on their carbon source utilization profiles

The clustering was done using sequential, agglomerative, hierarchical and nested (SAHN) method. The pair wise coefficient of similarity was used for clustering with the UPGMA algorithm using NTSYSpc2.02a software. The phenogram resulted

Table 3: Morphological and biochemical characterization of rhizosphere associated antagonistic *Bacillus* spp.

Strain(s)	Morphological tests			
	Grams staining	Shape	Spore formation	Motility
B-CK11	+	Rods	+	+
B-ML26	+	Rods	+	+
B-PV53	+	Rods	+	+
B-CL94	+	Rods	+	+
B-PL125	+	Rods	+	+
B-MV146	+	Rods	+	+
B-WM177	+	Coccobacilli	+	+
B-MM208	+	Rods	+	+
B-WV249	+	Rods	+	+
B-CV280	+	Rods	+	+
B-CM331	+	Coccobacilli	+	+
B-WL392	+	Rods	+	+
B-PK413	+	Ellipsoid	+	+
B-PM444	+	Coccobacilli	+	+

+ Positive result; - Negative result



Phenogram of antagonistic strains of *Bacillus* based on their carbon source utilization profile

into two major phenon at 0.76 similarity coefficient. The experiment was performed with three replicate(s).

Conclusion

The plant rhizosphere is a versatile and dynamic ecological environment of intense microbesplant interactions for harnessing essential micro and macro-nutrients from a limited nutrient pool. We focused on bacterial genera that are often found in large populations in soils with general disease suppression, such as Gram positive spore-forming species belonging to *Bacillus* and *Bacillus* derived genus. In this host and site specific explorative study, we used a cultivation based dual culture and well diffusion assay to characterize the *in vitro* antagonistic potential towards *FOC race 1*, *FS* and MP amongst dominant culturable strains of Bacillus collected from four different host rhizosphere (chickpea, wheat, potato and mustard) in four different sites (Mau, Varanasi, Lucknow and Kanpur) in Indo-Gangetic plains of India. The strains were taxonomically described as *L. fusiformis, Lysinibacillus* spp., *Bacillus cereus*, *B. subtilis*, *Bacillus* spp. and *B. thuringiensis* on the basis of 16S rRNA gene sequencing and subsequent molecular phylogeny analysis.

Project: Biocontrol of soil borne plant pathogen and growth promotion in vegetable crops

PI :Sudheer Kumar
Co-PI :Alok Kumar Srivastava

Rationale

Among soil borne plant pathogen, fungi are considered as important plant pathogens, particularly members of the genera *Fusarium* and *Rhizoctonia* which are able to infect a wide range of vegetable crops including tomato, potato and cucumber etc. The control of these diseases a big challenge as they form the resistance structure like spore.

Presently, the main focus on the exploitation of bacteria as biocontrol agent (BCAs) for the control of these pathogens and reduce the use of chemical agents. These PGPBs (plant growth promoting bacteria) present in the rhizosphere and in association with roots help in improving the extent or quality of plant growth directly or indirectly as they produce certain signaling molecules and sometime also induces induces systemic resistance.

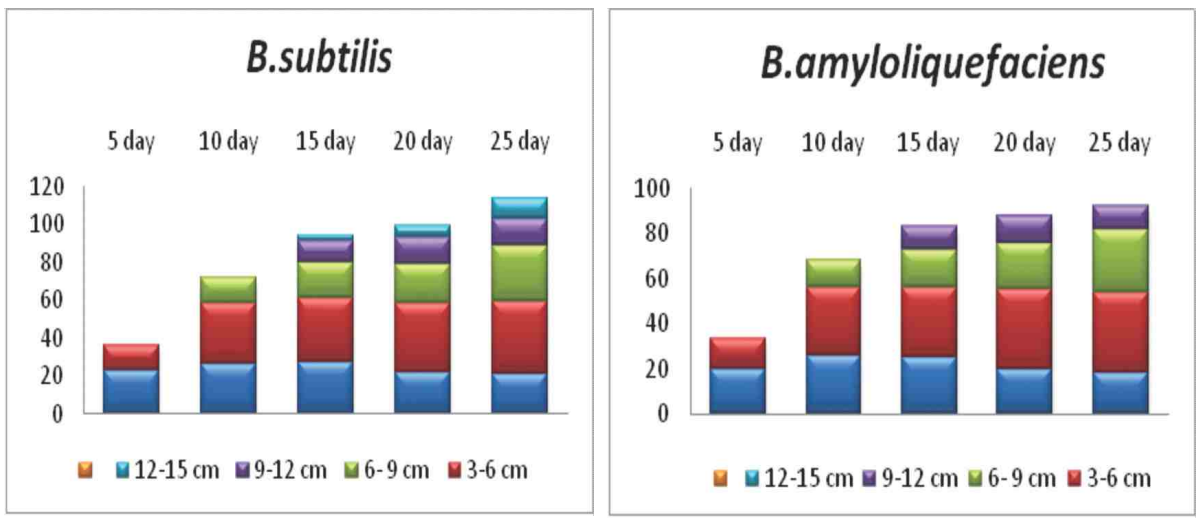
BCAs have disease control ability along with the growth promotion in vegetable crops. The most common used antagonistic bacteria are *Bacillus* and *Pseudomonas* species. Among them *B. subtilis* and *B. amyloliquefaciens were* found as potent biocontrol agents and growth promoter in vegetable crop system.

Objectives

- Isolation, identification and characterization of bacterial biocontrol agents.
- Study the rhizospheric competence and factors affecting it.
- Development of consortia of efficient isolates.

Significant achievements

- Soil samples were collected from salt affected soil of IGP region *viz* Lucknow, Kanpur, Allahabad, Mau, Varanasi. Isolation of bacterial strains from soil samples done by using standard methods. A total no. of 150 bacteria were isolated and evaluated against *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani a*nd after it 38 were found as antagonist action towards these pathogens.
- The bacterial isolates were characterized for biochemical mechanism like, siderophore, ammonia, HCN and chitinase production. All these selected isolates were further characterized on the basis of molecular studies and finally potent bacterial species identified by 16S rDNA sequencing.
- Antibiotic susceptibility to different antibiotics was also performed. A compatibility analysis showed compatibility among B-14, B-101 and P-2 strain.
- Two potent isolates identified as B. subtilis (B-14) and *B. Amyloliquefaciens* (B-101) were evaluated for growth promotion of tomato plant and effective root colonization of these strains were measure at different region of root.
- These selected strains B-14 and B-101 were also evaluated for biofilm formation tendency and EPS production which are important for root colonization and quantification for biofilm formation was done by crystal violet method and EPS (Exopolysaccharides) production were measured by phenol sulfuric method.
- The both strains are have good biofilm forming

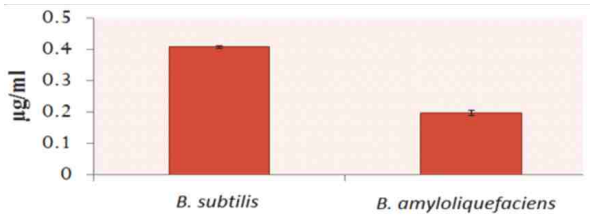


Root colonization in tomato plant were counted as cfu count of B-14 and B-101 at different regions of root during 5 days intervals and found more colonization at matured region by both biocontrol strains at 10^{-3} dilution

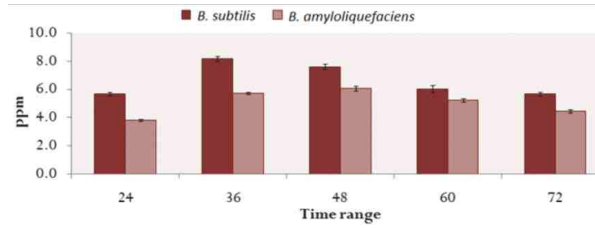
Table: Growth Promotion by selected biocontrol agents

Sample (n = 3)	Root Length (c.m.)	Shoot Length (c.m.)	Root Fresh wt.(mg)	Shoot Fresh wt.(mg)	Root Dry wt.(mg)	Shoot Dry wt.(mg)
B. amyloliquefaciens	12 ± 0.03	25.5 ± 0.04	350 ± 16	4060 ± 106	79 ± 5	1040 ± 98
B.subtilis	15 ± 0.02	29 ± 0.02	680 ± 29	6220 ± 190	102 ± 7	1198 ± 100
Control	6 ± 0.02	19 ± 0.02	247 ± 14	2197 ± 143	45 ± 4	536 ± 52

Biofilm formation capacity



EPS production



capacity and among them *B. subtilis* was found better biofilm former and EPS producer.

Conclusion

The potent biocontrol *B. subtilis* (B-14) and *B. amyloliquefaciens* (B-101) are good biofilm former and also produce EPS indicates that are the good root colonizer and may established in better way in the rhizosphere. These agents can be formulated as consortia to control fungal pathogen at primary level.

Project: Isolation of Oxalate Oxidase gene and decipherization of its potential to control *Sclerotinia sclerotiorum*

PI : Dilip K. Arora
Co PI : Alok Kumar Srivastava, Dr. Sudheer Kumar

Rationale

Sclerotinia sclerotiorum is among the world's most successful and omnivorous fungal phytopathogens, able to infect an extremely wide range of cultivated plants. 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 also are hosts. The dispersal, propagation, and long-term survival of this pathogen is mediated through the sclerotium, a pigmented, asexual, multicellular, and firm resting structure composed of condensed vegetative hyphal cells which become interwoven and aggregate together, and it is able to remain quiescent for extended periods under conditions that are unfavorable for vegetative growth.

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 polygalacturonas. Oxalic acid chelates calcium, resulting in a destabilization of pectate polymers allowing increased access and sensitivity to pathogen-produced pectolytic enzyme Oxalate oxidase (OXO) converts oxalic acid and oxygen to carbon dioxide and hydrogen peroxide.

Objectives

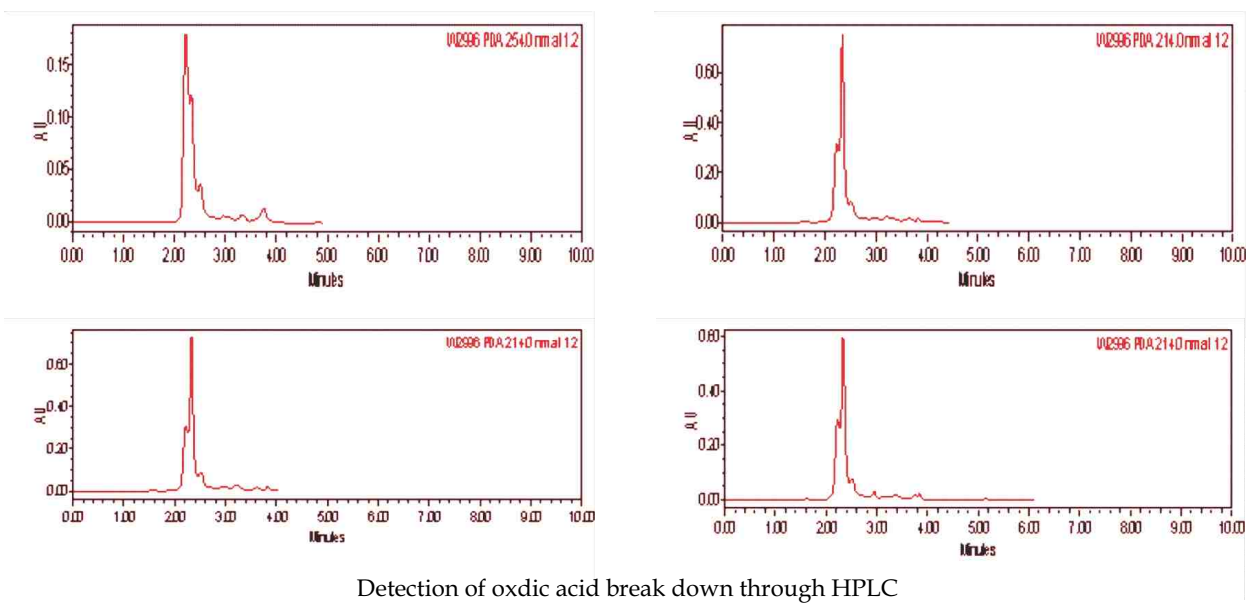
- Isolation and characterization of antagonistic fungi (against *S. sclerotiorum*) from vegetable rhizosphere.
- To screen potential isolates on oxalic acid amended media containing 10, 20, 30, 40 Ml concentration of oxalic acid
- Enzymatic estimation of oxalate oxidase enzyme in fungal extract.
- HPLC analysis for degradation of oxalic acid through oxalate oxidase present in fungal extract.

Significant achievements

- Isolates from the different vegetable rhizosphere soil of the Mau region were screened on different concentration of oxalic acid amended media, 24 cultures growing on 20mM oxalic acid containing media, 15 on 30mM oxalic acid containing media and 10 on 10mM oxalic acid containing media.
- These cultures are screened by biochemical test for oxalate oxidase, five culture are found positive.
- These positive culture are further analyzed through HPLC for degradation of oxalic acid through oxalate oxidase present in fungal extract at different time interval

Oxalate oxidase activity of the potential fungal isolates

Sample	Enzyme activity U /ml	Sample	Enzyme activity U /ml
F-1	1.25	F-13	1.04
F-2	0.74	F-14	0.59
F-3	0.86	F-15	0.79
F-4	1.27	F-16	0.98
F-5	1.22	F-17	0.76
F-6	0.56	F-18	0.48
F-7	0.85	F-19	1.38
F-8	0.13	F-20	0.16
F-9	0.85	F-21	0.22
F-10	1.31	F-22	0.24
F-11	0.77	*C.minitans*	1.19
F-12	0.49	*A. biennis*	1.06



Detection of oxdic acid break down through HPLC

Conclusion

Results of screening of oxalate oxidase enzyme in fungal biocontrol agents screened are encouraging, five fungal isolates are found positive for oxalate oxidase enzyme.

Project: Evaluation of endophytic fungus for growthpromotion and biocontrol

PI :Alok K. Srivastava
Co PI :Sudheer Kumar

Rationale

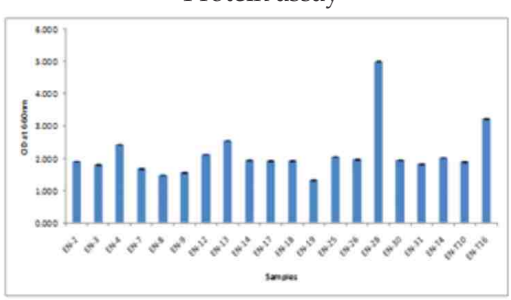
IGP and Western Ghat vegetation are part of highly productive ecosystems and have essential functions in habitat structuring. The complex communities that develop in these systems provide models for investigating plant-fungus interactions in a natural environment. Endophytes and parasites are the predominant fungi in these forest communities however, most of these organisms are primarily recognized as fungi that are active in terrestrial environments and include known endophytes. Fungal endophytes live within their host plants without causing any apparent disease symptoms. Endophytic fungi, residing almost ubiquitously inside the fresh healthy tissue of plants, have been accepted as a big but nearly untapped microbial reservoir that can be expected to provide a wide variety of structurally unique and/or biologically potent natural product.

Endophytes is a group of biotrophic fungi that form symbiotic associations with many plants. These endophytes systematically colonize the intercellular spaces of plant tissues. Through this association, the endophyte either produces a range of alkaloids or stimulates the host plant to synthesize alkaloids and other secondary metabolites, resulting a range of adaptations to biotic and abiotic stresses. Endophyte infection has also been shown to modulate growth, morphology, nitrogen assimilation, resource allocation, and mineral uptake of the host plant. The physiological mechanisms that are the basis for these traits are unknown. Keeping these points in view following objectives has been undertaken for the isolation of fungal endophytes with the potential as growth regulation and biocontrol.

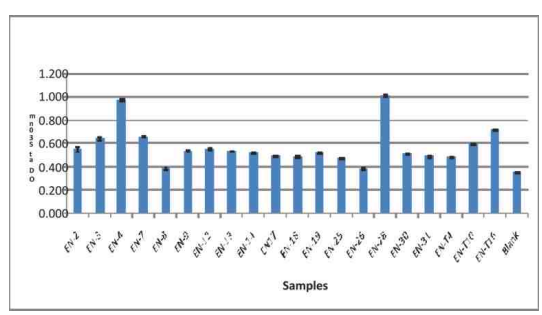
Objectives

- Isolation, identification and characterization of endophytic fungus from IGP and western Ghat.

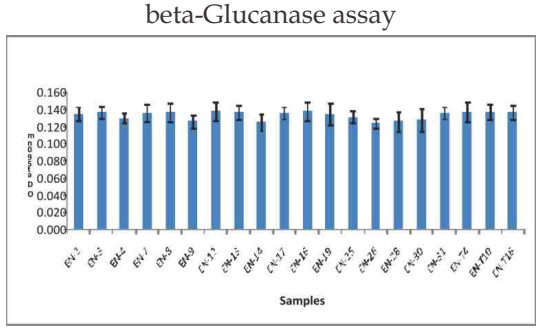
Protein assay



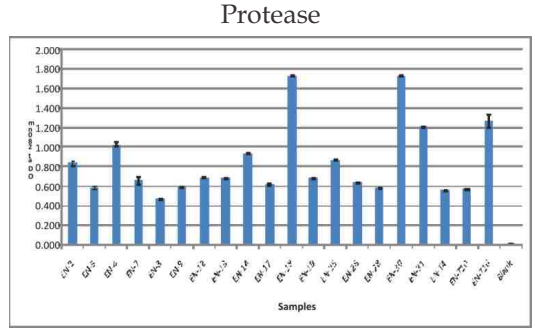
Chitinase



beta-Glucanase assay



Protease



- Molecular characterization of endophytic fungus.
- 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 endophytic fungi.
- Total 110 isolates of endophytic fungi were isolated from different plants from Indo-gangetic plains. The isolates were maintained at 28±2°C.
- The isolates were characterized on the basis of morphology, growth characteristics and production of various metabolites including IAA, siderophore, ammonia, and HCN.
- 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 in ITS region with this 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 and submitted to NCBI database.
- Enzymatic study of the identified endophytic cultures from Indogangatic plain was carried out for protein assay, β endoglucanase assay, proteinase and chitinase estimation.
- Endophytic cultures were checked for their ability to enhance plant growth and biocontrol in tomato plant under of pot experiment studies.
- After one month, plants were uprooted for measurement of root length, shoot length, fresh and dry weight of shoot and root for assessing the plant growth promotion in presence of endophyte.
- Studies were carried out to establish endophytic nature of fungi using plant leaves

Conclusion

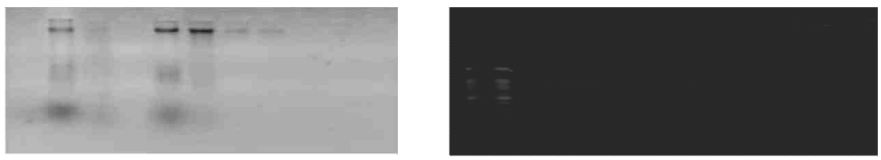
Considerably high endophytic diversity has been recorded from the vegetables of IGP region. The isolated fungi were identified by rRNA sequencing and characterized on the basis of PGP attributes and growth promotion under greenhouse.

Project: Harnessing arbuscular mycorrhizae for biofertilization in horticultural crops

PI :George V. Thomas
Co PI :Alok K. Srivastava

Rationale

Arbuscular mycorrhizal (AM) fungi are found in the soil of most ecosystems where they form mutualistic associations with a large number of terrestrial plant species. They are known as critical components of soil, and functional links between soil and plants. They can influence many important processes such as nutrient cycling, soil structure stabilization, organic matter transformation and accumulation and the turnover of organic residues in soil. AM fungi are important associates of plants and the composition of their community influences plant community structure, biodiversity, plant drought resistance, primary production and ecosystem dynamics. In recent years, molecular techniques have been used to study phylogenetic relationships of AM fungi, and attempts have been made to use ribosomal genes as a tool for the identification of AM fungal species. However, only few studies have been focused on the direct identification of mixed populations of AM fungi in actively colonized root using PCR based detection methods with specific primers. Helgason *et*



DNA extraction from soil and root samples
PCR amplification of endomycorrhizal SSU rDNA directly extracted from soil and roots with primers AM1 and Ns31

al. (1998) designed a primer pair NS31 and AM1 to detect AM fungal species composition in roots. The aim of this study is, therefore, to apply PCR based strategies to detect AM fungi in actively colonized roots and soil samples of different selected horticultural crops.

