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Indian Council of Agricultural Research

NBAIM
रा.कृ.उ.सू.ब्यू.

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

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Preface

The microbial world is the largest unexplored reservoir of biodiversity on the Earth. It is a frontier area in biology under intensive investigations. Microbes are essential for life since they perform numerous functions essential for the biosphere that include nutrient cycling and environmental detoxification hence the interest in the exploration of microbial diversity is gaining importance. The vast array of microbial activities and their importance to the biosphere and to human economies provide strong rationale for understanding their diversity, conservation and exploitation for society. Microorganism biodiversity is a crucial component of the functioning of the world's agro-ecosystems, and vital for the maintenance of their capacity to adapt to change. The number of species involved is almost incalculable, in the range of millions. In collecting and describing them, priority needs to be give to those of greater agricultural importance. In any case, ex situ conservation is only technically feasible and cost-effective technique for microorganisms. The key to conserve and managing the biodiversity of micro-organisms of importance to food and agriculture is therefore to design integrated sustainable management strategies that conserve this resource for the future and enhance the delivery of ecosystems services, such as soil health, pollination and biological control, which contribute to the livelihoods of farmers and rural communities. In the process of evolution, several valuable strains of microbes have been evolved and developed in diverse ecogeographical regions.

Bequeathed with remarkable inherent physiological and functional diversity, microbes have found application in agriculture, industry, medicine and environment. Much better known and exploited microbial activities are augmentation, supplementation and recycling of plant nutrients, so vital to sustainable agriculture. However, the realization of that potential will come after a great deal of research into these extraordinary microorganisms. The rapid development of microbial biotechnology encompasses important and far-reaching implications.

During the 20th century, microbes became model systems for genetic and biochemical research and as the 21st century begins, microbial genomics is becoming a major focus for research in the biological sciences that are vital for development of new technologies for industry, agriculture, and human health. Because of the high cost of genome sequencing and limited public resources, it is essential to identify and prioritize microbes that are of agricultural/industrial importance or which have the prospects for environment and human health management.

I am thankful to our esteemed Director General, ICAR and Secretary DARE, Dr. S. Ayyappan for guidance and support. The consistent cooperation received in the farm of encouragement, suggestions and constructive criticism from Deputy Director General (CS) Dr. Swapan K. Datta and Assistant Director General (PP) Dr. T.P. Rajendran is gratefully acknowledged. I am also thankful to the colleagues at NBAIm involved in compilation, editing and formulation of documents.

June 28, 2013

Arun Kumar Sharma
Director

Executive Summary

Sustainable agriculture involves the successful management of agricultural resources to satisfy changing human needs while maintaining or enhancing the environmental quality and conserving natural resources. The continuous decline in soil organic matter levels due to continuous cropping without recycling enough crop or animal residues, and insufficient application of nutrients has led to serious nutrient imbalances, impaired soil health and declining factor productivity. Thus there is an urgent need to recycle all available organics in a more efficient way and improve and expand biofertilizer usage. Microorganisms are utilized in agriculture for various purposes; as important components of organic amendments and composts, as legume inoculants for biological nitrogen fixation as a means of suppressing insects and plant diseases to improve crop quality and yields, and for reduction of labor. All of these are closely related to each other. An important consideration in the application of beneficial microorganisms to soils is the enhancement of their synergistic effects. This is difficult to accomplish if these microorganisms are applied to achieve symptomatic therapy, as in the case of chemical fertilizers and pesticides. The plant rhizosphere is a versatile and dynamic ecological environment of intense microbes–plant interactions for harnessing essential micro-and macro-nutrients from a limited nutrient pool.

The uniqueness of microorganisms and their often unpredictable nature and biosynthetic capabilities, given a specific set of environmental and cultural conditions, has made them likely candidates for solving particularly difficult problems in the life. The various ways in which microorganisms have been used over the past 50 years to advance medical technology, human and animal health, food processing, food safety and quality, genetic engineering, environmental protection, agricultural biotechnology, and more effective treatment of agricultural and municipal wastes provide a most impressive record of achievement.