Objective

Molecular characterization of efficient arbuscular mycorrhizal fungi Strains (associated with selected horticultural crops) collected by different centres.

Significant achievements

- Total 10 (5 soil and 5 root) samples were collected from different crops (Tomato, Marigold, Banana, Papaya, and Capsicum).
- Root colonization was evaluated by the grid line intersect method and out of 5 crops only 4 crops showed successful root colonization by AMF [% root colonization Marigold (33) >Tomato (32) >Papaya (30)>Capsicum (29)>Banana (28)].
- Spores of AMF was isolated by rhizospheric soil samples of these 4 collected crops by wet sieving method and number of mycorrhizal propagules per 100g soil was determined by the Most Probable Number technique.
- These isolated AMF strains were maintained in tomato and maize plants by pot culture method with different potting mixtures (Sand+Soil, Soil+Sand+Compost ,Soil+Sand+FYM Replications: 3 and AMF Dose: 500 spores/pot).
- Genomic DNA from soil samples of Tomato and Maize plant was extracted using the PowerSoil DNAisolation kit (MoBio Laboratories, Carlsbad, CA) and from root samples using a modification of the method described in Edwards *et al* (1991) after 2, 4, 6 and 8 weeks (plant age).
- These 8 isolated DNA samples was subjected to a first polymerase chain reaction (PCR) amplification using primers ITS1 and ITS4 and the second stage primers used were AM1(Helgason, *et al.* 1998) and NS31 (Simon, *et al* 1992). All soil DNA showed successful amplification with primer ITS1 and ITS4 but only four soil DNA showed successful amplification with primer AM1 and Ns31.

Conclusion

Results suggest that a PCR-based technique could be useful to detect AM fungi in actively colonized root and soil samples of different selected horticultural crops.

Agrowaste Management, Bioremediation and PHT

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 : Dilip K. Arora
Co-PI : Kamlesh K. Meena

Rationale

The pulp and paper industry release a large quantity of effluent (72-225 m^3/ ton paper), for which proper disposal is required. Pulp and paper mill effluent contains several elements including important plant growth regulators such as nitrogen (N), phosphorus (P) and potassium (K), which can help contribute to higher crop yields when applied to nutrient deficient soils.However, other elements (magnesium, sodium, chlorides, and 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, decreases root expansion capabilities, and reduces water percolation and soil aeration. Further more, the addition of such a "mixed bag" of compounds may induce changes in physiochemical properties of the soil and also create significant shifts in structure and function of the associated microbial community, which in turn may ultimately affect the soil viability for agriculture purposes.

Soil microbial communities play a vital role in nutrient cycling, the decomposition of organic matter, carbon sequestration and more general effects on soil genesis, such as effects on structure and consequently water retention of the soil. Though pulp and paper mill effluent application to soil is known to increase the organic matter content in the soil, thereby increasing the populations of soil microorganisms. By practicising culture independent analysis of metagenome of effluent and analysis of 16S rRNA gene cloning directly may provided more insight into changes occurring in the structure of the microbial community as a whole .

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

- Cluster analysis of combined 16S rRNA gene restriction pattern based on Jaccard's similarity index, grouped all 128 isolates under 20 distinct groups. For phylogenetic analysis, one representative isolate from each ARDRA group was investigated with partial 16S rRNA gene sequencing. A total of 9 genera were identified from the WIF and EIF soil samples, viz., *Bacillus*, *Brevibacillus*, *Amycolaptosis*, *Staphylococcus*, *Flavobacterium*, *Streptomyces*, *Arthrobacter*, *Rheinheimera*, and *Pseudomonas*.
- From the bacterial 16S rDNA clone library, 258 clones (115 from WIF and 143 from EIF) were subjected to PCR-RFLP. A total of 82 (37 from WIF and 45 from EIF) different banding patterns were detected. DNA isolated from a single representative clone from each RFLP group was PCR-amplified and sequenced on both strands. After excision of 3 chimeric sequences, a total of 79 (35 from WIF and 44 from EIF) different phylotypes were obtained.
- A total of 71 OTU were detected on based on sequences having similarity of ≥ 97%. The sequenced clones were affiliated with sequences from 10 phyla of which one (OP10) is unculturable of the domain Bacteria. Other phyla were α-, β-, γ-, and δ- subdivisions of the *Proteobacteria, Acidobacteria, Firmicutes, Bacteroidetes, Planctomycetes, Cyanobacteria, Chloroflexi, Gemmatimonadates,* and *Aquificae.*
- OTUs corresponding to the phylum Proteobacteria were most abundant in all libraries (55%). Among them, the subdivision β-Proteobacteria was detected in the majority of both WIF and EIF soil library. The acidobacteria related phylotypes delivered the second most abundant (24%) number of clones in the library

Conclusion

In conclusion, we found that microbial communities in pulp and paper mill effluent irrigated fields were more diverse in both structure and function. This increase in diversity and function supports the continued use of pulp and paper effluent as the main source of irrigation water for crops in regions where clean freshwater is scarce. We showed that the different methods of microbial fingerprinting gave similar results when samples were processed consistently and compatible statistical methods were used. An integrated multi-technique approach where biochemical and molecular methods are combined can yield results that will allow more confidence than the use of a single method. This approach is important for ecological studies, especially for determining the impact of anthropogenic activity, where a single method will not provide data in which researchers can have absolute confidence in their validity when applied and used in larger perspective ecological conclusions.

Microbial Management of Abiotic Stress

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

PI : Dilip K Arora
Co- PI : Kamlesh Kumar Meena

Rationale

Environmental stresses represent the most limiting factors for agricultural productivity. Apart from biotic stress caused by plant pathogens, a number of abiotic stresses such as extremes temperature, drought, salinity, heavy metals and radiation which all have detrimental effects on plant growth and yield. Drought is very detrimental to all types of plant growth. When there is no water in the soil there are not very many nutrients to support plant growth. Salinity affects plant growth and development adversely and exerts negative impact on critical ecological balance in the agro ecosystem to disturb biological stability. Metabolic imbalances caused by ion toxicity, osmotic stress and nutritional deficiency under saline conditions may also lead to oxidative stress. It has been claimed by one study that abiotic stress causes the most crop loss of any other factor and that most 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. Microorganisms have been implicated in alleviating the effects of abiotic stress by different mechanisms. They can alleviate salt and drought stress by production of growth promoting substances and also involved in production of antioxidants to prevent injury to the plant due to stress. Bacterial exo-polysaccharides have been implicated in providing protection from environmental stresses and host defenses.

Objective

- Survey of salt and drought affected area of India.
- Isolation of microorganisms from rhizotic zones of cereal crop grown under salt stress and drought stress
- Screening of salt & drought tolerant bacteria at different NaCl and PEG concentration
- Evaluation of selected micro-organisms in the rhizosphere of cereal crop on the basis of phytotron studies
- Biochemical & molecular characterization of selected microorganisms
- Development of consortium of microorganisms that can alleviate the effect of salinity and drought to improve the growth and yield of cereal crop (wheat)
- Field evaluation of consortium of microorganisms for improvement of wheat growth and yield
- Osmoprotectant studies (proline, glycine betaine) on salt tolerant and drought tolerant bacteria

Significant achievements

- The isolates obtained from the salt and drought affected regions have been identified using 16SrRNA gene sequencing.
- On the basis of biochemical screening the potent isolates were selected for further screening in depth and plant microbial interaction studies.
- The potent isolates were subjected to develop the HPLC profiling to identifying the osmoprotectent produced in response of the stresses. Potent isolates on the basis of HPLC profiling were selected to further characterization for their PGP traits.
- The potent osmoprotectent producing bacterial isolates *i.e. Bacillus pumilus* (EU 927414), *Pseudomonas mendocina* (EU 927412), *Arthrobacter* sp. (EU 927410), *Halomonas* sp. (FJ984532), and *Nitrinicola lacisaponensis* (FJ973520) were selected for further characterization for PGP traits. The results showed that All isolates were found to be IAA-producers and possess different PGP-traits like p-solubilization, siderophore and ammonia production and salt-tolerance capabilities varied from 0-22% NaCl concentration.

- Bacterial strains (AS 121, AS 40 and AS 18) were isolated from the rhizosphere soils collected from Mau Ghazipur and Ballia districts of Uttar Pradesh (India) and other two isolates SL-9, SL-11 isolated from sambhar salt lake were used for pot experiment.
- The effect of individual treatment was studied in the form of chlorophyll, carotenoid and protein content of the wheat leaves at different stages. Apart from this other parameter were also considered like root, shoot length, fresh weight and dry weight.
- Treatments with bacterial inoculants supported plant growth as assessed after 15 and 30 days after inoculation (DAI) as compared to non-inoculated control showed significantly Maximum shoot and root length was recorded with the inoculation of *B. Pumilus*.
- Total chlorophyll and protein content was maximum in *B. pumilus* inoculated plant leaves (17.47 and 19.21 mg^{-g} fresh wt. after 15 and 30 DAI) as compared to control (11.36 and 14.94 mg^{-g} fresh wt.).
- Carotenoid content was maximum in plants inoculated with *Halomonas sp.* (806.8 and 912.9 mg^{-g} fresh wt.) as compared to control (682.7 and 726.4 mg g^{-1} fresh wt.) after 15 and 30 *DAI. Arthrobacter sp.* caused maximum accumulation of total protein **(**2.45 and 2.62 mg g^{-1} fresh wt.) in comparison to control (1.33 and 1.72 mg g^{-1}fresh wt) at 15 and 30 DAI.
- Inoculation with bacterial isolates increased the levels of total flavonoids and total phenol content in wheat leaves. In comparison to control, proline and reducing sugar accumulation was maximum in *N. lacisaponensis* (1.74 µmole/ g and 268.6 µg g^{-1} fresh wt. respectively) after 15 DAI and (2.28 $\mu mole^{-g}$ and 419.9 μg^{g-1} fresh wt respectively) in *B. pumilus* after 30 DAI. *Halomonas sp., favoured maximum accumulation of total soluble sugar (*283.8 and 375.5 µg g^{-1} fresh wt.) as compared to uninoculated control (1.12 and 1.54 μg^{g-1} fresh wt.) after 15 and 30 DAI.
- Inoculation with *B. pumilus* resulted in maximum accumulation of individual phenolics (gallic, caffeic, syringic, vanillic, ferulic and cinnamic) after 15 DAI. When root exudates from the inoculated plants and control were assessed, higher level of phenolics was again recorded and interestingly, in the root exudates we were able to analyse the presence of a flavonoid, quercetin.

Conclusion:

The results showed that the microbes which can tolerate up to 15 -20% NaCl, 25%PEG concentration could be utilized to alleviate the effect of salt and drought stress for growth and yield of wheat crop. Particularly isolate number AS-121*Bacillus pumilus* SL 11 *Nitrinicolalacis aponensis* as a great potential as it has all the attributes of *p*hysical, biochemical and PGP traits as it is evident from the growth kinetics and HPLC studies that bacteria-mediated presence of phenolics and quercetin in the root exudates and rhizosphere played an essential role in the enhanced interaction of bacterial inoculants on the plant roots which finally played a cumulative synergistic role in systemic accumulation of stress-tolerance biochemical in leaves and enhanced plant growth promotion. Cultivable isolates of salt and drought tolerant isolates further explored for consortia developmental studies as a bioinoculant under alleviated abiotic stress for growth and yield of wheat crop.

Utilization of actinomycetes to alleviate salt and drought stress in cereal crops

PI :Mahesh Yandigeri
Co- PI : Kamlesh Kumar Meena

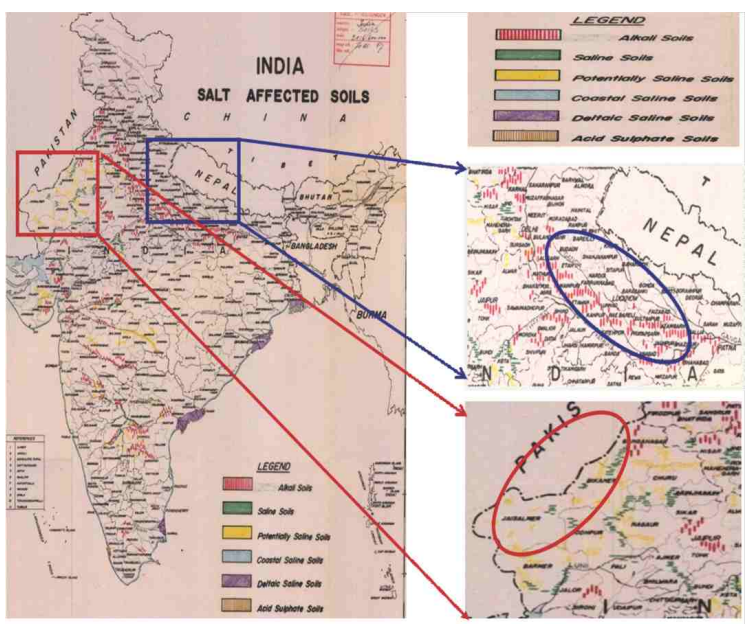
Rationale

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.

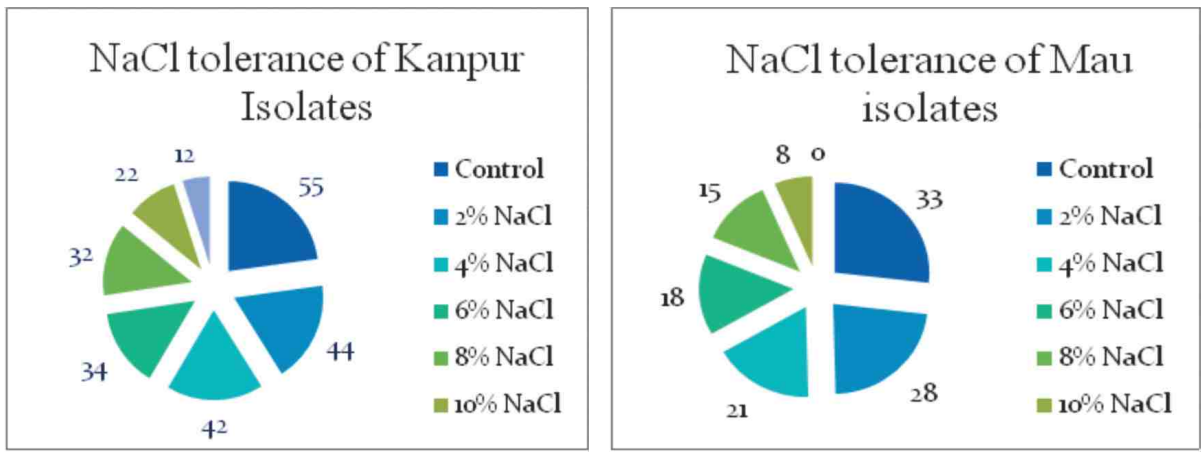
Actinomycetes are found in neutral to saline soils. Most of actinomycetes are tolerant to alkaline conditions and in alkaline soils, 95% population may be actinomycetes. Most of the actinomycetes possess inherent capacity to tolerate salt stress (especially *Streptomycetes* genera, *Nocardiopsis* sp., *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

- Isolation and screening of actinomycetes from different salt affected regions of India
- Diversity analysis studies from salt stressed regions
- Characterization of the isolates for the accumulation of sugars, sugar alcohols, amino acids, osmolytes and secondary metabolites
- Evaluation of the promising actinomycetes under pot/ field experiments and study of plant microbial interactions during salt stress
- Development of consortia of actinomycetes to



Map depicting the sampling sites of salt affected regions of Kanpur district and drought affected regions of Rajasthan



Pie diagram representing salt tolerance capacity of Kanpur and Mau isolates against different NaCl concentrations

alleviate the effect of salinity in crop plants.

Significant achievements

- Soil sampling survey was carried out for the salt affected regions of Kanpur, Fatehpur, Auraiya, Etawah, Mainpuri and Mau Districts of Uttar Pradesh for the isolation of salt tolerant actinomycetes. A total of twenty three soil samples were collected from Kanpur, Etawah, Auraiya, Mainpuri and ten soil samples from Mau district.
- The pH of soil samples ranged from 7.5 to 11.0 and electrical conductivity from 0.30 to 13.33 dS/m. A total of 112 morphotypes were obtained from saline soils of Kanpur and Mau districts of Uttar Pradesh using different media and enrichment methods.
- Actinomycetes isolated from the Uttar Pradesh saline soils region were evaluated on different salts (NaCl, KCl and $MgSO_4$). The Actinomycetes isolated from Kanpur regions had shown significant results on NaCl. A total of five isolates shown growth on 4% NaCl concentration, twenty isolates were shown growth at 10% KCl and 30 isolates were shown growth at 10% MgSO4 while the isolates from Mau region showed tolerance up to only 10% NaCl concentration.
- Salt tolerant actinomycetes from Uttar Pradesh showed significant PGP traits *viz.*, IAA production, ammonia production, siderophore production, HCN production, H_2S production and P-solubilization and extracellular enzyme activity.
- Salt tolerant actinomycetes have been subjected for osmolyte production (proline) and estimation of osmolytes.
- The actinomycetes have been subjected to molecular identification using universal 16S rDNA primers and restriction fragment length polymorphism is under progress for clustering and identification of representative isolates for sequencing.

Conclusion

Among all the isolates obtained from the salt stressed regions most of the isolates showed tolerance upto 6-8% of NaCl concentration along with considerable extent of PGP attributes and extracellular enzyme activity. These isolates can be taken to further assess their potential for seed germination and promotion of plant growth under stress conditions as well as production of compatible solutes to cope up with stressed environment. Promising isolates will be further taken for pot and then field assay in saline soil for assessing their potential as stress alleviators and in development of consortia along with other microbes.

Microbial Genomics

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

PI :Dilip K. Arora
Co- PI : Alok K. Srivastava

Rationale:

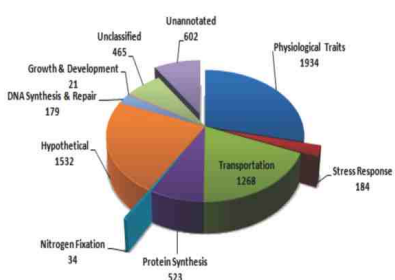
Mesorhizobium ciceri ca181 was selected for whole genome sequencing as it is a nodule forming chickpea rhizobia with very specific and high qualities like, efficient nitrogen fixer shows good nodulation competitiveness and performed well at different locations in different agro-climatic regions, different soil types in All India Coordinated trials. The whole genome sequencing of this bacterium 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 and Operons and proteins.

Objective

- Complete genome sequencing of *Mesorhizobium ciceri* Ca 181

Significant achievements

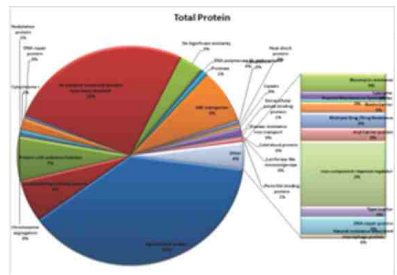
- Genome Assembly and Annotation: Sequencing of the Genome was done by 454 Next Generation pyrosequencing as well as Sanger technology. A total of 6461 genes have been predicted and annotated for the functions they perform in *Mesorhizobium ciceri* Ca181. Filtration of Annotated Genes according to their functional categories (Stress, Biosynthesis, Regulatory, and Signaling) is in progress.
- Nitrogenous products accumulate in plants when soil nitrogen level is high and readily available but the plant is unable to utilize it. Nitrate level can go up and go in plants. After harvesting plant nitrate gets converted in nitrite that is ten times more toxic in comparison to nitrate. Above 5000 ppm of nitrate, it is dangerous for the growth of plants and it checks the formation of root nodules in the legumes. So the assessment of utilization of toxic level of nitrate in the soil is planned.
- Experiment has been set up for the assessment of Soil Nitrate and Nitrite utilization ability of the strain Ca181. Four sets of experimental combinations have been made.
- The experiment is set up for the competitiveness of *M. ciceri* Ca181 with other Chick pea rhizobia strains. A total of eight different rhizobium strains have been taken including *M. ciceri* Ca181. Twenty eight combinations have been made and experiment was setup with 5 replication including negative control.



Genes involved in different functions in the Genome of *M. ciceri* Ca181

Conclusion

After assembly, the genome was search for the similarity in genome database available at NCBI Genome browser and it found about 35% similar from its closest organism *M. loti.* These results show potential that after complete analysis of the genome, it will give some new and unique genes and process 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.



Protein involved in different functions in the Genome of *M. ciceri* Ca181

Project: Microbial Genomic Resource Repository

PI : D. K. Arora
Co. PI : Alok Kumar Srivastava, Sudheer Kumar, Mahesh Yandigeri, K.K. Meena

Significance of MGRR

Microbial genetic materials (e.g. genomes, plasmids, vectors, cDNAs) are the tools for biotechnology and underpin the life sciences. The vast majority of microorganisms and their gene pool around the globe still remain hidden and need to be explored, identified, conserved and utilized for the benefit of humankind. Microbial genetic resources are established in many countries around the world having a variety of purposes. These range from small, specialized collections that support small groups of researchers to the large international public service repositories that provide reference materials and services to the scientific community and bio-industries. The huge gap between the discovery of new microorganisms and their potential numbers in nature has stimulated an interest in microbial diversity and the harnessing of their genes, properties and products. The operations of microbial collections have changed over the last twenty years as a result of the advancement of bioinformatics and the facility to present electronic data over the internet. This makes even the smaller collection resources more accessible.

Indian Council of Agricultural Research has taken up an initiation to establish Microbial Genomic Resource Repository (MGRR) at National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau Nath Bhanjan. MGRR is a facility that preserves and conserves the genetic material of microorganisms, maintained in selected hosts or cloned and maintained in plasmids, accompanying the data details. This new organizational structure indicates the high importance and visibility that NBAIM places on our role as custodians of microorganisms and its related genetic resources. The policies and procedures represent evaluation, maintenance, regeneration, distribution and documentation of genetic resources at MGRR. MGRR maintains genetic materials like whole genome shotgun and cDNA/EST libraries, PAC/BAC/YAC clone vectors, competent cells from sequencing projects, promoter DNA-fragments with reporter genes, RFLP probes specific for different microbes and expression vectors.

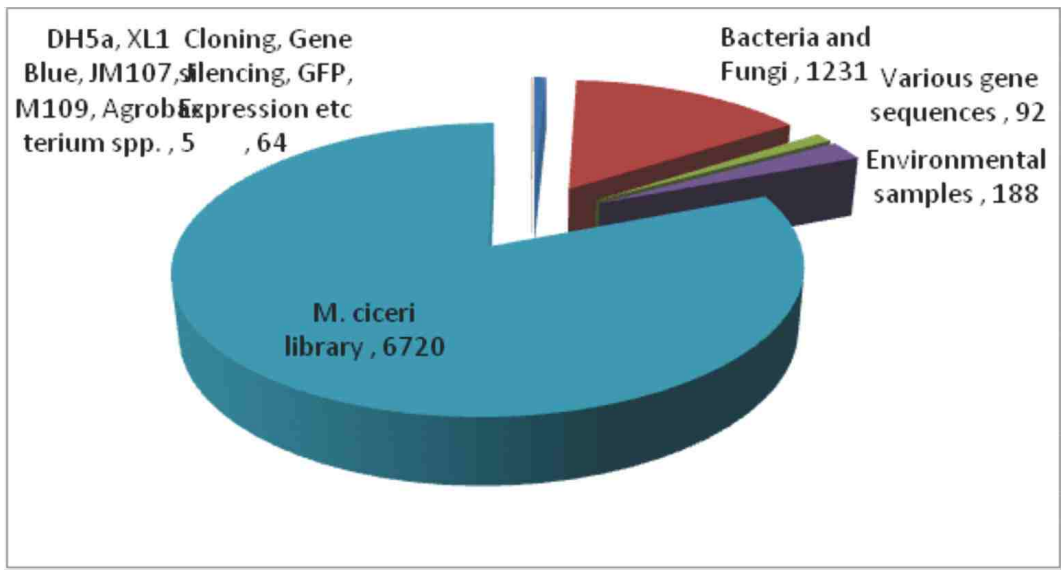
Genetic materials at MGRR can be deposited free of charge in the public collections and will be available for any third parties under the terms of the material transfer agreement. MGRR provides a safe deposit to the genes and genetic elements with associated information for long-term stable preservation of microbial genetic resources. It is equipped with improved infrastructure and techniques for conserving diverse genetic materials. To deposit the genetic material send the genetic material along with dully filled pass port data or MTA form. We can store your DNA samples at our secure, environmentally controlled facility for many years.

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 genetic resources/DNA.
- Development of linkages between research institutions/Universities, and researchers
- Technology and protocols development for isolation and long-term preservation of the Microbial Genetic Resources
- Technology and Protocols development for collection/transportation of microbial samples
- Development of infrastructure facilities for the preservation and maintenance of genetic resources
- Collection of environmental samples from different Agro climatic Regions and exploration of non-culturable microorganisms
- Development of databases/ information bank for Microbial Genomic Resources



- Documentation and electronic cataloguing of Microbial Genetic Resources.
- Exploration of non-culturable microorganisms and direct DNA isolations from environmental samples.
- Development and implementation of genome projects to explore non-culturable microorganisms.

Significant achievement

Presently, the center is enriched with genomic DNA of different groups of fungi which include Myxomycetes, Mastigomycetes, Zygomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes in addition to bacteria, actinomycetes and blue green algae (BGA). It is also enriched with total genomic shot gun library of *Mesorhizobium ciceri* ca 181 along with its genome sequences, alongside metagenomic libraries,significant fluorescent marker like GFP (Green Flourescent Protein), YFP (Yellow Flourescent Protein), RFP (Red Flourescent Protein), gene silencing vectors like pSilent-1 for characterization and identification of unknown fungal genes, His-tagged expression vectors for functional studies of an known or unknown gene, and various other gene constructs of Bt gene from Bacillus thurengensis etc. Each genetic material is preserved by at least two methods according to the type of genomic material, either under short term , or long term storage at 80 °C.

Conclusion

MGRR collaborates with various institutions working in this field to collect and preserve the DNA materials and to provide necessary DNA samples for an active research study. It builds a database library of anonymous microbial information which can provide clues for a research investigation. In future, MGRR will be efficiently utilized by the scientific community to facilitate innovative researches in both functional and applied genomics of microorganism for sustainable agriculture.

National Agricultural Innovation Project

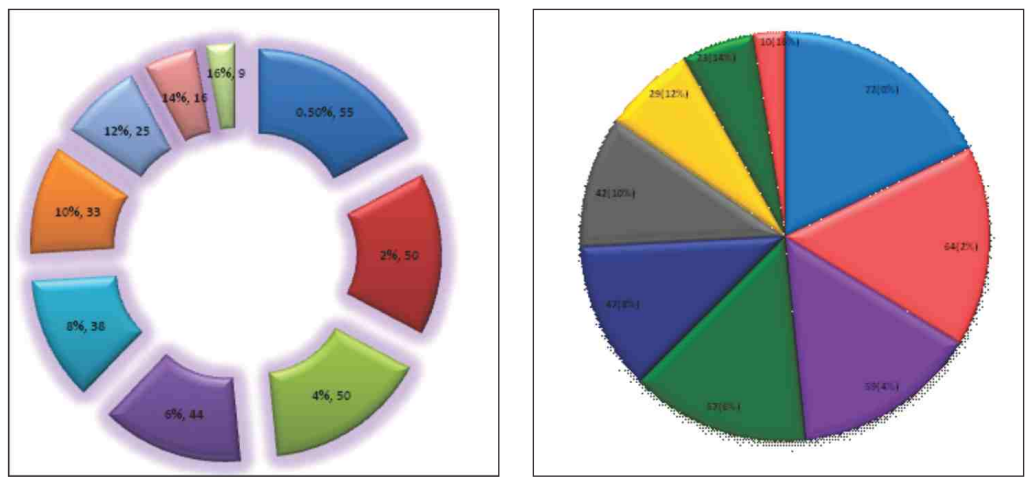
Project: Diversity analysis of *Bacillus* and other predominant genera in extreme environments and its utilization in Agriculture

Consortium Leader : Dilip K Arora
Consortium PI : Sudheer Kumar
Coordinating Scientists : Alok K Srivastava

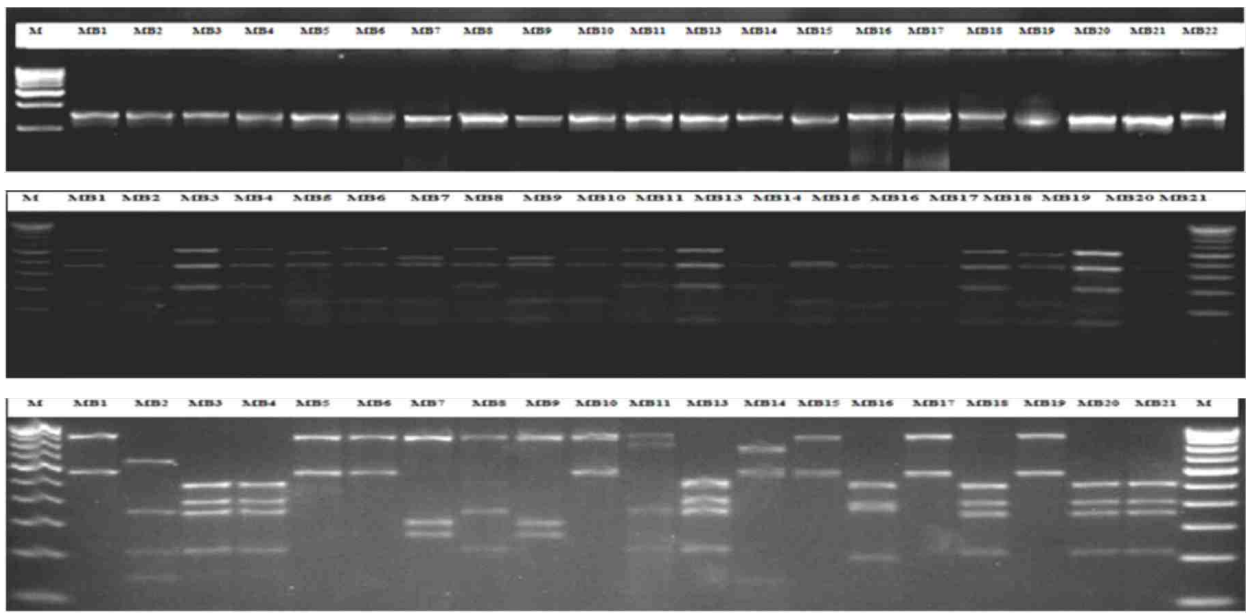
Rationale

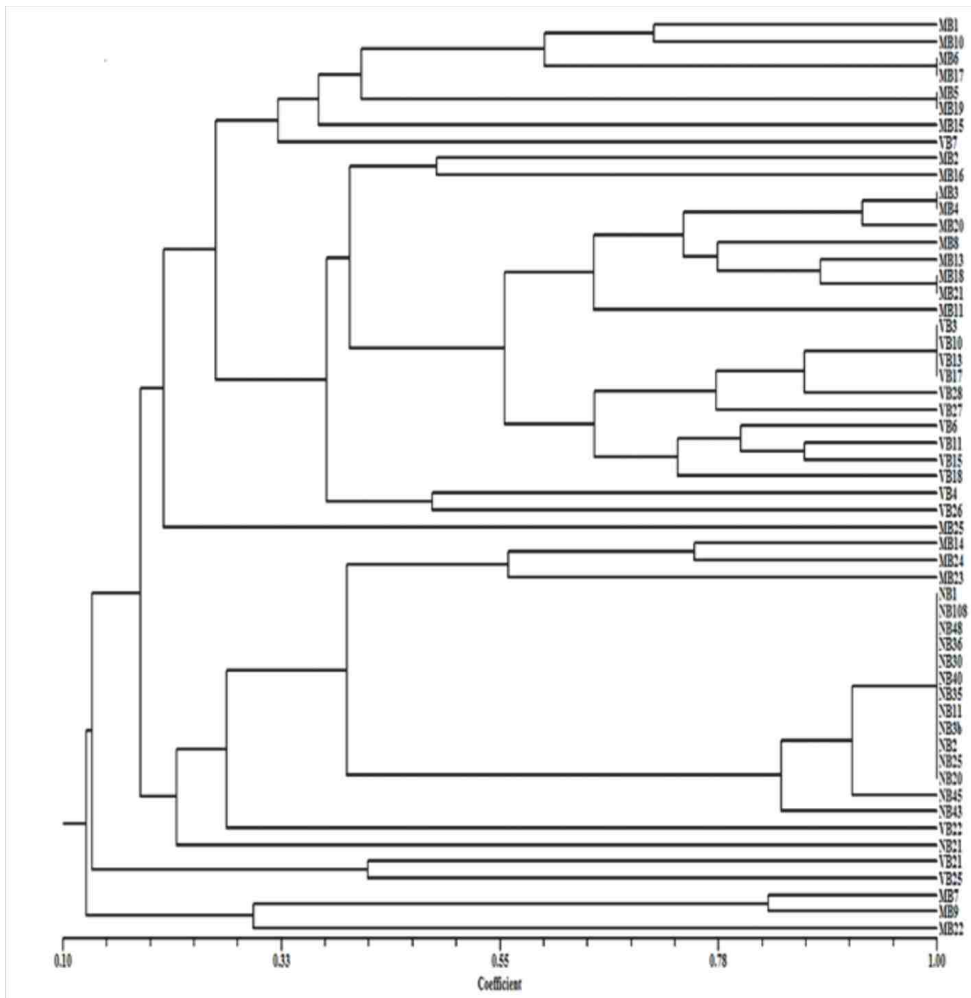
Extreme environment (such as salinity, drought, high or low temperatures and acidity) causes a serious problem of reduced agricultural production in over 100 countries especially in India and other countries. In India, there is a widespread saline soil problem, commonly known as the formation of Usar land which results in low income and poverty of farmers. Salinization is predicted to result in 30% of farmable land loss globally within the next 25 years, and up to 50% by the year 2050. In developing countries like India the problem could be more serious due to the increasing demand for food. High salt concentrations lead to a decline in soil fertility by adversely affecting the soil microbial flora, and therefore further decreasing crop productivity. The most appropriate solution in such conditions is to use salt tolerant microbial inoculants that may prove useful in developing strategies to facilitate plant growth in saline soils. Microbes from the extreme environments have tremendous potential as they have developed and developed many mechanisms for their growth and development. Lesser attention has been paid to explore diversity of microbes in extreme environments like saline, acidic, and drought. Microorganisms present in the rhizosphere reported to alleviated the salinity stress by different mechanisms. Emphasis therefore, is being given to manage abiotic stress through the application of microbial inoculation.

Bacillus and other predominant genera from extreme environments are focusing major attention in recent years primarily due to their nutritional versatility & survival under extreme conditions. *Bacillus* 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. *Bacillus* and *Bacillus* derived genera (BBDG) are employed in industry as a source of enzymes, in agriculture as inoculants (PGPR) and biocontrol agents. 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. The use of BBDG as biofertilizers, biocontrol agent and bioremediators will help Indian agriculture by reducing the



Salt tolerance of *Bacillus* and other predomiant genera





Combind dendrogram showing percentage similarity using UPGMA among of isolates based on 16S & 16-23S ARDRA with *Alu* I, *HaeIII* & *Taq*I

dependence on chemical inputs and protecting the environment salinity and drought stress to attain optimum yield of field and horticultural crops.

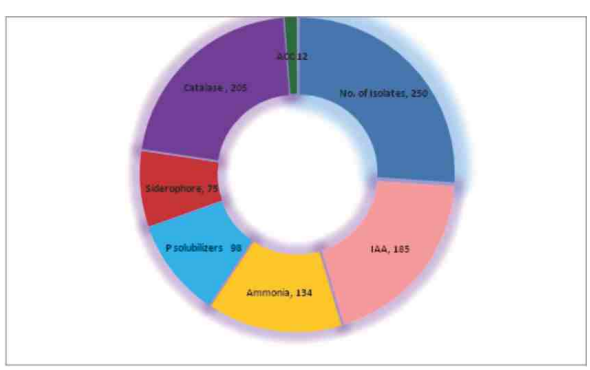
Diversity analysis of *Bacillus* or *Bacillus* derived genera and other predominant genera under extreme conditions would provide a database that is currently unavailable and will enrich the Indian microbial culture collection. The work in this project deals with the screening of different salt tolerant and PGPR *Bacillus* 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.

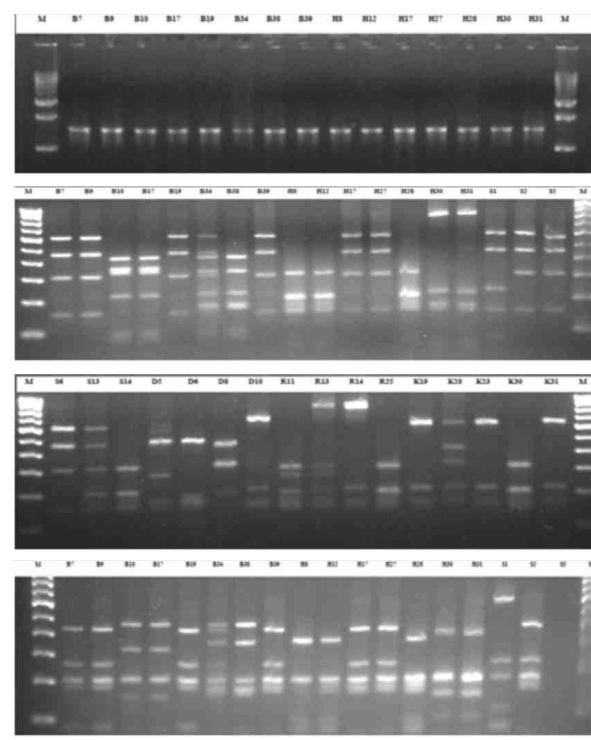
Objectives

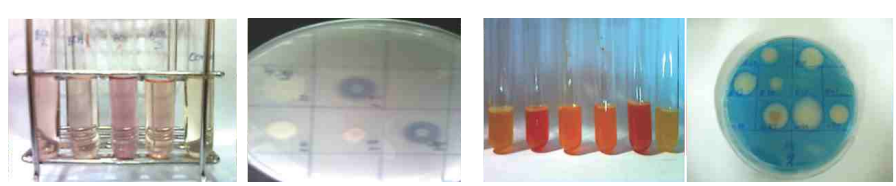
- Diversity analysis and identification of *Bacillus* and other predominant genera from extreme conditions of salinity from different regions of Eastern Uttar Pradesh.
- Study of the diversity of *Bacillus* and other predominant genera associated with plant species under extreme environments and evaluating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