The application of beneficial microorganisms to soil can help to define the structure and establishment of natural ecosystems. The greater the diversity of the cultivated plants that are grown and the more chemically complex the biomass, the greater the diversity of the soil microflora as to their types, numbers and activities. However, Microbial diversity is the largest untapped resource for both understanding how biological systems function as well as for new biotechnologies . It is essential to enhance the activities of microbes that benefit plant nutrition, control diseases and assist plants to cope with a variety of abiotic stresses to sustain and improve global food production in future climate scenarios while maintaining environmental health. A diverse range of beneficial microorganisms have been found but their reliable use in field environments is yet to be fully realised. New knowledge on soil microbial diversity can lead to the discovery of new generation inoculants as well as improve survival and performance of beneficial microbes in situ following their introduction into foreign environments.

Microbiology has long relied on diverse methods for analysis but with the development of technology like genetic engineering, many mutants are developed which are capable of performing extra with respect to production quality and quantity as compared to their wild types. Recently, metagenomics provide the tools to balance the abundance of knowledge attained from culturing with an understanding of the uncultured majority of microbial life.

National Bureau of Important Microorganisms (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

cyanobacteria 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 equipment's and Agricultural Research Information Service (ARIS) cell etc. Looking at the prospects of the most modern research trends including microbial ecology, genomics, bioprospecting, gene-mining and bio product development, the Bureau has taken a lead in research and development in these areas, and has acquired genome sequencing units, DNA fingerprinting unit, Shotgun Cloning Lab, Sequencing Laboratory and Genoinformatics Centre, Confocal and SEM microscopy, HPLC and GC units and a separate unit for computerized and digital documentation. A Local Area Network and Website of NBAIM as per ICAR Guidelines for Uniformity of Websites of ICAR Institutes have been created, and all the units of the NBAIM are linked with various ICAR institutes and research organizations all over the country. The National Agriculturally Important Microbial Culture Collection (NAIMCC) has developed state-of-the art short-term conservation of AIMs based on culture and mineral oil techniques. Using these techniques, AIMs can be conserved for 5-10 years. The NAIMCC has high capacity lyophilizers for long term preservation of AIMs (20-25 years) under vacuum at–60°C. A software 'Microbial culture Collection Database' developed by the bureau in XIth plan lists out characteristics of AIMs in terms of origin, ecology, morphology, physiology and biochemical parameters, pathogenic/nonpathogenic nature, detailed available information about specific properties and molecular tools used for the characterization of AIMs. Cyanobacteria and actinomycetes units were strengthened in the culture collection in the plan period. A dedicated growth chamber was established for cyanobacteria. The NAIMCC has reached to the about 4500 microbial culture holdings, for safeguard of these cultures from any unforeseen menace situation; a safety storage facility has been created to NBPGR, New Delhi.

NBAIM is now upgrading the facilities to meet the current and future requirement for the conservation and characterization of AIMs in the country. At present the Bureau is actively involved in characterization of microbes, not only from the point of view of protecting the important gene-pool resource, but also for supporting integrated pest and disease management programs, abiotic stress management, management of agro-residues, bioremediation of contaminated and degraded soils and bioprospecting for novel genes, alleles and metabolic pathways.

. Director, NBAIM

कार्यकारी सारांश

Infrastructure

The Bureau was established in IXth plan period by Indian Council of Agricultural Research at New Delhi for exploration, evaluation and conservation of agriculturally important microorganisms (AIMs) and then moved to National Institute of Sugarcane and Sugarcane Technology (NISST) at Mau, Uttar Pradesh in June, 2004. 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. It is expected that NBAIM continues to fulfil its mandate to make Indian agriculture locally, regionally and globally competitive.

The Bureau 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. The campus as well as laboratories has been put under the electronic surveillance system.

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.

National Agricultural Important Microbial Culture Collection (NAIMCC)

Bureau conserves and characterizes the microbial accessions of agriculturally important microorganism as a mandated activity under its unit NAIMCC. The cultures are maintained in mineral, glycerol storage, and lyophilization and now it has all the conservation facility including cryogenic storage under ultra low temperature (-196°C). NAIMCC is well equipped will microbial imaging systems like light microscopy, scanning electron microscopy and laser confocal microscopy. The NAIMCC has state of art facility for isolation, conservation, maintenance and storage facility for nearly 100000 microbial holdings.