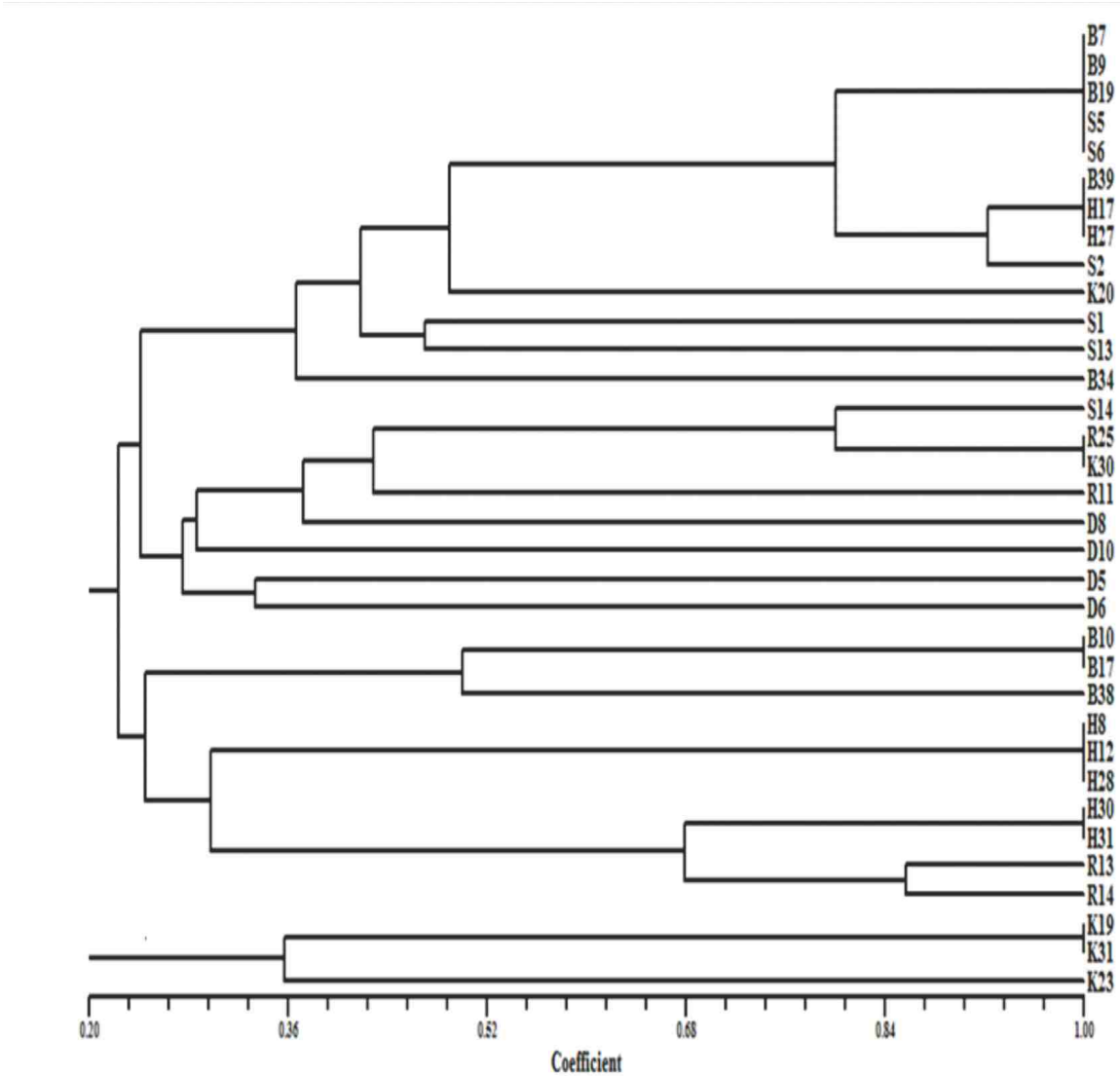
Significant achievements

- Extensive exploration, survey and collection of soil and chickpea rhizosphere soil samples were carried out from eastern U.P.
- On the basis of morphological characteristics a total 55 *Bacillus* and 70 other predominant genera were isolated from the saline soil of eastern U.P.
- 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 14 and 16% (w/v)). Out of the 55 *Bacillus* isolates only 9 isolates and from 70 predominant genera, only 10 isolates were able to tolerate NaCl up to 16 % .
- Molecular characterization of the isolates was carried out using 16S & 16-23S rDNA PCR-RFLP analysis with three restriction endonucleases *Alu*I, *Hae* III and *Taq* I. A combined dendrogram of 55 bacilli isolates were grouped in to 37 clusters.
- A total 250 isolates were isolated form chickpea rhizospheric soil from salt affected area of eastern U.P.
- All the isolates were screened for *in vitro* PGP activities like IAA, ammonia,P-solubilization, siderophore, and ACC deaminase. Out of 250 isolates, 185 isolates were recorded as IAA producers, 134 Ammonia producers, 98 phosphate solubilisers, 75 siderophore









Dendoram showing percentage similarity using UPGMA of PGPR isolates of Eastern Uttar Pradesh

producers, 205 catalase positive and 12 isolates were positive for ACC deaminase activity.

- Of 250 isolates, 34 isolates were selected on the basis of multiple PGPR attributes for pot & field experiments of Chickpea. 16SrDNA PCR-RFLP analysis with three restriction endonucleases *Alu*I, *Hae* III and *Rsa*I grouped 34 isolates from Chickpea rhizosphere into 22 clusters

Conclusion

Studies made so far revealed the presence of salt tolerant *Bacillus* and predominant genera isolated from eastern U.P. Bacteria isolated from chickpea rhizosphere of salt affected area of eastern U.P. showed multiple plant growth promoting attributes.

Project: Georeferenced soil information system for land use planning and monitoring soil and land quality for agriculture

Consortium Leader :NBSSLUP, Nagpur
Partners : CICR, Nagpur, NBAIM, Mau (UP), WTCER, Bhubaneswar
CCPI :Alok Kumar Srivastava, NBAIM, Mau

Rationale

Agriculture is the one of the oldest and most important practice of mankind. The soil health is one of the foremost important parameter, and plays decisive role in agricultural practices and sustainable agriculture and should be monitored regularly. Thus there is a need to develop a land information system for assisting land use planning, monitoring land quality changes, spotting of the important indicators and fixation of minimum criteria that help in forewarning the consequences of non-compatible land uses on land quality.

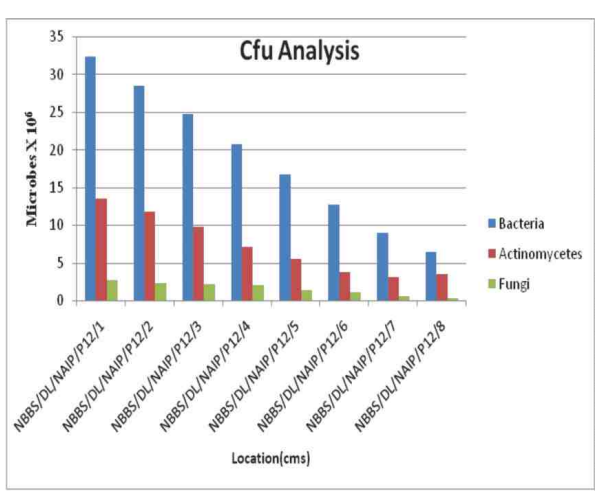
Soil microbes contribute to the exchange of the matter and energy in terrestrial environment. The good population soil microorganisms are indicator of soil health and which contribute significantly in maintenance of soil fertility. Any terrestrial soil has unique biochemical enzymatic pattern because of soil microorganism's constituency. As they secret or have many enzyme which help in the conversion of many complex compound to simpler product. Soil enzyme assays are process of determining the potential of a soil to degrade or to transform substrate. 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. Dehydrogenase activity serve and an indicator of the microbiological redox system and may be considered a good measure of microbial oxidative activities in soil.

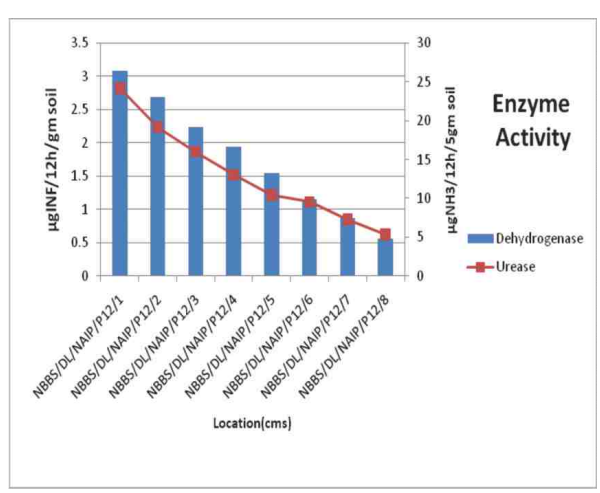
Urea is one of the most important chemical N fertilizers and its application increased over the time. Urea hydrolysis in soils is an enzymatic decomposition process by the enzyme urease.

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. Therefore the project was conceptualized under NAIP.

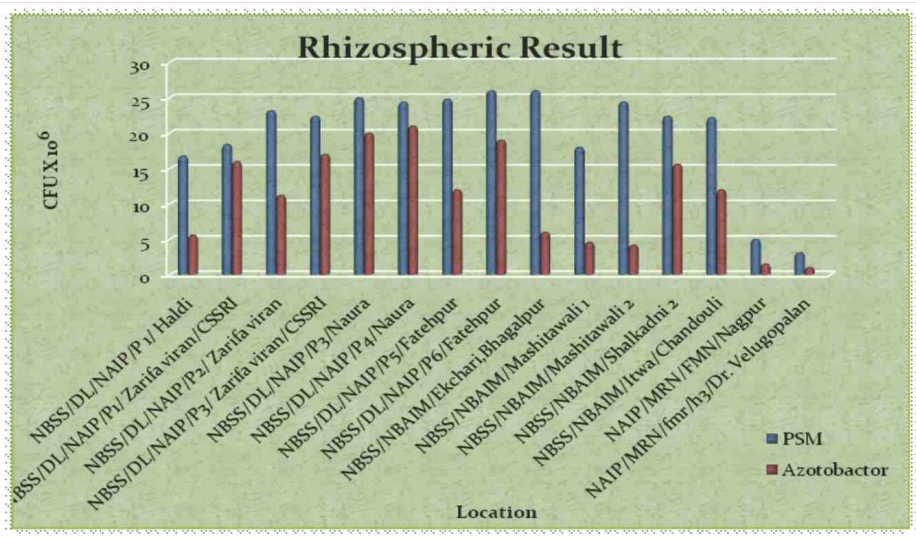
NBAIM is one of the partners mainly contributing in microbiological analysis of soil samples with following objectives:



Population of Microorganisms in TANDA Forest, Tehsil Rudrapur, Dist- Udham Singh Nagar (UK)



Microbial enzyme activity in TANDA Forest, Tehsil Rudrapur, Dist- Udham Singh Nagar (UK)



The Rhizospheric soil analyzed for Phosphate Solublizing Bacteria

Objectives

- Determination of CFU for different microorganisms of provided sample
- Determination of soil Dehydrogenase activity (Dehydrogenase activity in soil provides co relative information on the biological activity of microbial populations in the soil)
- Determination of soil Urease activity (indirect indicator of N mineralization)
- Determination of P-Solubilization Microbes and *Azotobacter*

Significant achievements

- 223 soil samples have been received from different NBSS&LUP regional centers. 100 % of total sample have been processed to achieve the above mentioned objectives of project.
- All the soil samples were analyzed for the quantitative analysis of Fungi, bacteria and Actinomycetes.
- The soil of benchmark spots from Uttara Khand showed higher population of bacteria followed by actinomycetes and least of fungi. Other soil samples also exhibited similar pattern.
- Higher level of dehydrogenase was recorded in the top soil as an indicator of active microbial biomass which reduced significantly along with the depth
- Exponnetial decrease in enzyme activity was found at 1 meter of depth.
- Wheat rhizosphere was evaluated for the phosphate solublizing bacteria, maximum population was recorded in Fatehpur soil followed by Ekchari soil samples. Least population was found in Haldi and Mashitawali samples.

Conclusion

The soil microbial population estimation (Cfu analysis) of different soil samples gives the significant information of culturable microbial community. There is usual sharp and relevant decrease in Cfu of single bench mark spot. Enzymatic trend of urease and dehydrogenase enzyme was estimated and analyzed. It has been found that urease and dehydrogenase enzymatic activity decrease as the distance increase from reference of Rhizospheric level, which is expected trend. Presence of *Azotobacter* and PSB microorganism in soil indirectly indicate the health of soil and crop yield.

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

Consortium Leader :T. Mohapatra, NRCPB
Partners :Alok K. Srivastava
Co-PI :Kamlesh Kumar Meena

Rationale

Microbes are important for the Earth System, playing a very important role in maintaining the wellbeing of our global environment. Despite the obvious importance of microbes, very little is known of their functional diversity. Recent progress in mining the rich genetic resource of culturable and non-culturable microbes has led to the discovery of new genes, enzymes, and natural products. The impact of gene and allele mining is witnessed in the development of commodity and fine chemicals, agrochemicals and pharmaceuticals where the benefit of enzyme-catalyzed chiral synthesis is increasingly recognized. Recovery of metabolic pathways and gene clusters involved in biosynthesis of antibiotics and bioactive molecules has increased the prospect of identifying useful natural and synthetic products for drug development. The discovery of biocatalysts operating optimally with high efficiency in conditions amenable to industrial processes requirements are key to successful development of food products, detergent additives, bioactive compounds, fuel alcohol and biodiesel, as well as optically active intermediates for chemical and drug synthesis. The microbes are known to thrive well in abiotic stress environment and are good repertoire of the genes could contribute a lot in abiotic stress management in plants which can further be used for extensive activity screens and a number of the recent applications in the field of agricultural biotechnology.

The study area under the project is located in all the extreme parts of India where only few microflora were found. Kutch Eco region has high salt concentration and low water availability. Manikaran and Rajgir regions has high temperature water. The proper exploration of diversity of microflora and exploration of adaptation strategy will be helpful in allele mining. A diverse and wide range of germplasm still remains to be explored for abiotic stress tolerance. 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.

Objectives

- 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
- Development of highly competent groups of scientists drawn from various disciplines and institutions of international standard for undertaking research in genomics and its application for improvement of agricultural species

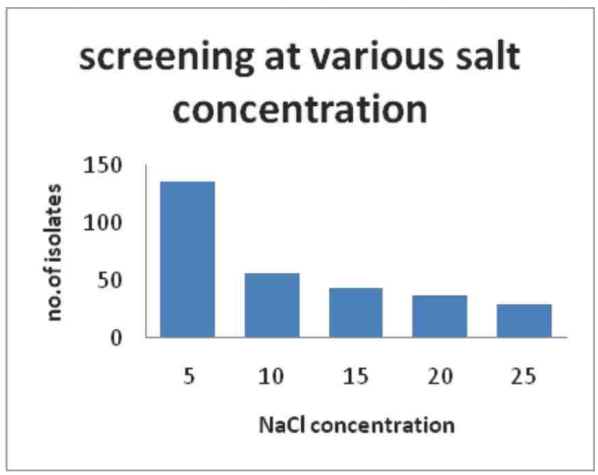
Significant achievements

1. Survey and sampling from different extreme environment for survey and collection of soil, water, and sediment samples:

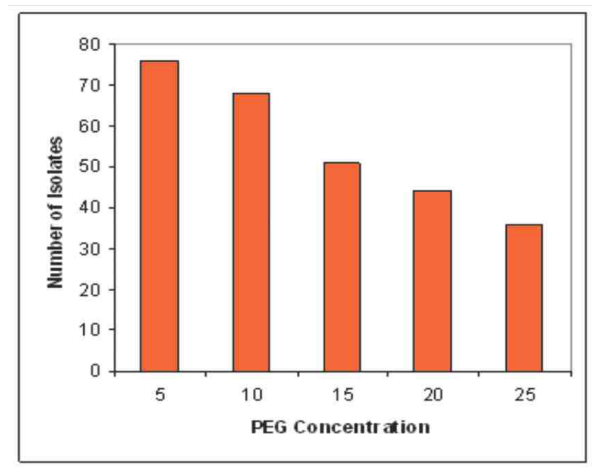
Sampling and survey from Kutch (Gujarat), Manikaran (Himanchal pradesh), Leh (Jammu and Kashmir) and Sāmbhar lake (Rajasthan) has been done, and Physico chemical analysis of soil, water and sediment sample had been done .

2. Analysis of diversity of predominant genera from various extreme environments

- Isolation of fungi for salt stress and desiccation: Isolation of fungi was done using different enrichment technique on the different media (MEA, CMA, SDA, V8JA, CDA) general and specific for salt stress and dessication was done and 89 isolates of fungi from Kutch soil samples and 35 isolates from sambhar salt lake have been isolated.
- Isolation of high temperature resistance fungi: Different general and specific media was used on different temperature incubation for isolation. (YPSS media, PDA media, yeast starch media, Malt extract agar). 24 fungal isolates had been isolated from Manikaran , and 56 isolates of fungi was isolated growing at a temperature range of 0 to 40 temprature from Leh soil samples .



The isolate showing halotolerance



The number of isolates tolerant to moisture stress

3. Screening of isolates for different abiotic stress tolerance using different selection pressure:

- **Osmotolerant test**: Kutch isolates were screened on different NaCl concentration 10%, 15%, 20%, 25%, 30% amended in malt extract broth. 29 fungi out of 89 were growing at 25% salt concentration. 5 isolates from Sambhar lake are able to grow at 30% of salt concentration.
- **Desiccation tolerance:** Kutch isolate were also screened on different concentration of PEG 5%, 10%, 15%, 20%, and 25% using PEG infused Malt extract agar plates and Malt extract broth methods creating. 38 fungi growing at 25% PEG concentration. 10 kutch isolates were showing cross resistivity for salinity and dessication tolerance.
- **Thermal tolerance:** Manikaran and Rajgir isolates were screened at temperature range from 40˚C to 65˚C. for screening. 10 fungal isolates were growing at 55°C.
- **Low temperature:** Fungal isolates from Leh region were screened by incubating them at a temperature range of 0 to 40 C and14 fungi are growing at 4°c were obtained.

4. Sequencing of rDNA genes for identification

i. DNA Isolation and Amplification-: G-DNA isolation and ITS amplification:

Genomic DNA of all isolates was extracted by the Prep method. PCR amplification of ITS region was done using two universal primer ITS1 AND ITS 4.

ii. RFLP and clusturing

Sequences of relevant fungus were downloaded from NCBI for detection of restriction enzymes which may be used for RFLP on the basis of softwares like cleaver and neb cutter. The restriction enzymes chosen for RFLP are *Hae*III ,*Alu*I , and *Eco* RI.

Degenerate primers will be used to detect presence of

Screening of isolates for temperature tolerance

Sample sites	50°c	40°c	28°c	10°c	4°c
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
Tanse02	NIL	1	3	3	1
Indus sangal	NIL	NIL	3	1	1
Pangong	NIL	1	2	1	1
Pangong Thso	NIL	NIL	2	NIL	NIL
Changla	NIL	2	1	1	2
Chumathang	NIL	1	NIL	1	NIL

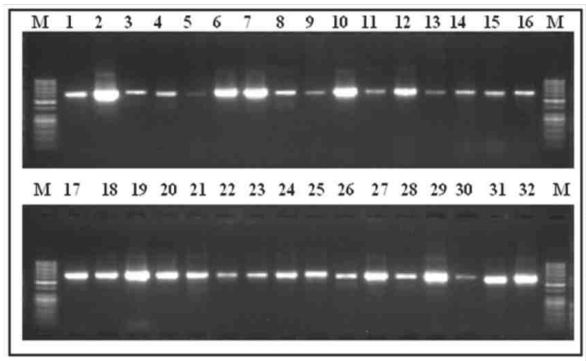


Fig: Amplification of rDNA (ITS region) from Kutch isolates

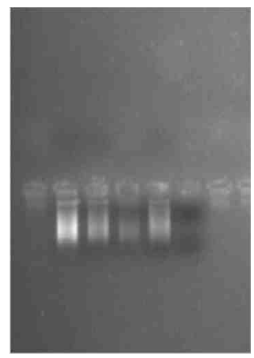


Fig: Amplification of rDNA (ITS region) from Kutch isolates

osmotolerant genes in microbes followed by construction of genomic library in suitable vector and screening of clones for identification of novel genes.

4. Primer designing and Insilco validation of primers:

7 primers were validated *in silico*: i.e. : Trehalose 6 phosphate synthase, Trehalose 6 phosphate phosphatase, proline, codA, for salt and drought tolerance and Tre1, hsp17 and glycerol phosphate dehydrogenase for high temperature. Wet lab validation of these primers is under process.

5. Metagenomic DNA isolation:

- Metagenomic DNA from soil samples of kutch eco region was isolated and their ITS amplification and DGGE profiling is done.
- 14 bands were obtained after DGGE profiling and out of which 6 were different on the basis of their size and sequencing of these bands is in process.
- The metagenomic DNA was also amplified with the primer of Tps1 gene.
- The amplified product is then cloned using TA cloning and the sequencing of these clones is under process to get allelic variability among them.

List of primers being validated *in silico* for amplification of metagenomic DNA

Name of the Primer	sequence (5'-3')	Length
TP-S0-GC	(CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCC CGG GGG CGG GAT CCG ACC CCA TCA TGT CTA CG)	**65**
TP-S0	(CGG GAT CCG ACC CCA TCA TGT CTA CG)	26
TPA-S0	(CGG GAT CCG GGT GGG AAA AGC TAC)	24
otsA-GC	(CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCC CGG GGG GTA TTC TTT GGC CAC CAR GTT)	60
otsA	(GTA TTC TTT GGC CAC CAR GTT)	21
otsA	(CCT TTC ACG TAA TCC ASC CG)	20
GiTPS2F2-GC	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCC CGG GGG GAA CTG CTA TAA TGG TAA ATC CTT GG)	65
GiTPS2F2	(GAA CTG CTA TAA TGG TAA ATC CTT GG')	26
GiTPS2R2	(GAT CGG TGT TAA TGT TCC ATC ATA ATC)	27
HAT1F-GC	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCC CGG GGG CCG GAA TTC ATG AGT GTT GCT GCT GAA)	66
HAT1F	(CCG GAA TTC ATG AGT GTT GCT GCT GAA)	27
HAT 1R	(CCG GAA TTC CTA TTT ACC GAT ACA GA)	26
MA-GC	(CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCC CGG GGG TTT CTG GCA AAC CAT CTC TG)	59
MA	(TTT CTG GCA AAC CAT CTC TG)	20
MB	(GAA CAT ACT TGT TGC CAG AG)	20
ITS 1-GC	(CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCC CGG GGG TCC GTA GGT GAA CCT GCG G)	58
ITS1	(TCC GTA GGT GAA CCT GCG G)	19
ITS4	(TCC TCC GCT TAT TGA TAT GC)	20

Project : Establishment of National Agricultural Bioinformatics Grid (NABG)

Consortium Leader :Anil Rai, IASRI
CCPI :D. P. Singh, Alok K. Srivastava, Mahesh Yandigeri, Kamlesh Kumar Meena

Rationale

The network of institutions based on two-tier architecture in bioinformatics will bridge the gap between genomic information and knowledge, utilizing statistical and computational sciences. It will also open up new vistas for downstream research in bioinformatics ranging from modeling of cellular function, genetic networks, metabolic pathways, validation of drug targets to understand gene function and culminating in the development of improved varieties and breeds for enhancing agricultural productivity. Although, activities related to bioinformatics were initiated at different ICAR institutions at small scale in isolated mode, hardly any coordinated efforts were made to integrate these activities at national level in the field of agriculture. The research on bioinformatics needs establishment of large genomic databases, data warehouse, software & tools, algorithms, genome browsers with high-end computational power to extract information and knowledge from cross-species genomic resources. Also, there is hardly any consolidated efforts are made for collection, compilation, storage and knowledge mining of indigenous agricultural genomic resources. In order to keep pace with the research and developments in agricultural bioinformatics at global level, country needs expertise and exposure in this area of research. Therefore, there is an urgent need to establish NABG which will help in developing databases, data warehouse, software and tools, algorithms, genome browsers and high-end computational facilities through systematic and integrated approach in the field of agricultural bioinformatics.

Objectives

The specific objectives of the centre will be as follows:

- Development of agricultural bioinformatics grid for the country
- Creation of local databases and Bioinformatics Data Warehouse (BinDW) for genomic resources across species
- Human resource development in agricultural bioinformatics
- Create and promote inter-disciplinary research groups with focus on agricultural bioinformatics.
- Identification and prioritization of research areas of bioinformatics.

Significant achievements

- Priority areas in microbial bioinformatics research in the country were identified and data generation resources are being targeted.
- Compilation of a resource book of bioinformatics comprising some 1000 pages on tools, methods, techniques, softwares, languages and literature resources is under way.
- Codon usage pattern was analyzed in *Thermosynechococcus elongatus,* a thermophilic cyanobacterium and a model organism for the study of photosynthesis. Codon usage variations are widely used for quantitative prediction of gene expressivity and our results suggest that mutational bias is the major factor for thermophilli in *T. elongatus* genome but translational selection and accuracy also influenced the codon usage variation.
- A database on stress-related microorganisms has been developed. Various forms of stresses, whether biotic or abiotic with their underlying mechanisms have tremendous impact on microbes that have developed strategies to tolerate them. The database 'STRESSMICROBESINFO" includes species and strains of a number of microbes undergoing different stresses, their morphological behavior, mechanisms, biochemistry, genes, proteins, enzymes, metabolites and other products including the bibliography.
- Capacity building program under NABG project included organization of one Partner Meet (one day), Sensitization training program (7 days) and subject matter training (12 days) during the year 2010-11. In these training programs, 35 trainees from NARs system have participated.

Outreach programme on phytophthora *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

Rationale

Fusarium species are the most diverse and widely dispersed plant-pathogenic fungi, a soil-borne, saprophyte, facultative pathogen with a worldwide distribution, causes vascular wilt and diseases in a wide variety of economically important crop. It has the ability to survive in most soils like tropical, desert, cultivated and non-cultivated. These morphological features serve as the basis for formal descriptions of these taxa that meet the rules of the International Code for Botanical Nomenclature, but are not necessarily easily applied in a diagnostic and characterization. The molecular characterization of *Fusarium* will give the clear picture of species / races present and its host range in India.

SSRs are highly versatile, PCR-based markers, usually associated with a high frequency of length polymorphism. They have been found in both coding and non-coding DNA sequences of all higher organisms. These EST-SSRs have been applied successfully in studies of genetic variation, linkage mapping, gene tagging, evolution and sequencing of several plant genomes. Such sequences are widely dispersed in eukaryotic genomes including those of fungi. Simple sequence repeats refer to the sequences that are one to six-nucleotides repeated in tandem in a genome. SSRs have many advantageous features for various biological studies: SSRs are ubiquitous and abundant in a genome, highly variable and suitable for high throughput applications. Applications for microsatellite markers include genome mapping, paternity and kinship analyses, powerful tool for taxonomic and population genetic studies including estimations of population size and assignment of inbreeding coefficients. Compared with randomly amplified polymorphic DNA (RAPD) PCR, restriction fragment length polymorphism, and isozyme markers, microsatellite markers can be analyzed with considerably less material, even if it is old or partially degraded. For studies of plant-associated fungi, microsatellite markers have the added advantage that the analysis can be performed directly in plant if the flanking primers are sufficiently specific.

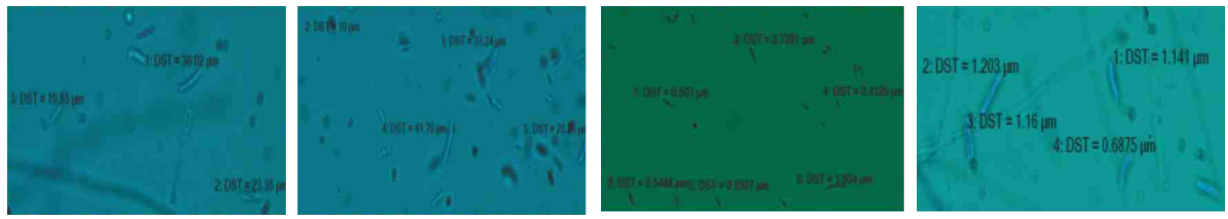
The project is aimed to develop a repository of *Fusarium which is* lacking at present. In this network project a wide collection of *Fusarium* will be generated and a data base shall be developed regarding information like place of origin, pathogenicity, virulence, races and DNA fingerprinting. It will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

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. 102 different cultures of *Fusarium* species, including *Fusarium oxysporum* f. sp. *lycopersici* (28) from tomato root, *Fusarium solani* (34) from chilli root, *Fusarium udum* (20) from stem of pegionpea and rest 20 species of *Fusarium oxysporum* f. sp. *ciceri* from the root of the chickpea, were collected from various agro ecological zones vary in the morphological, cultural, pathogenic characters .
- All isolates have been preserved for short term as well as long term conservation in mineral oil, glycerol (kept at -80^{0}C) and lyophilized.
- All 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.
- *Fusarium oxysporum* f. sp. *lycopersici* produced micro-conidia 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, *F. solani* produced micro-conidia of 8-14.5 x 2-4.5µm and macro-conidia ranges from 22.5-34 x 4-6 µm, *F. udum* produced micro-conidia 10.2 20 x



Variability in spore size of *Fusarium* species (*F. oxysporum* f. sp. *lycopersici*, *F. solani*, *F. oxysporum* f. sp. *ciceri* and *F. udum* respectively)

1.8 3.8 µm and macro-conidia ranges from micro-conidia 20 31 x 2.6 4.5 µm with 3-8 septa and *Fusarium oxysporum* f. sp. *ciceri* produced micro-conidia of 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.

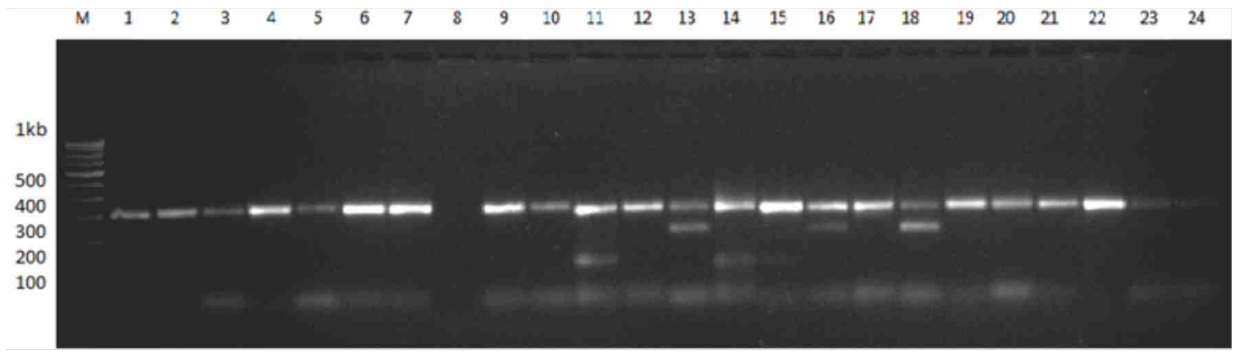
- Human resource development in agricultural bioinformatics

Molecular characterization

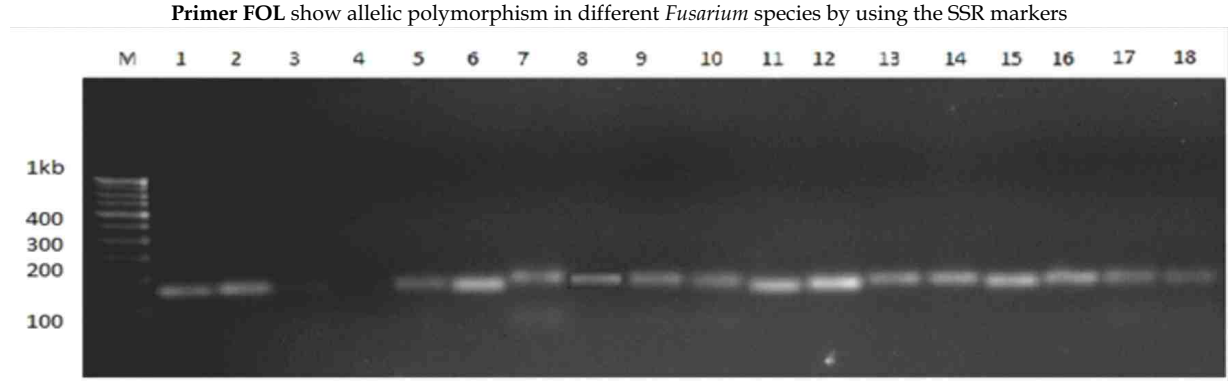
- For the development of SSR marker to study the variability in Fusaria, downloaded twenty seven thousand EST sequences of various *Fusarium spp.* from NCBI databank/ Broad Institute database.
- Freely available internet software WEBSAT has been used for spotting/marking the microsatellite repeat and these repetitive microsatellites has been used to design primers.
- A set of 30 SSR primers were designed and validated *in silco*.
- Twenty four diverse *Fusarium* formae speciales collected from different agro climatic zone under the network project which are different geographical locations of India and used in the present study to validate the SSR markers. In which six cultures of *Fusarium oxysporum* f. sp. *melonis*, six cultures of *Fusarium oxysporum* f. sp. *cucumerium*, six cultures of *Fusarium oxysporum* f. sp. *lycopersici*, three cultures of *Fusarium oxysporum* f. sp. *cubence* and three cultures of *Fusarium oxysporum* f. sp. *ciceri* respectively.
- Characterization and cross species amplification of microsatellite markers from EST of *Fusarium oxysporum* could be show the transferability in *Fusarium udum*.

Conclusion

Different isolates of *Fusarium* species showed considerable variation in conidial size as well as



Primer FOL show allelic polymorphism in different *Fusarium* species by using the SSR markers



Primer FOM show allelic polymorphism in different isolate of *Fusarium* species by using the SSR markers

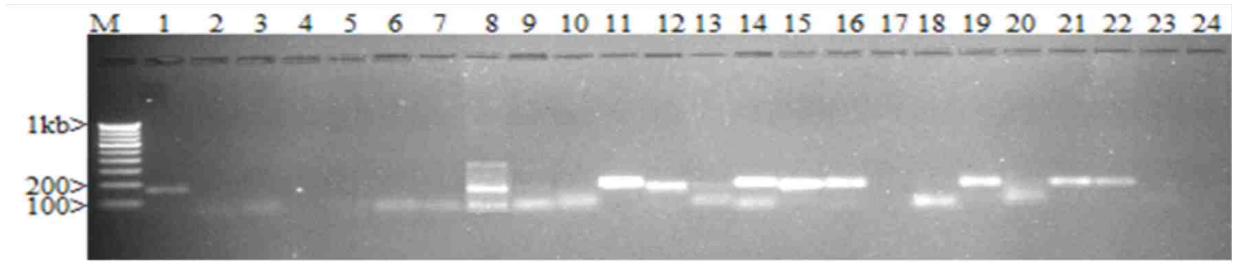
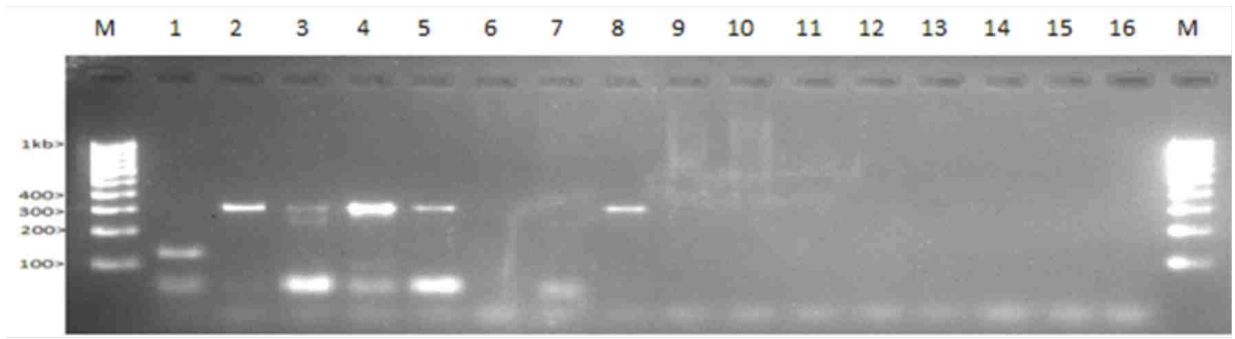


Fig.5 :**Primer FOC** show allelic polymorphism in different isolate of *Fusarium* species by using the SSR



Amplification of FOC/FOM/FOL SSR sequences in *Fusarium udum* shows the genetic transferability

growth and pigmentation. The SSR markers generated in the present study offer potentiality for its use in characterization and genetic diversity analysis of related species. The high rate of cross species transferability of the generated SSR markers might be due to the sequence conservation in the ESTs of related species. 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.

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-PIs : Alok Srivastava, Kamlesh K. Meena

Rational:

Leaf spot pathogens caused a number of important diseases in a wide range of hosts including cereals, legumes, vegetables, perennial crops worldwide. Infection by leaf spot pathogens is favoured by conditions of high relative humidity and 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. The broad host range of many species has caused problems for plant pathologists who need to identify specific plant pathogen to control disease. Accurate species identification is critical to understand disease development or epidemiology and also to develop effective control measures.

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

Scanning Electron **Microscopy** of variability in spore size and shape among same species of *Colletotrichum.*

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

- Eighty four cultures of Alternaria species, including *Alternaria brassicae* (26) from mustard, *Alternaria solani* (28) from tomato, *Alternaria brassicicola* (10), *Alternaria porri* (10) *Alternaria sesame* (10) were isolated from different crop plant and ninety nine cultures of *Colletotrichum* species including *Colletotrichum gloeosporioides* (59) isolated from mango, *Colletotrichum falcatum*(15) from Sugarcane and other *Colletotrichum* spp. (25) isolated from grapes were obtained. All the isolates of *Alternaria* and *Colletotrichum* have been preserved under long term conservation.
- All the isolates of Alternaria and Colletotrichum were characterized morphologically on the basis of colony colour, pigmentation, shape, size and arrangement of spores. Large numbers of variations were recorded within and between species.
- The genus Colletotrichum is one of the most important pathogenic fungi group, mainly in tropical and subtropical areas of the world. The identification of Colletotrichum species is difficult due to their large morphological variation, because there few reliable characters and many of these characters are plastic, dependent upon methods and experimental conditions.
- Conidial and mycelial appressoria are described in Colletotrichum species using a slide culture technique with incubation in the dark.

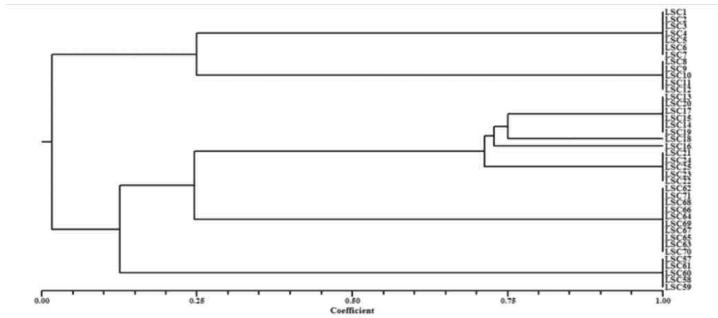
Molecular Characterization

- Genomic DNA of 84 cultures of different species of *Alternaria* and 99 cultures of different species of *Colletotrichum* were isolated and amplified by exploiting internal transcribed spacer (ITS).
- Phylogenetic analysis of *Colletotrichum* species for 40 isolates. The amplification of the r-DNA by the ITS1 and ITS4, digestion of the ITS region (internal transcribed spacer region) using restriction enzyme AluI. Eight different morphotypes of *Colletotrichum* species from various locations.

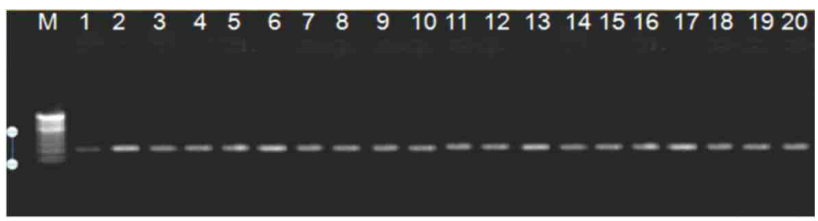
Species specific primers

Species specific primers were designed for the detection of *Alternaria* species.