Safety duplicates storage facility at NBPGR:

The NAIMCC has reached to the about 4500 microbial culture holdings, for safeguard of these cultures from any unforeseen menace situation; a safety storage facility has been created to NBPGR, New Delhi. Some of the important and foremost required equipment for storage of microbial holdings has been transferred to the storage facility. A set of lyophilized cultures and glycerol stock has been kept as safety duplicate of the collection. The NBAIM Culture Storage Facility inaugurated by the Hon'ble Director General, Dr. S. Ayyappan, at NBPGR, New Delhi on 1st Jan, 2013 in presence of Dr. T.P. Rajendran, Assistant Director General (PP), Dr. J.S. Sandhu, Assistant Director General (Seeds) and Dr. K.C. Bansal, Director, NBPGR, New Delhi, Arum Kumar Sharma, Director, NBAIM, Dr. Alok K. Srivastava, Dr. Sudheer Kumar along with others scientist from NBPGR and IARI, New Delhi on 1st January, 2013.

NAIMCC recognized as the National Repository for microbial germplasm by Ministary of environment and forestry. It 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 bureau follows strict quality control and biosafety standards in the culture collection as well as in laboratories. Various types of microorganism 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. At present NAIMCC has a collection of more than 4500 microbial cultures. It has published its first catalogue of strains in 2009 and second edition in 2011. 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.

NAIMCC have been digitized and put-up all the information about cultures in a database in retrievable format. 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 format is organized to permit rapid searches and to facilitate communication between database and user.

Research facilities

The bureau has different 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.

Library and Agricultural Research Information System (ARIS) CELL

The NBAIM library has subscribed many periodicals related to microbial research and also has 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 has facility to access many national and international journals through Consortium of e-Resources in Agriculture (CERA).

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

The new website of NBAIM (www.nbaim.org.in) designed based on the ICAR guidelines for uniformity of website, contents 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.

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 utilize 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)

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 Kumar Srivastava
Co PIs : Sudheer Kumar and Prem Lal Kashyap

Rationale:

Plants have developed mechanisms to successfully co-exist in the presence of pathogenic organisms. However, because of lack in the resistance in plants to most of the pathogens, often the diseases limit the productivity. In most of the cases fungicides are used to control the diseases but looking into the environment safety, other ecofiriendly measures are also being explored for disease management. Hypersensitive responses during the interactions between plants and pathogens form the basis of systemic resistance. The concept of Induced Systemic Resistance (ISR) are based on recognition of specific elicitor molecules from avirulent pathogen races (*avr* gene products), which is described in the gene-for-gene resistance theory. Another type of resistance is multigenic (horizontal) resistance, which is a less well-studied phenomenon that depends upon multiple genes in the plant host. All plants possess resistance mechanisms which can be induced upon pre-treatment of plants with a variety of organisms or compounds. This general phenomenon is known as induced systemic resistance (ISR). In induced resistance processes, more than one biochemical pathway appears to be activated, based on the requirement of different signal transduction pathways depending on salicylic acid (SA) or jasmonic acid and ethylene. At least in some plant species, ISR depends on the timely accumulation of multiple gene products, such as hydrolytic enzymes, peroxidases or other gene products related to plant defences. The pre-treatment of plants with an inducing organism or compound appears to incite the plant to mount an effective defense response upon subsequent encounters with pathogens, converting what would have been a compatible interaction to an incompatible one. In some of the cases the onset of induced resistance in cucumber was found to be accompanied by the accumulation of several phenolic compounds with antifungal activity. In continuation with the approved work plan following objectives were undertaken.