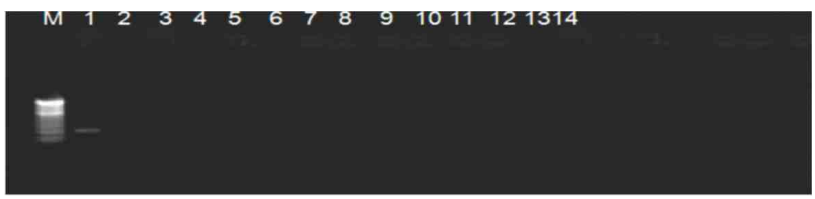
- For the development of species specific primers for rapid detection of *Alternaria solani,* the sequences were downloaded from NCBI and aligned using Bio Edit software. Based on the results of the alignment, sequences were picked up from the hypervariable regions to design the primers. A total of 6 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*
- For the wet lab validation, gradient PCR was set up to optimize the cycling parameters for the designed primers. To test the specificity



Dendrogram using RFLP-PCR polymorphic bands of *Colletotrichum* species isolates



PCR product banding patterns amplified from 20 *Alternaria solani* cultures (A) Lane M: 100 bp ladder Lane 1 to 20: *A.solani*



PCR product banding patterns amplified 14 different species of Alternaria M: 100 bp ladder Lane 1: *A. solani* Lane 2: *A. arborescence* *Lane 3: A. brassicae* Lane 4: *A. brassicicola* Lane 5: *A.cassiae* Lane 6: *A. gossypina* Lane 7: *A. polenderi* Lane 8: *A.porri* Lane 9: *A.poneasis* Lane 10: *A. sesame* Lane 11: *A.alternata* Lane 12: *A.tennuissima*, Lane 13: *A.triticola* and Lane 14: *A. zinnae* using *A.solani* specific primer NBAIMalt

towards *Alternaria solani* isolates, the primers designed were first used in a PCR reaction with genomic DNA extracted from 30 *Alternaria solani* pure cultures received from different geographical and biological origins. DNA was extracted from 14 known positive isolates of different species of Alternaria (*A. alternata*, *A. arborescence*, *A. brassicae*, *A. brassicicola*, *A.cassiae*, *A. gossypina*, *A. polenderi*, *A.porri*, *A.poneasis*, *A.sesame*, *A. solani*, *A.tennuissima*, *A.triticola* and *A. zinnae*) obtained from culture collection centre of National Bureau of Agriculturally Important Microorganisms (NBAIM) and was amplified with all the 6 sets of species specific primers designed. The universal primer pair ITS1/ITS4 was used as a positive control to assess the quality of extracted DNA. PCR product of the expected size were obtained from Alternaria solani isolates only. No cross hybridization was observed with other species of Alternaria.

- Primers were also validated at field level under the controlled conditions of glass house. Tomato seedlings were grown in pots and were infected artificially by *A. solani* culture. After 7 days DNA was extracted and was amplified with the designed species specific primer.Work is under progress for the quantitative authentication and accuracy of the developed species specific primer of *A. solani* through RT-PCR.

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 species specific primers will help for detection of *A. solani.* Molecular methods, such as PCR-RFLP and rDNA sequence, have been very useful for phylogenic studies of the pathogen.

Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)

Significant Achievements

Theme 1: Microbial Diversity and Identification

- A total of 245 isolates were isolated from the soils of Trans (Punjab) and Central IGP (U.P) regions. 20.5% of the isolates from Central IGP region were found to produce siderophores, whereas from Trans IGP region, a total of 22.5% isolates showed siderophores production. Molecular characterization of Central and Trans IGP region isolates by using PRA analysis of 16S rRNA with three restriction enzymes confirmed their identity as *Lysinibacills fusiformis*, *Paucisalibacillus globulus*, *Brevibacillus parabrevis*, *Bacillus humi*, *B.clausii*, *B.farraginis*, *B.arbutinivorans*, *Pontibacillus sp.*, *B.casamancensis*, *B.oleronius*, *B.circulans*.
- A total of 53 thermophilic bacteria (growing at 45-65°**C**) from Yumthang and Yumesamdong hot spring in India having pH 6.5 and exhibiting distinct colony characteristics were charactersied by using 16S rDNA gene clustered in 22 groups , having five **d**ifferent classes: *β-,-Proteobacteria, Firmicutes* and *Actinobacteria*.
- *Pseudomonas fluorescens (*116), *Pseudomonas cichori (*20), *Pseudomonas putida* (39), *Pseudomonas aeruginosa* (69), *Azotobacter* (310), *Azospirillum* (88), *Beijerinckia* (287) and 94 Phospahte solubilisaing bacteria belonging to *Pseudomonas*, *Bacillus*, *Alcaligens*, *Enterococcus*, *Acetobacter*, *Gluconobacter*, *Aminobacter*, *Rhizomonas*, *Acenitobacter*, *Phenylobacterium*, *Acetobacterium*, *Azomonas*, *Flavomonas*, *Flavobacterium* and *Xanthomonas.* were isolated from 54 grids of the central Western Ghats.
- A total of 35 N_2 fixing bacteria, 85 P-solubilizers, 9 *P. fluorescens*, 25 cellulose degraders, 19 lignin degraders and 9 *Trichoderma* and *8 P solubilising fungi were isolated* from Aryankavu, Achan Kovil and thenmala forest range of Kollam district and Nilambur range of Malappuram district.
- A total of ten nitrogen fixing cyanobacteria have been isolated, purified and identified as *Nostoc* sp. LCRNK6, *Nostoc* sp. LCRNK9, *Calothrix* sp. LCRNK10, *Nostoc* sp. LCRNK11, *Calothrix* sp. LCRNK12, *Calothrix* sp. LCRNK13, *Tolypothrix* sp. LCRNK14, *Tolypothrix* sp. LCRNK15, *Nostoc* sp. LCRNK16 and *Nostoc* sp. LCRNK17.
- Seven hundred thirty three bacteria were isolated from ten soil samples of Arunpur, Chilika, Haripur, Humma, Indrakhi (Orissa), Kalipatnam, K.P. Palem, Gondhi, Shankaraguptam and Undi (Andhra Pradesh) using seven media. Forty-two isolates which produce >50 µM ml^{-1} IAA and 34 ACC deaminase were tested for ammonia, siderophore and HCN production, P-solubilization and N_2 fixation.
- Fifty-eight Bt isolates were bioassayed along with two formulations against rice leaf folder (*Cnaphalocrocis medinalis)* in the field during rabi and kharif seasons. Eight indigenous isolates were effective having LC_{50} 3.162×10^6 - 1.259×10^9 spore-crystals/ml. Three isolates were more effective (LC_{50} about $3x10^6$ - $5x10^7$ spore-crystals/ml) than the formulations (LC_{50} about $3x10^8$- 10^9 spore-crystals/ml). Sixteen Bt isolates showed intrinsic salt tolerant and grew in presence of >18% NaCl. SOD, catalase, proline and amino acids imparted intrinsic osmotic and anoxic stress tolerance to the organisms.
- During the surveys in the rainy season of 2010, 111 wild mushroom species viz. *Leucocoprinus*, *Leucoagaricus*, *Schizostoma*, *Limacella*, *Leucopaxillus*, four spp. of *Termitomyces*, *Amanita pantherina*, *A. vaginatae* were collected from different forest areas of, Jodhpur (Rajasthan), Ranchi (Jharkhand) Agartala (Tripura) and Himachal Pradesh i,e Khada Pathar, Chail, Kufri, Narkanda. Edible *Termitomyces* spp. (3 spp.), *Russulla* sp and *Boletus* species were collected from Ranchi.

- A new edible mushroom species *Macrocybe giganteum* which is a tropical species was successfully domesticated on pasteurized wheat straw.
- Application of salt tolerant fluorescent pseudomonads and *Pantoea dispersa* has been found to alleviate salt stress in plants by modulating enzymes involved in reducing the impact of reactive oxygen species in groundnut.
- A total of 56 bacterial isolates collected from different fish farms were screened for protease production on skimmed milk agar plates. Out of these, nine isolates showed protease activity at 4^0C, 20^0C and 37^0C. The protease activity of these isolates was quantified and subjected to molecular identification by 16s rDNA sequencing. The results showed that the isolates belonged to genus i.e. *Bacillus*, *Aeromonas* and *Pseudomonas*. Out the three, *Bacillus* group showed maximum activity (14 units) followed by *Aeromonas* (8 units) at 37^0C.
- *P. aeroginosa, P.putida, P.flourosence* and *P.streuzi* strains collected from crop rhizosphere of *Glycine max, Cicer Arietinum, Trigonella foenum graecum and Cajanus Cajan* of Raisen, Hoshangabad, Betul, Shajapur, Bhopal, Shahdol, Chhindwara, Rajgarh districts of Madhya Pradesh were assessed in term of plant growth promotion assay and phosphorous solubilisation.
- Seven isolates (BK-2, MDb-15, LGI-4, LGI-19, NFB-22, MNFB-23 and MNFB-24) isolated from arid and semi-arid zones of Haryana and showing better performance in cotton & pearl millet were identified as *Sinorhizobium, Rhizobium* sp., *Agrobacterium tumefaciens, Rhizobium etli, Stenotrophomonas maltophilia, Ralstonia* sp. and *Leptothrix cholodnii*, respectively. The isolates from each phylogenetic group were characterized for Gram staining, IAA production, P- solubilization, ammonia excretion, NaCl tolerance and were identified on the basis of partial 16S rDNA gene sequencing. Most of the isolates showed 99-100% similarity with *Agrobacterium, Sinorhizobium, Pseudomonas, Ensifer, Rhizobium*, *Bacillus, Paenibacillus, Streptomyces, Microbacterium, Stenotrophomonas* species.
- *Achromobacter* sp. KRD9 and *Providencia* sp. KRD23 showing significant amount of protease production was obtained from Gibbon Wild Life Sanctury, Jorhat, Assam.
- Soil samples collected from rhizosphere of *Cinnamomum cassia, Citrus reticulata, Hevea brasiliensis* and *Cucurbita moschata* of Darjeeling districts of North Bengal yielded 103 bacterial isolates and 22 fungal isolates. Among them 15 isolates were phosphate solubilizers; 26 were chitin degraders whereas 10 showed siderophore production; 26 showed protease activities; 31 showed amylase activities and 5 isolates showed antifungal activities.
- *Bacillus altitudinis* (BRHS/P 22) isolated from rice rhizosphere showed *in vitro* antagonistic activity against *Sclerotium rolfsii* and *Rhizoctonia solani* and could be utilized for plant (*Phaseolus vulgaris*) health improvement in the field condition.
- Fifty (50) cyanobacterial strains belonging to 16 genera were isolated from different ecological habitats of Arunachal Pradesh, India were screened and identified for biochemical characterization for Chl-a, phycobiliproteins (phycocyanin, phycoerythrin and allo-phycoerythrin) and carotenoids for value additions.
- A total of 22 *Trichoderma* spp *viz*. *T. atroviride* (5), *T. konigii* (1), *T. hamatum* (1), *T. minutisporum* (1), *T. polysporum* (1), *T. brevicompactum* (1), *T. virens* (4), *T. crassum* (1) and *T. harzianum* (1) isolated from Middle and North Andaman and Hut Bay were characterized on the basis of cultural, morphological and antagonistic properties.
- Twenty one ammonia oxidizing bacteria like *P aeruginosa, Citrobacter spp., Morganella morganii, P fluorescens, Alcaligens feacilis* and *Prividencia vermicola* isolated from freshwater ecosystems were able to reduce nitrate to nitrite. In addition, these isolates were also positive for cellulase, Lipase, Protease and Phosphatase and also grow in presence of arsenate and arsinite.
- From hot spring *Bacillus licheniformis, Brevibacillus limnophilus, Klebsiella pneumoniae. Paenibacillus popillae. Leclercia spp.Pseudomonas aeruginosa* were isolated which produce proteases and celluase enzymes at 45-55^0C.
- A total of 180 diazotrophs identified as *Stenotrophomonas maltophilia, Bacillus amyloliquifqciens Bacillus circulans, Pseudomonas aeruginosa, Paenibacillus sp., Paenibacillus panacisoli, Azotobacter vinelandii, Paenibacillus amyloliticus, Pseudomonas putida, Bacillus subtilis*

isolated from Central plain region of India were found postive for P solubilization and antagonism.

- *Paenibacillus amyloliticus* was isolated from soil having pH 5.3 and higher ammonical nitrogen as 110 mg/kg whereas *Azotobacter vinelandii and Pseudomonas putida* were isolated from soil having high ammonical nitrogen 119 mg/kg.
- Phenotypic characterization completed for 38 bacterial strains, isolated from the skin, gills and gut of Silver ribbon fish *Lepturacanthus savala, Parascolopsis aspinosa Gerres filamentosus,* Indian scad *Decapterus russeli* and *Priacanthus hamrur,* and for 5 bacterial strains from the gut of prawns, Kadal shrimp *Metapenaeus dobsoni* and *M. monoceros*.

Theme 2: Nutrient Management, PGPR, Antagonists, Biocontrol Agent and Disease Management

- A total 480 bacterial strains were obtained from the rhizosphere of different crops from Indogangetic plain regions (Mau, Varanasi, Lucknow and Kanpur) of India. All the strains were tested against three plant pathogenic fungi (*FOC race 1*, *FS* and *MP*). Out of them, only fourteen bacterial strains *viz. Lysinibacillus fusiformis* (B-CK11, B-CL9 and B-MV146), *Lysinibacillus* spp. (B-ML26, B-WV249, and BRL392), *Bacillus cereus* (B-PV53 and B-CV280), *B. subtillus* (B-RM 177, B-CM331 and B-TM444), *B. thuringiensis* (B-TK433) and *Bacillus* spp. (B-PL125, B-MM208). (B-CK11, B-ML26, B-PV53, B-CL94, B-PL125, B-MV146, B-WM77, B-MM208, B-WV249, B-CV280, B-CM331, B-CL392, B-PK413 and B-PM444) showed significant inhibitory effect on mycelial growth against all the three plant pathogenic fungi. Strain B-CM331, B-TM444, and B-WM177 were the most efficient antagonists and showed 38.33, 37.03 and 36.80 mm inhibition zone against *FOC race 1*, respectively.
- Total 110 isolates of endophytic fungi isolated from different plants from Indo-gangetic plains were characterized on the basis of morphology, growth characteristics and production of various metabolites including IAA, siderophore, ammonia, and HCN.
- A total of 150 bacteria were isolated from salt affected soil of IGP region *viz* Lucknow, Kanpur, Allahabad, Mau, Varanasi and evaluated against *Fusarium oxysporum* f. sp. *Lycopersici and Rhizoctonia solani.* Strain B-14 and B-101 showed strong tendency for forming biofilm and EPS (Exopolysaccharide) production.
- Fifteen elite PGPR strains including *Lysinibacillus fusiformis* and *Dyella marensis* increased the soybean yield by 18% and 10 elite rhizobial strains increased the grain yield of soybean by 15% in vertisol field.
- *Bradyrhizobium japonicum* ISR-33 and PGPR-*Bacillus megaterium* ISP-3 were supplied for mass production to JNKVV Biofertilizer production centre, Jabalpur. 6,06,6766 inoculant packets were prepared with these strains and supplied all over Madhya Pradesh since 2009.
- B105+P17+*Rhizobium* formulation showed good growth promotion of pigeonpea in pots where as B87+P17+*Azospirillum* formulation showed good growth promotion of sorghum in pots, when compared to single and dual inoculations. P17 was compatible with fungicide Carbendazim 50%WP-1%, Mancozeb 75% WP- 0.5%, Metaxyl 35% WP- 1%, Copper oxy chloride 50% WP-0.5% and Captan 50% WP 0.5%.
- Eight bacterial consortium developed from five elite cold tolerant P solubilizing *Pseudomonas* strains were evaluated under pot condition and significantly enhanced nutrient content (N, P, K) of wheat (VL Gehun 804) upto 1.61, 1.26 and 1.64 folds respectively. Inoculation with bacterial consortium significant enhanced nutrient parameters (shoot dry weight, shoot length and root length by 2 folds, 1.3 folds & 1.65 folds respectively) and cold stress response (phenolics, proline, starch, EC & relative water content) under field condition.
- In field condition four bacterial consortium enhanced P content of wheat (VL Gehun 804) in the range of 11.1 to 33.3% in stover and 17.2 to 31.0% in seed and significantly increased wheat yield 16.9 to 39.4% over the uninoculated control. At various locations of Himachal Pradesh two potent bacteria (RT5RP2 & RT6RP) showed significant increase in yield of wheat (4.0-13.0%) & pea (26.6 -28.2%)
- The rhizobacterial strains GRB 68(*Serratia marcescens)* and GRB 35(*Bacillus amyloliquefaciens*) from ginger were found to enhance the sprouting of rhizomes besides reducing the soft rot and

bacterial wilt in ginger. Three rhizobacterial isolates (BRB 3, BRB 13 and BRB 23) from black pepper with different combinations of NPK was found to promote the growth in black pepper plants. These isolates have significantly enhanced the levels of mineral N, Bray P and exchangeable K in soils. The results on growth parameters revealed that the treatments 100% N + 100% P + 100% K+ BRB 3 and 100% N + 100% P + 100% K+ BRB 13 showed maximum height followed by 100% N + 100% P + 75% K + BRB 3.

- Five *Bacillus subtilis* isolates (CSB 8, KGEB 10, PEB 2, PEB4 and VEB 17) from cocoa rhizosphere could tolerate a maximum temperature of 60^0 C and were able to grow on TSA medium amended with 12% NaCl. Three *Bacillus* spp. (*B. subtilis* CSB 16, *B. subtilis* CEB 9 and *Bacillus* sp. PS2 VEB 4) from cocoa showed intrinsic resistance to 12% of NaCl in TSA. The cocoa PGPR isolates *Pseudomonas putida* KDSF 23 and *Pseudomonas* sp. KDSF 7, isolated from Kidu, Karnataka exhibited pH tolerance from 5.2 to 9.0.
- Twenty one isolates of entomofungal pathogens belonging to *Beauveria bassiana* (7 isolates), *Metarhizium ansiopliae* (7 isolates), *Nomuraea rileyi (3 isolates), Lecanicillium lecanii* (2 isolates), *Ascheronia aleyrodis* (1 isolates) and *Paecilomyces farinosus (1 isolate) were isolated from soils and insect hosts from Gujarat, Meghalaya, Himachal Pradesh, Kerala and Karnataka,* Soybean oil EAO, Mineral oil EAO, Groundnut oil EAO and Sunflower oil EAO showed higher conidial germination of *B. bassiana* (81-97% at 48hrs) and *M. anisopliae* (85-94% at 48hrs).
- Field based evaluation of a combinational set of PR7, PR3 and CR1 (T7) along with basal application of 2/3 N+PK, recorded highest grain yield and plant biomass, as well as microbial parameters followed by PR3+CR1+CR2 (T8) in rice crop. Treatments involving inoculation with both single strain and multi-strain consortia enhanced wheat grain production, harvest index, quality and yield, besides savings of N (40-60Kg N/ha) and micronutrient enrichment.
- 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. Under glass house experiment, *Bacillus megaterium* supported growth in presence of two out of the ten rock phosphates i.e. North Carolina and Gafsa as the only P source, comparable to that of single super phosphate (SSP).
- Dual inoculation of *Gluconacetobacter* with mycorrhizal fungus (*Glomus intraradices*) in maize significantly increased soil available Fe (M- 1.9; M+ 2.1 mg kg^{-1}) and Zn (M- 4.16; M+ 4.50 mg kg^{-1}) in both calcareous and non-calcareous soils. Acid phosphatase activity increased by 20-40% due to mycorrhizal colonization that acidify the rhizosphere besides improving the availability of Zn.
- Two potential isolates (*Bacillus amyloliquefaciens* - BA1 and *B. subtilis* -BS2) evaluated against wilt and collar rot of tomato under field conditions reduced the disease (56.8 %) and enhanced the yield (25.1 %). Among different combination of consortium with potential rhizobacterial isolates tested, a consortium partner of Bs2 with T2-2 or T2-7 found effective in reducing the wilt and enhancing the growth of tomato under green house conditions.
- Application of NPK containing biofertilizers along with *Pseudomonas fluorescens* and biocontrol agent, *Trichoderma* in the *Amorphophalus* revealed the possibility of substituting chemical fertilizer to the tune of 25-75% level of the recommended dose of N, P and K chemical fertilizer.
- *Trichoderma harzianum*-Th4d (oil formulation 1ml/lit), *Pseudomonas fluorescens-Pf2 (Talc formulation 5g/lit)* and consortia oil formulation of *Trichoderma harzianum*-Th4d + *Trichoderma viride*-Tv5 1ml/l were effective against *Alternaria leaf blight* of sunflower and *Botrytis* grey rot of castor with maximum disease reduction over pathogen check (55-65%) and showed better persistence.
- The four MHBs isolated from AM spores of coconut based cropping systems were identified as *Corynebacterium coyleae, Bacillus subtilis, Bacillus cereus and Bacillus ern by B amyloliquefacience* based on their carbon utilization pattern based on BIOLOG. The MHBs isolated from sapota cropping system were identified as *Brevibacillus parabrevis, B. choshinensis, Psudomonas putida, and P. aeruginosa by molecular method.*

Theme 3: Microbial Management of Agro waste, Bioremediation, Microbes in Post Harvest and Processing

- A microbial consortium consisting a bacteria (*Serratia marcescens* L-11), an actinomycete (*Streptomyces rochei* PAH-13) and a white rot fungus *Phanerochaete chrysosporium* VV18 was found to be very effective for degradation of PAH mixture even up to 200ppm concentration. Supplementation of yeast extract (0.1%) improved the degradation of PAH.
- A new method of total indoor compost production standardized in 10 days time escaping phase-I conditions altogether using consortium of thermophlic fungi (*S. thermophilum, H. insolens, H. grisea*). Such composting procedure besides being an express method (10 days) is environment friendly, less labour consuming and steam requirement for pasteurization is almost nil and end product is most suitable for the growth of mushroom mycelium giving higher productivity of mushroom.
- Simultaneous saccharification and fermentation (SSF) using crude cellulolytic enzyme produced by a strain of *Aspergillus niger* and a thermotolerant strain of *Pichia kudriavzevii* cells produced ethanol at a concentration from 34 g/l.
- Recycling of *Pichia kudriavzevii* cells in a galactose medium over three cycles helped in enhanced ethanol production at temperatures in the vicinity of 40 °C and 45 °C.
- Two chlorpyriphos degrading bacteria of CRRI soil were identified as *Labrys* spp. Strain CH23 (PCR2) and *Methylobacterium* sp. Strain PCR CH13. Optimum temperature for chlorpyriphos degradation for the potent isolate CH23 was 35°C and about 99.88% of the chemical was detoxified by the bacteria after 15d incubation.
- The crude consortium Cb (*Bacillus pumilus, B. subtilis, B. endophyticus and B. megaterium*) has given the best results with higher xylanase activity compared to that of individual members. Cellulase activity of *B. subtilis* was significantly higher, and that of *B. megaterium* was slightly higher than that of the consortium.
- Optimum conditions like time, inoculum level were worked out for efficient fungi (*T. longibrachiatum, T. fasciculatum*) and bacteria (*B. cereus*, *Bacillus.* sp.) for maximum removal of heavy metals (Pb, Cd and Ni) under laboratory conditions. Consortium of five fungi (*Aspergillus Niger, A. terreus, T. longibrachiatum, T. fasciculatum, A. awamori*) and two bacteria (*B. cereus*, *B. sp.*) grown on pressmud indicated encouraging result for removal of heavy metal (Cr, Cu and Ni) from industrial effluents.
- The isolates of *Lactococcus lactis* ssp. lactis have been identified and screened as nisin producer for enhancing the strength of industrial important strains for the application as starter cultures.
- The anti-bacterial activity of fabric dye (2, 4 bis (1, 1 dimethylethyl) - phenol and pentanoic acid 5 hydroxyl est) with yellow pigment of *Thermomyces* sp. showed a maximum bacteria reduction.

Theme 4: Microbial Management of Abiotic Stress

- Actinomycetes isolated from the Uttar Pradesh saline soils region were evaluated on different salts (NaCl, KCl and $MgSO_4$). The Actinomycetes isolated from Kanpur regions had shown significant results on NaCl. A total of five isolates have showed growth on 4% NaCl concentration, twenty isolates at 10% KCl and 30 isolates at 10% $MgSO_4$ while the isolates from Mau region showed tolerance up to 10% NaCl concentration.
- Consortia comprising *Azospirillum* (ES-173), *Azotobacter* (S63(1)R), PSB (S125R) and fluorescent pseudomonad (S4(1)S) gave significant improvement in the plant growth and yield of cotton (cv RAHS-14) at all the soil EC levels. Consortia recorded 16.5 per cent increase in seed cotton yield over UIC at soil EC 10 dS/m. Seed inoculation of sunflower (KBSH-1with selected salt tolerant isolates and their consortia gave 10 to 14, 14 to 21 and 28 to 39 per cent increase in seed germination; 16 to 30, 21 to 41 and 22 to 60 per cent increase in biological yield; and 16 to 70, 18 to 68, 26 to 97 per cent increase in grain yield over UIC at soil EC of 4.5, 7.9 and 10.3 dS/m respectively.
- Two bacterial consortia *viz.* P7+B30+G12 for sorghum and P45+B17+G12 for sunflower with inorganic and organic fertilizers amendments increased grain yield in sorghum as well as in sunflower plants compared to 75% chemical + Inoculation; 50% chemical +8tons FYM+ Inoculation; 75% chemical + 4tons FYM+

Inoculation; 75% chemical + 4tons FYM and 100% Inoculation.

- Cold tolerant *Pseudomonas* strain NARs9, PBRs5, PPERs23 and PGERs17 enhanced wheat yield by 19.2, 17.1, 16.0 and 13.5% respectively, over the uninoculated control under field condition. Among the bacterial consortium C4 (*P. fluorescens* PPRs4, *Pseudomonas* sp. PCRs4, *P. jessenii* PGRs1) recorded maximum (29.5%) wheat yield over the control under field condition.

Theme 5: Microbial Genomics

- Sequencing of the genome of *Mesorhizobium ciceri* Ca181 was done by 454 Next Generation pyrosequencing as well as Sanger technology. A total of 6461 genes have been predicted and annotated for the functions they perform in *Mesorhizobium ciceri* Ca181.
- Comparative profiling of three potential strains (i.e. JAZ1, AAB8 and PAS1) by 2 D gel electrophoresis and MALDI-TOF mass spectrometry identified protein A, GroES protein, Alkyl hydro peroxide reductase subunit C and GroEL protein, respectively as Universal stress proteins expressed by these strains.
- In fluorescent pseudomonad *P. putida* S11W, a two component regulatory system involved in regulation of siderophore pyoverdine synthesis was identified and characterized.
- End sequencing of 1824 fosmid clones have been done by using Newbler Assembler, Velbet and Lasegene DNA Star software and generation of 23 sequence contigs. Fosmid end-sequences were used for creating scaffolds of 23 sequence contigs and 14 gaps between contigs have been filled.
- Two genes (*end* 1 and 2) encoding hydrolytic enzymes from *A. laxa* (RPAN8) which showed β-1, 4 (*end* 1 and 2) and β-1, 3 (*end* 2) endoglucanase activities were identified, purified and characterized.
- Out of 50 *Xanthomoans oryzae* pv. *oryzae* strains, belonging to 11 different pathotypes, 10 strains were found to have the BXO8 type of LPS gene cluster.

Theme 6: Microbial Genomic Resource Repository

- Three hundred genomic DNAs from bacteria, fungi and actinomycetes were isolated, purified, quantified and deposited in the DNA bank.
- Digitization of the genomic DNA data has been completed

Human Resource Development

Activities being carried out to strengthen human resource

- Organising National level training programmes on "Molecular Characterization" of agriculturally important microorganisms at NBAIM for researchers, research students and scientists.
- Developing Memorandum of Understanding (MoU) between NBAIM and different Universities for academic exchange among research students pursuing Ph.D. Programmes in order to share the facilities and expertise available in each other's institutions.

Specialized Training organized

The Bureau is gaining nation-wide recognition for organizing specialized training programmes 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 2010-11, following specialized trainings were organized.

- National training programme on "Microbial Identification and Gene Mining: A Bioinformatics Approach" from September 1-10, 2010

- National training programme on "Metagenomics: Methods and Applications in Microbiology" from January 11-20, 2011.

- Sensitization workshop on "Bioinformatics and Computational Biology in Microbiological Research" from January 29- February 4, 2011.

- National training programme under NAIP on "Data Mining and Computational Methods in Bioinformatics for Microbial Research" from March 4-15, 2011 at NBAIM, Mau

Meetings and Visits

Meetings/ Conference/ Training organized by NBAIM Organized

- Half yearly review meeting of AMAAS project on January 9, 2011 at NBAIM, Mau.
- NAIP CIC meeting on January 18, 2011 at NBAIM, Mau.
- Research Advisory Committee meeting on February 18, 2011 at NBAIM, Mau.
- Institute Management Committee meeting on February 19, 2011 at NBAIM, Mau.
- Half yearly review meeting of AMAAS project on February 21, 2011 at NBAIM, Mau.
- Fifth annual review meeting of AMAAS project on 14-15 May, 2011 at NBAIM, Mau.

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

- Attended the CIC-NAIP meeting on 07.04.2010.
- Attended the National Consultation on Agro-Biodiversity on 26-27th May 2010 at NASC complex, New Delhi
- Attended the Director Conference and VC Conference at NASC Complex New Delhi from July 15-18, 2011
- Attended the meeting with DG, Biodiversity International 27.07.10 to 29.07.10
- Attended the National Biodiversity Authority (NBA) at NASC Complex New Delhi from 25.08.10 to 27.08.10
- Attended the interface meeting for data sharing, management, strengthen and integration of databases at NASC Complex New Delhi from 22-25.11.10
- Attended the 4th CIC and CAC meeting of NAIP-II held at NBSS&LUP Nagpur from December 13-14, 2010.
- Attended the 51st Annual Conference of AMI held at Birla Institute of Technology, Mesra Ranchi from 14-17 December 2010.
- Attended the 5th National Conference on KVK 2010 at Maharana Pratap University of Agricultural and Technology, Udaipur from 20.12.10 to 24.12.10
- International Conference on Microbes in waste water & waste treatment, bioremediation and energy production (MWT 2011) held at Goa, from January 24-27, 2011
- National workshop on MDS of Health indication for Soil Resources under Agroclimatic condition at DWM, Bhubaneshwar from January 29-30, 2011.
- Annual Thematic Workshop of NAIP III held at CISH Lucknow from February 1-2, 2011.
- Panelist, in Agriculture Science Congress at NBFGR organized by National Academy of Agricultural Sciences from 07.02.11-11.02.2011
- Short term training programme on "Application on Genomics & Bioinformatics in Phytopathora/ Ralstonia Research" at IISR, Calicut from February 8-17, 2011
- 10th Agriculture Science Congress held at NBFGR Lucknow from February 10-12, 2011 " Interface meeting of VC, DAC, DADR & Director of ICAR Institutes/ Bureaus, NRC and PC at NASC Complex, New Delhi from 23-24 February 2011
- Attended 15th Annual Convention of ADNAT International Symposium on "Genomics and Biodiversity" held on February 23- 25, 2011 at Centre for Cellular and Molecular Biology (CCMB), Hyderabad- 500 007, A. P. (India).
- National conference on Microbial diversity and its applications in agriculture, health and industry from 5-6 March, 2011 at ICAR Research Complex, Goa.
- Attended Division-wise interaction meetings with the Chairs of RAC at NASC Complex, New Delhi on 01.03.2011.
- Attended National conference on Microbial diversity and its applications in agriculture, health and industry from 5-6 March, 2011 at ICAR Research Complex, Goa
- Annual Thematic Workshop of NAIP-1, held at IIHR Bangalore from March 7-8, 2011.
- International Conference on Microbial diversity held at CCMB, Hyderabad from 23-25 March 2011.

Visits Abroad

- Foreign deputation to Dr. Kamlesh Kumar Meena, Scientist, in the area of Molecular Microbial Taxonomy at University of Washington, Seattle, U.S.A. from October 0, 2010 to January 02, 2011.
- Foreign deputation to Dr. Sudheer Kumar, Senior Scientist, in the area of Molecular Diagnostics at Ohio State University, Ohio from December 09, 2010 to March 08, 2011.

Award/Distinction

- Anjney Sharma, M. Kumar, Sudheer Kumar, A.K. Srivastava, and D.K. Arora (2011) Isolation and Characterization of Plant Growth Promoting (PGP) Bacilli from Saline Soil of Eastern U.P. In 10th Agriculture Science Congress at NBFGR Lucknow from February 10-12, 2011 **(Awarded as Best Poster Presentation)**

Publications

Research Paper

- Mahesh S. Yandigeri, Kamlesh Kumar Meena, Srinivasan, R., and Sunil Pabbi (2011) Effect of mineral phosphate solubilization on biological nitrogen fixation by diazotrophic cyanobacteria. Indian Journal of Microbiology, 51(1):48-53.
- Srinivasan R., Ajjanna R. Alagawadi, Mahesh S. Yandigeri, Kamlesh Kumar Meena and Anil K. Saxena (2011) Characterization of phosphate-solubilizing microorganisms from salt-affected soils of India and their effect on growth of sorghum plants [Sorghum bicolor (L.) Moench]. Annals of Microbiology, 10.1007/s13213-011-0233-6.
- Gulzar Singh Sangera, Prem Lal Kashyap, Gurpreet Singh and Jaime A. Teixiera da Silva (2011) Trangenics: Fast track to Plant Stress Amelioration. Transgenic Plant Journal 5(1):1-26
- Thind T.S., Goswami S, Kaur R, Raheja, S and Mohan C. (2011). Development of metalaxyl resistance in Psuedopernospora cubensis and management options with novel action fungicides. Indian Phytopath. 63(4) 387-392.
- Rashmi Aggarwal, Lalit L. Kharbikar and Renu (2011). Identification of *Bipolaris sorokiniana*-responsive differential transcripts in wheat (*Triticum aestivum* L.) . Indian Phytopath. 64(1) 24-27.
- Sahay H, Singh S, Kaushik R, Saxena AK & Arora DK (2011). Characterization of halophilic bacteria from environmental samples of Pulicat brackish water lake, India, Biologia (In press)
- Tiwari S, Meena KK, Yandigeri M, Singh DP & Arora DK (2011) Salt tolerant rhizobacteria-mediated induced systemic tolerance in wheat (Triticum aestivum) and chemical diversity in rhizosphere enhance plant growth. Biology and Fertility of Soil (Accepted).
- Solanki MK, Singh N, Singh RK, Singh P, Srivastava AK , Kumar S, Kashyap PL & Arora DK (2011). Plant defense activation and management of tomato root rot by a chitin fortified Trichoderma formulation. Phytoparasitica. (Accepted).
- Solanki MK, Kumar S, Panday AK, Srivastava S, Singh RK, Kashyap PL, Srivastava AK & Arora DK (2011). Diversity and antagonistic potential of *Bacillus* spp. associated to the rhizosphere of tomato for the management of *Rhizoctonia solani*. Biocontrol Science and Technology (Accepted).
- Singh, D. P., Ratna Prabha, Yandigeri, M. and Arora D. K. (2011) Cyanobacterial inoculation in rice (Oryza sativa) enhance phenylpropanoids and phytohormones to support plant growth and stress tolerance, Antonie van Leuwenhoek. (Accepted for publication).

Papers/posters presented in National/ International Conference:

- Anjney Sharma, M. Kumar, Sudheer Kumar, A.K. Srivastava, and D.K. Arora (2011) Isolation and Characterization of Plant Growth Promoting (PGP) Bacilli from Saline Soil of Eastern U.P. In 10th Agriculture Science Congress at NBFGR Lucknow from Feb10-12, 2011.
- Manoj K. Solanki, Supriya Srivastava, Alok K. Srivastava, Sudheer Kumar, Prem Lal Kashyap, Akhilesh K. Panday and Dilip K. Arora (2011). "Diversity and antagonistic potential of Bacillus spp. associated to the rhizosphere of tomato for the management of Rhizoctonia solani". National conference on Microbial diversity and its applications in Agriculture, Health and industry from 4-5 March, 2011 at ICAR Research Complex, Goa.
- Udai B. Singh, Dhananjaya P. Singh, Alok K. Srivastava and Dilip K. Arora (2011). Evaluation of functional gene enrichment in a wheat rhizospheric soil: A means to access and use genes from uncultured microorganisms. In: Xth Agricultural Science Congress on "Soil- Plant- Animal Health for Enhanced and Sustained Agricultural Productivity" Organized by National Academy of Agricultural Sciences in collaboration with NBFGR, IISR and CISH on February 10- 12, 2011 at National Bureau of Fish Genetic Resources(NBFGR), Lucknow, U.P., India.
- Kamlesh K. Meena, Manish Kumar, Shri Krishna, Ratna Prabha, Udai B. Singh, Alok K. Srivastava and D. K. Arora (2011). Genetic diversity and phylogeny of methylotrophic community from phyllosphere and rhizosphere in different tropical crop plants. In 15th ADNAT Convention and three days Symposium on Genomics and Biodiversity held on 23- 25 February, 2011 at CCMB, Hyderabad (A.P.), India.
- Sukumar M., Achala Bakshi, Udai B. Singh, B. Kishor Babu, Sangeeta Saxena and D. K. Arora (2011). Genetic markers in detection of variations among Indian isolates of *Fusarium udum* infecting pigeonpea. In 15th ADNAT Convention and three days Symposium on Genomics and Biodiversity held on 23- 25 February, 2011 at CCMB, Hyderabad (A.P.), India.
- Udai B. Singh, Dhananjaya P. Singh and Dilip K. Arora (2011). Environmental Whole-Genome Amplification to Access Biodiversity of Type II Polyketide Synthase Gene in Wheat Rhizosphere. In National Symposium on "Harnessing Biodiversity for Biological Control of Crop Pests" going to be held on 25- 26 May, 2011 at National Bureau of Agriculturally Important Insects (NBAII), Bangalore, (Karnataka), India.
- Harmesh Sahay, Surendra singh Anil K. Saxena and D.K Arora (2011) Diversity assessment and industrial application of thermophilic bacteria from Manikaran Hot spring, India Poster presentation in the symposium on "Genomics and Biodiversity" at CCMB as part of 15th ADNAT Convention from 23-25th February 2011.
- Udai B. Singh, Dhananjaya P. Singh and Dilip K. Arora (2011). Frequency and biodiversity of 2,4-diacetylphloroglucinol producing bacteria from the wheat rhizosphere. In 3rd Global Conference on "Plant Pathology for Food Security" going to be held on 10- 13 January, 2012 at Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur- 313 001, India.(accepted).
- Singh RN, Srivastava AK, Arora DK (2011). Diversity of psychrophiles of Leh glaciers: a molecular approach. Accepted for 4th Congress of European Microbiologists Geneva, Switzerland to be held from 26 to 30th June, 2011.