Objectives:

- 16S rDNA, rpoB, asd and glnD gene sequencing of rhizobacteria
- Induction through rhizobacteria and histochemical staining of phenolic compounds
- Extraction of glycoside-linked phenolics and Bioassay and analysis of glycoside-linked phenolic compounds

Significant Achievements:

- The genomic DNA of the selected rhizobacterial isolates were amplified for 16S rDNA, rpoB, asd and glnD Gene using repective primers.
- Restriction analysis of the 16S-rRNA gene of the bacteria used for the identification and characterization was performed and phylogenetic analysis was done for 15 isolates. 16S rRNA gene sequencing of 53 bacteria was done. The same was identified through BLAST search. The rpoB gene has been amplified, sequenced and identified as *Bacillus megateranium*, *Pseudomonas* sp., *Burkholderia* sp., *Bacillus pumilus*, *Serratia marcescens*, *Enterobactercloacea*, *Pseudomonas* sp., *Enterobacter* sp. *Pseudomonas nitroreducens*, *Enterobactercloacea*, *Bacillus* sp., Acinetobacterra

dioresistens, *Agrobacterium tumefaciens*, *Burkholderia cepacia*, *Burkholderia cenocepacia*, *Burkholderia* sp., *Enterobacter cloacae*, *Pseudomonas putida*, *Enterobacter* sp., *Pseudomonas denitrificans*, *Pseudomonas fluorescens*, *Burkholderia* sp., *Bacillus pumilus*, *Agrobacterium tumefaciens*, *Rhizobium* sp.

- The level of phenolic compounds in cucumber leaves inoculated with rhizobacteria and cross inoculated with *R. solani* was studied. One and three days after inoculation with rhizobacteria the formation of phenolic compounds was significantly higher in plants treated compared with that in DW-treated plants.
- The conjugated phenolics extracted from the leaves of different aged cucumber plants inoculated with the rhizobacteria and challenge inoculated with *R. solani* were tested for their antifungal activity *in vitro* against fungal pathogens i.e. *M. phaseolina, F. solani* and *R. solani*. The activity was shown by the size of halo zones observed on plates. The size of antifungal spots tended to increase with time following treatment with DW with or without the challenge inoculation of the pathogen. According to these results, the accumulation of antifungal compounds appears to have a relationship with the age of cucumber leaves.

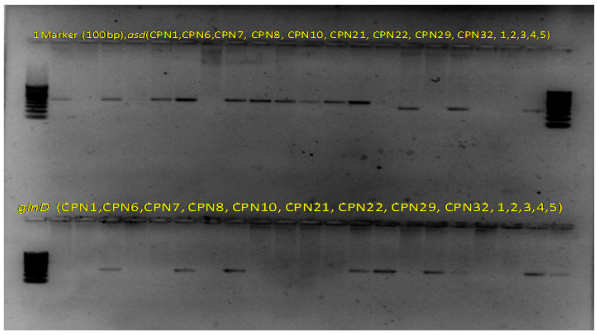


Fig 1: Amplification of asd and glnD from the genomic DNA of selected rhizobacteria

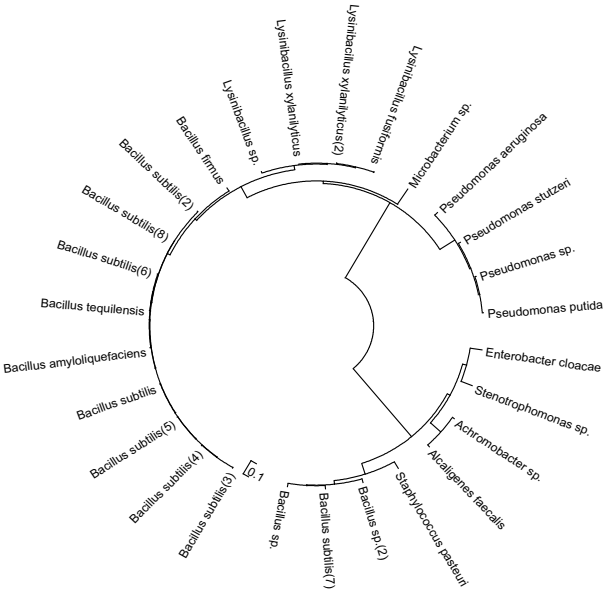


Fig 2: The phylogentic tree of the selected rhizobacteria based on 16s rRNA gene

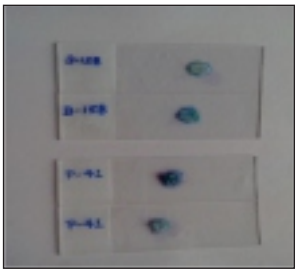


Fig 3: Inoculation of cucumber plants with rhizobacteria challenge inoculated with *R. solani* and histochemical staining of leaves.

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

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

Rational

The management of plant disease especially soil borne disease are very difficult. The host resistance and pesticides are being used for the management of these diseases. However, resistance sources for soil borne disease are very rare, and if available, that express low level of resistance. Indiscriminate use of pesticides is causing environmental and human health hazards. Therefore, an environmentally friendly approach to protecting plants from fungal pathogens through rhizobacterium-mediated biological control is in practice. There are number of reports on management of plant disease through application of rhizobacteria including species from the genera *Pseudomonas*, *Bacillus, Azospirillum*, *Klebsiella*, *Enterobacter*, *Alcaligens*, *Arthobacter*, and *Burkholderia*. As biocontrol agents, isolates of *Pseudomonas fluorescens* and *Bacillus* have been the most studied and exploited. However, the performance to successful management of soil borne disease through biological control agents at field level is limited.

The success of any biocontrol agent to manage the disease under field condition depends on its antagonistic potential along with its ability to colonize the rhizosphere, competition with other resident microflora and edaphic factors. Other then these, the performance of antagonistic bacteria may also be attributable to various crops rhizosphere that influence growth of antagonistic bacteria and as a result, affect their ability to exert a beneficial effect on the plant. To be an effective antagonistic strain, bacteria must be rhizospherically competent, and capable of surviving and colonizing the rhizospheric soil.

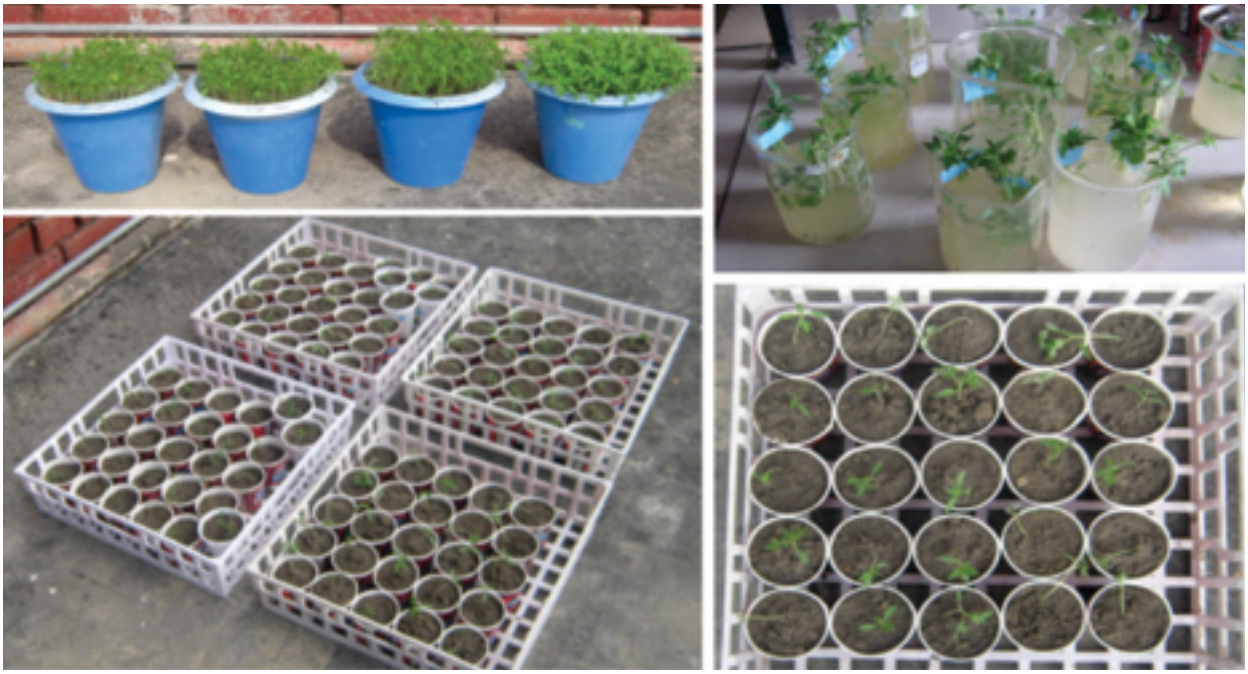
The relationship between antagonistic bacteria and plants is very feeble and can be unstable under adverse conditions. To achieve optimal growth conditions for promoting interaction between antagonistic bacteria and crop plants, it is important to determine how rhizobacteria exert their effects on plants and whether these effects are influenced by various factors like there rhizospheric competence, ability to produce various antibiotics, and other environmental factors, including the presence of other microorganisms. 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. Therefore, to develop efficient biocontrol strains that can be effective under field conditions. One possible approach is to explore soil microbial diversity for selection of promising antagonistic bacteria with plant growth promoting activities as such bacteria are assumed to be well adapted to the soil environment from which they are isolated. The present investigation is undertaken to understand the host preference in 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