Library Information and Documentation

Books

Administration		58
Bacteriology		80
Biochemistry		32
Bioinformatics		35
Bioinstrumentation		01
Biotechnology		153
Botany		10
Environmental Sciences		14
Integrated Pest Management		12
Microbiology		415
Molecular Biology		171
Phycology		12
Plant Pathology		115
Plant Virus		14
Mycology		147
Genetics	35	
Genomics		25
Intellectual Property Rights		45
Proteomics		05
Virology		20
Enzymology		07
Others		22
Biocontrol		04
Biostatistics		04
Biofertilizers		03
Total		**1439**

Periodicals

Annual Review of Microbiology (Vol 47 to 56, 58 & 64) Annual Review of Phytopathology (Vol 30-41 & 44-48)

Applied and Environmental Microbiology (Vol 1 to 12)

Miscellaneous Literature

Annual Reports of ICAR Institutes

Complete Solution for Biotech Research

Advanced Biotech

Hindi Books

Current Contents of Life Sciences

Catalogues

Dictionaries

ICAR News/ Bulletins

Journal List

1. Applied and Environmental Microbiology
2. Asian Journal of Microbiology, Biotechnology and Environmental Sciences
3. Biology and Fertility of Soils
4. Canadian Journal of Microbiology
5. Clinical Microbiology
6. Current contents
7. Current Science
8. FEMS Microbiology Ecology
9. Fungal genetics and Biology
10. Indian Journal of Experimental Biology
11. Indian Journal of Microbiology
12. Indian Journal of Sugarcane Technology
13. Indian Phytopathology
14. Journal of Biochemistry and Biophysics
15. Journal of Biosciences
16. Journal of Biotechnology
17. Journal of Eco-friendly agriculture
18. Journal of Indian Institute of Science
19. Journal of Mycology and Plant Pathology
20. Journal of Scientific and Industrial Research
21. Journal of the Indian Institute of Science
22. Molecular Plant Microbe Interaction
23. Molecular Plant Pathology
24. Mycobiology
25. Mycologia
26. Mycological Research
27. Nature
28. Pestology
29. Plant Diseases
30. Soil Biology and Biochemistry
31. The Journal of the Indian Botanical Society
32. Eukaryotic Cell

Linkages and Affiliations

Local Institute

The NBAIM has effective linkages with different ICAR research institutions and SAUs situated in U.P. The Bureau is actively participating Networking with NRCs on microbial biodiversity along with other Institutes having expertise. These linkages 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 Innovation Project, ICAR approved projects and Bioscience

RAC and IMC

Research Advisory Committee (RAC)

Chairman
Dr. D.J. Bagyaraj

Members
Dr. T. P. Rajendran
ADG (PP), ICAR

Dr. H. Shekhar Shetty
Department of Studies in Applied Botany
University of Mysore, Mysore

Prof. Sudheer Meshram
Director
Rajiv Gandhi Biotechnology Centre
Nagpur University, Nagpur

Dr. Banwari Lal
Director
TERI, New Delhi

Dr. D.L.N. Rao
Principal Scientist & Network Coordinator
Indian Institute of Soil Science, Bhopal

Recommendations

- RAC recommended that there may be a strong representation of NBAIM institute in the taskforce of DST, DBT and other funding institutes to unravel some aspects on bio-agents and bi-control through projects.
- RAC appreciated the work on *Magnaporthe grisea* and advised to focus on the component of "Exploration of pathogenicity gene(s) of *Magnaporthe grisea* in hot spot regions of India
- High throughput and advanced cryopreservation facility need to be developed at the Bureau.
- Research on exploration of sulphur oxidizing microbes, Arthrobacter, and oligotrophic microbes in agriculture, Metabolite profiling of bioactive molecule from agriculturally important actinomycetes, bio-priming of seeds with microbial formulations and development of nanoparticle based delivery system needed to be initiated as major component in the AMAAS project.
- More scientists from the NBAIM may get trained in metagenomics, community analysis, annotation of

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Pratima Hospital
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Prof. D. K. Arora
Director, NBAIM

Member Secretary
Dr. Alok K. Srivastava
Senior Scientist, NBAIM

metagenomic data and other specialized areas of agriculturally important microorganisms at abroad and ICAR should be requested to manage the same.

- A separate building and infrastructure for the MGRR may be developed to strengthen the facility in 12th five year plan.
- The culture collection unit of the Bureau may be equipped necessary facilities for the conservation and maintenance of obligate parasites of national importance with the help of experts.
- Director may be authorized by ICAR to appoint consultant for short term (2 -4 weeks) and the services may be extended thereafter.

Institute Management Committee (IMC)

Chairman
Prof. Dilip K. Arora
Director, NBAIM

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

Dr. S. P. S Ahlawat
Former Vice Chancellor
Vikram University, Ujjain

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Dr. V. N. Singh
Pratima Hospital
Mau, Uttar Pradesh

Member Secretary
Dr. Mahesh Yandigeri
Senior Scientist, NBAIM

Recommendations

- The Action Taken Report was approved and accepted.
- IMC appreciated the efforts done by NBAIM in the field of research and infrastructural development.
- IMC appreciated the efforts for maintenance and housekeeping of revenue receipt.
- IMC was satisfied with replies of the para related to use of equipments and other issued related to audit.
- IMC recommended the Common Security for NBAIM-DSR Campus.
- IMC recommended the Residential Research Facilities for the working women at NBAIM but the matter should be forwarded to Council.
- The dedicated power supply issue may be pursued in the XIIth plan as major items. The matter may be taken up and sorted at the SMD level whether the provision made for the same in the DSR could be resorted.
- IMC took note of proceedings and action taken report of the issue and strongly recommended pursuance of early implementations.
- The proposal for purchase of NanoDrop, HPLC Automation and other minor equipments was considered and IMC suggested for the purchase for the equipments costing less than 5 lakhs under minor equipments under equipment head of the budget.

General

- The Action Taken Report on the recommendations of the 6th IMC was presented and approved.

- The chairman and members appreciated that all the points have been taken up and Bureau is concentrating research work, HRD and infrastructure development.
- An overview of the research achievements of the ongoing institute projects was given. The Committee expressed its satisfaction on the progress of ongoing work in the bureau.
- Campus maintenance in the light of proper drainage system, cleaning of the channel, improvement of the water supply pipelines and sump well, boring of a new tube-well for drinking water supply was appreciated and suggested for undertaking maintenance by revenue.
- IMC recommend that the facility of Post-Office should be created. It also suggested consult Postmaster General, Lucknow to convert post box to sub-office.
- IMC felt that in 12th plan post of more scientists may be given to Bureau looking in the magnitude of the mandates of Bureau. IMC has also taken a serious note on crises of technical staff at the bureau and proposed to send a request letter to the council to redeploy the technical and scientific staff to the Bureau.

NBAIM Personnel

Scientist

Dilip K. Arora
Director

Alok K. Srivastava
Senior Scientist

Sudheer Kumar
Senior Scientist

D. P. Singh
Senior Scientist

Mahesh Yandigeri
Senior Scientist

Renu
Senior Scientist

Anurag Chaurasia
Scientist

Kamlesh Kumar Meena
Scientist

Udai Bhan Singh
Scientist

P. L. Kashyap
Scientist

Lalan Sharma
Scientist

Sanjay Goswami
Scientist

Dipak T. Nagrale
Scientist

Staff

Samar Nath Yadav
Shyamji Shukla
Manish Kumar Jain
Ashok Kumar
Sudesh Kumar
Satish Pal
Pratap Singh
Manish Roy
Mahesh Yadav
Rajkumar Meena
Pillu Meena
Anchal Kumar Srivastava
Alok Upadhyay
Amit Rai
S. S. Reddy
Ashutosh Rai
Amar Nath Singh Patel
Manoj Kumar
Bali Ram
Chetan Singh
Rekha Gupta
Ram Gopal
Ram Avadh Singh
Chandra Kishore
Anil Kumar Rana
Asheesh Kumar
Ajay Vishwakarma

कार्यकारी सारांश

हमारे भौगोलिक पर्यावरण की खुशहाली में महत्वपूर्ण भूमिका का निर्वहन करने वाले सूक्ष्मजीव भू-तन्त्र के लिए महत्वपूर्ण होते हैं। सूक्ष्मजीवों का प्रत्यक्ष महत्व होने के बावजूद, उनकी विविधता और पर्यावरण में उनकी सक्रियता का हमें बहुत कम ज्ञान है। सूक्ष्मजीव पृथ्वी पर सबसे महत्वपूर्ण जीव प्रारूप हैं और बहुत से क्षेत्रों में उनका कोई विकल्प नहीं है। पर्यावरण बदलाव में, जैव-भू-रासायनिक चक्रों में, पादप अन्योन्यक्रियाओं और फसल सुधारों में, खाद्य श्रृंखला, मृदा उर्वरता प्रबन्धन, कृषि अपशिष्टों की चक्रीय व्यवस्थाओं, और जैविक पौध रोग प्रबंधन उपचार में, पर्यावरण प्रसंस्करण, और विकसित जीवों ;पौधौद्ध के साथ होने वाले संबंधों की भूमिका में वे मूक कार्यकर्ता की तरह अपने कर्तव्यों को बिना किसी प्रतिफल के दिन-रात निभाते रहते हैं।

कृषि उपयोगी सूक्ष्मजीवों को खोजने, पहचानने, संरक्षित और अनुरक्षित रखने और कृषि की खुशहाली के लिए उन्हें उपयोग में लाने के लिए राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो (एन0बी0ए0आई0एम0) अपने कार्य अधिपत्र के अनुरूप कार्य प्रतिपादित कर रहा है। ब्यूरो का उद्देश्य पर्यावरण और कृषि में उच्च आर्थिक गुणवत्ता वाले परम्परागत और आकस्मिक उत्पादों के जीन्स को ढूँढ़ निकालने और उनके उपयोग में अपनी अपनी कार्यकुशलता को विकसित करना है। भारतीय कृषि को स्थानीय एंव भौगोलिक रूप से प्रतिस्पर्धारक बनाने के लिए राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो संकल्पित है।

ब्यूरो में सुसज्जित शोध प्रयोगशालाएँ, केन्द्रीय उपकरण प्रयोगशाला, पृथक कवक और जीवाणु प्रयोगशालाएँ, आण्विक जीव वैज्ञानिक प्रयोगशाला, अत्याधुनिक उपकरणों से सुसज्जित जीनोमिक्स प्रयोगशाला, लाइफोलाइजेशन यूनिट, सायनोबैक्टीरिया यूनिट सहित सूक्ष्मवर्धन संग्रह केन्द्र, नव-स्थापित एम0जी0आर0आर0 प्रयोगशाला, डी0एन0ए0 अनुप्रतिमुद्रण प्रयोगशाला, बायोइन्फार्मेटिक्स प्रयोगशाला, प्रशासनिक खण्ड, वैज्ञानिक कक्ष, पुस्तकालय, अत्याधुनिक दृश्य-श्रृव्य उपकरण सुविधाओं से युक्त छोटे-बड़े सभागार सम्मेलन, कृषि अनुसंधान सूचना सेवा प्रको ठ (एरिस सेल) और पी0एम0ई0 सेल आदि सुव्यवस्थित तरीके से कार्यरत है। वर्ष 2010-11 के प्रमुख बिन्दु निम्न है।

- 10,000 कृषि उपयोगी सूक्ष्मजीवों की सभंरण क्षमता वाले ?राष्ट्रीय कृषि उपयोगी सूक्ष्म-वर्धन संग्रह केन्द्र' (एन0ए0आई0एम0सी0सी0) की स्थापना एक एतिहासिक उपलब्धि है। एन0ए0आई0एम0सी0सी0 संग्रहालय के पास उपलब्ध ??कैटलाग आफ स्ट्रेन्स-2011'' का द्वितीय नवीनतम संस्करण प्रकाशित हो चुका है और पूर्ण रूप से गुण-चिह्नित एवं प्रलेखित 4,000 सूक्ष्मजीवो का विशाल संग्रह उपलब्ध है। हर अवाप्ति (असेसन) के साथ वर्धन से संबंधित सूचनाएँ यथा- उनके पृथक्करण का स्रोत, परिवेश, विकास की दशाएँ, निवेशक का नाम और निवेश का वर्ष भी दिया गये है। सूची में विभिन्न सूक्ष्म-जीवाणुओं के लिए उपयुक्त पोष पदार्थ के संयोजन, वर्धन निक्षेपण का प्रारूप, दीर्घावधि संग्रहण के नियम, सूक्ष्मजैविक वर्धन पंजीकरण के लिए सूचनाएँ, आई0पी0आर0 मामले, सूक्ष्म संग्रहण के लिए डब्लू0एफ0सी0सी0 के बारे में जानकारी, भारत और विश्व के विभिन्न सूख्म-संग्रह केन्द्रों की सूची और उनके पास उपलब्ध सूक्ष्म जीवियों की सूचनाएँ दी गयी हैं।
- ब्यूरो के पास उपलब्ध सभ्पूर्ण सूक्ष्मजैविक डाटा को प्रलेखित किया जा चुका है और संशोधनात्मक प्रारूप में रखा गया है। कृषि उपयोगी सूक्ष्मजीवों के प्रलेखन के लिए ?एम0सी0सी0डी' साफ्टवेयर को विकसित किया गया है। यह साफ्टवेयर माई एसक्यूएल प्रणाली पर आधारित है। इसमें विविध प्रकार के डाटा जैसे वर्धनों के पासपोर्ट डाटा सूचनाएँ, उनकी भौगोलिक अवस्थिति, निवेशक का नाम, संस्था का नाम, वर्धन विवरण, अनुरक्षण का प्रारूप आदि एकसाथ रखे जा सकते हैं। इसमें चित्रों के लिए स्थान, लाइफोलाइजेशन के लिए वर्धन सूचीै, बार कोड्सै एंव वर्धनों के पुनर्जीवन के लिए संचेतक आदि हैं।
- ?अमास' (एप्लीकेशन आफ माइक्रोआर्गनिजम्स इन एग्रीकल्चर एण्ड एलाइड सैक्टर्स) टीम की अगुआई में भारत में पहली बार मेसोराइजोबियम साइसेरी ;ब्रं81द्ध की आनुवंशिक संरचना के पूरे जीन अनुक्रम का पता लगाने में सफलता प्राप्त की गयी है। जीन विज्ञान के गहन अनुसंधानिक क्षेत्र की यह एक एतिहासिक सफलता है जिसमें जीन अनुक्रम के एकत्रीकरण और उनकी व्याख्या की अद्यतन तकनीकों को शामिल किया गया है। मेसोराइजोबियम साइसेरी ;ब्रं81द्ध के जीनोम का अनुमानित आकार 6ण्47 उइच है। और इसके 4116 प्रतिरूपों में 6742 जीन होते हैं। इनमें से कई समरूप (34 जीन) जैविक नत्रजन स्थिरीकारक और (184 जीन) प्रभाव सह्य होते हैं। यह खोज जैव उर्वरक अनुप्रयोगों के लिए राइजोबियम प्रजाति के जीन को विकसित करने की दिशा में तेजी के कार्य करने के अवसर प्रदान करती है जिससे परिष्कृत और उन्नत आनुवंशिक राइजोबियम प्रजातियों को तैयार करके दालों की पैदावार और उपज को बढ़ाया जा सकता है।
- एक्टीनोजीवाणु संबंधी वनस्पतियों के सूक्ष्मजीवों को अलगाने उन्हें गुण-चिह्नित करने तथा उनकी मैपिंग करने के लिए भारत के छः कछार वनस्पति-पारस्थितिकी क्षेत्रों यथा- सुन्दरवन (पश्चिम बंगाल), भीतरकणिका नेशलन पार्क (उड़ीसा), गुजरात के कछार, कोरिगा कछार (आन्ध्र प्रदेश), पुलीकट कछार (तमिलनाडु) और गोआ के कछारों का सर्वेक्षण किया गया। विभिन्न सम्वर्धन तकनीकों और मीडिया का प्रयोग करके 167 एक्टीनोमाईसीट्स संरचनाओं को विलगित गया है। ये सूक्ष्म-वर्धन आकृतिमूलक, सूक्ष्मजीवीय-रासायनिक और क्रियात्मक विशेषताओं से संबंधित थे।
- सूक्ष्मजीवों के जैव-भूगोल, सामुदायिक संगठन एवं पर्यावरण प्रक्रिया, जिसमें उनके विकसित होने के लिए स्थान विशेष का

निर्माण होता है, को समझने के लिए सूक्ष्मजैविक आवादी का मूल्यांकन विभिन्न पारिस्थितिक प्रकोस्ठो में किया गया है। ये विशेष प्रकोस्ठ सूक्ष्मजीवों को आश्रय देते हैं और आदिकालीन होने के कारण सम्भवतः आखिरी शरणस्थली के रूप में होते हैं ऐसे जीवों की जैव तकनीकी सम्भावनाएँ अभी तक अनुछुई रह गयी हैं। इस वर्ष भारत के विभिन्न समुदायों और प्रजातियों के 223 अन्त्यपारिस्थितिक सूक्ष्मजीवों को उनके परिवेशों से पृथक किया गया जिनमें क्रमशः गोआ कछारों से 55 - 40 होलोफिलिक फंजाई और बैक्टीरिया के सूक्ष्मजीव, टिपोंग कोल माइन्स से 50 एसिडोफिलिक सूक्ष्मजीव, सिक्किम गर्म-जल प्रपातों से 53 थर्मोफिलिक सूक्ष्मजीव और लेह प्रदेशों से 25 साइक्रोफिलिक एक्टीनोमाईसीट्स सूक्ष्मजीव शामिल हैं। वे तरह-तरह की आकारिक विविधता और क्रियात्मक विविधता जैसे पी0जी0पी0 विशेषताएँ (फास्फेट विलायक, साइड्रोफोर, अमीलेस, सेल्युलेस और लाइपेज एन्जाइम उत्पादक) आदि को प्रदर्शित करते है।

- इन सूक्ष्मजीवों में 3.0 -5.0 पी0एच0 पर 20 प्रतिशत विकास दर वाले 53 सूक्ष्मजीवों को संग्रह केन्द्र में संरक्षित किया गया है। ये परिणाम पुनः संकेत देते हैं कि वर्धनयोग्य सूक्ष्मजीवों का पृथक्करण सार्थक रूप से इन एक्सट्रीमोफिलिक क्षेत्रों की स्थानीय प्रजातियो की जानकारी प्रदान कर सकता है।
- गुजरात, पश्चिम बंगाल, तमिलनाडु, आन्ध्र प्रदेश, गोआ और उड़ीसा के कछारों से पृथक किये गये एक्टीनोमाईसीट्स सूक्ष्मजीवों की सबसे अधिक आवादी आन्ध्र प्रदेश के कछारों से मिली एक्टीनोमाईसीट्स की मारफोटाइप आधारित संरचनात्मक विविधता तमिलनाडु, गुजरात, गोआ और उड़ीसा के कछारों की तुलता में पश्चिम बंगाल और आन्ध्र प्रदेश कछारी पर्यावरण तंत्र में अधिक देखी गयी। पृथक किये गये एक्टीनोमाईसीट्स पादप वृद्धि वर्धक जैसी बहुत-सी विशेषताओं से युक्त थे। इन विशेषताओं को फसल उत्पादन और पादप रोगों के विरूद्ध जैव नियंत्रक गतिविधियों के लिए उपयोग में लाया जा सकता है। इसके अतिरिक्त एक्टीनोमाईसीट्स को कछारों के अनुसार संरचनात्मक और विविधता के अनुरूप मानचित्रित किया जायेगा।
- ब्यूरो ने भारत में (प्राचीन) सूक्ष्मजैविक समुदाय की संरचनाओं और विविधताओं पर काम शुरू किया है। ठण्डे रेगिस्तानों, गर्म-जल प्रपातों, लवणीय और क्षारीय दशाओं के (आर्किया) पर अभी तक बहुत कम काम हुआ है। यह परियोजना आर्कियल समुदायों और विभिन्न पर्यावरणीय क्षेत्रों, विशेष रूप से प्रचुर खनिज अयस्कों और भारी धातु संदूषित औद्योगिक जल प्रवाहों से उनकी जैव विविधता की पहचान करने में सहायक होगी। उनका गुण-चिह्नांकन परियोजना से उत्पन्न आर्कियल जैव विविधता और समुदायों के बारे में बहुत उपयोगी सूचनाएँ देगा जो ब्यूरो द्वारा चलाये जा रहे मौजूदा सूक्ष्मजैविक विविधता अध्ययन को आगे बढ़ाएगा
- ब्यूरो के अन्तर्गत आने वाली मानव संसाधन विकास इकाई के माध्यम से इस वर्ष मैटाजीनोमिक्स और बायो-इन्फार्मेटिक्स के क्षेत्र में 4 प्रशिक्षण कार्यक्रम आयोजित किये गये। राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो (एन0बी0ए0आई0एम0) की एक वेवसाइट ूण्दइंपउण्वतहण्पद बनाई गई है। वेव सूचनाओं की एकरूपता के लिए आइसीएआर दिशा निर्देशों के अनुरूप इसे डिजाइन किया गया है। एन0बी0ए0आई0एम0 की सभी इकाईयॉ भारतीय कृषि अनुसंधान परिषद (आई0सी0ए0आर0) के विभिन्न अनुसंधान संस्थानों से जुड़ी हुई है।