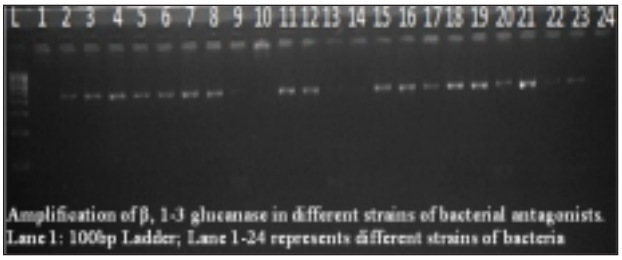
Achievements

- The promising bacterial antagonistic isolates preliminary screened under laboratory against different soil borne pathogens of vegetable crop and further under green house condition against the Rhizoctonia root rot of tomato were selected for further study its root colonization response to different vegetable crops as well as for the presence of different antibiotic genes.
- These isolates were characterized on the molecular level for variability, genetic relatedness and identified on the basis of 16S rDNA sequencing.
- These isolates were evaluated for their comparative root colonization in different vegetable crops (tomato, chilli, bringal and cucumber)
- Different isolates showed the differential preference to the host rhizosphere like isolate PM1 has more root colonization potential over other tested isolates and showed more preference toward solanaceous crops and less preference to cucumber.

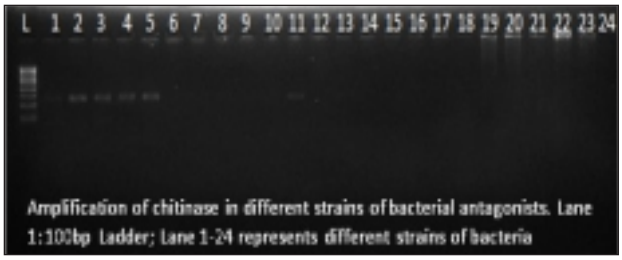


Detection of antibiotic related genes in bacterial antagonists:

- These bacterial antagonists were evaluated for the presence of different antibiotic gene important in suppression of plant pathogens. The PCR condition has been optimized through gradient PCR for the amplification of chitinase, β, 1-3 glucanase, bacillomycine, and phenazine. A total 24 bacterial isolates were screened for the presence of these genes.
- Amplification of chitinase and β, 1-3 glucanase: Among twenty four strains, 6 and18 isolates were found positive for chitinase, β, 1-3 glucanase, respectively.

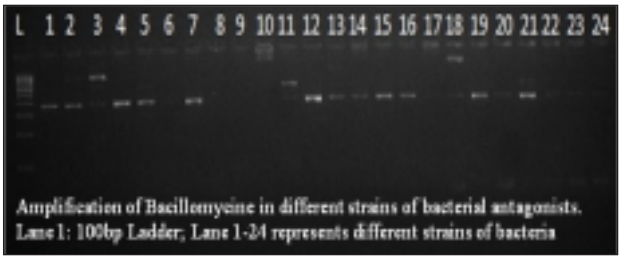


Amplification of β, 1-3 glucanase in different strains of bacterial antagonists. Lane 1: 100bp Ladder; Lane 1-24 represents different strains of bacteria

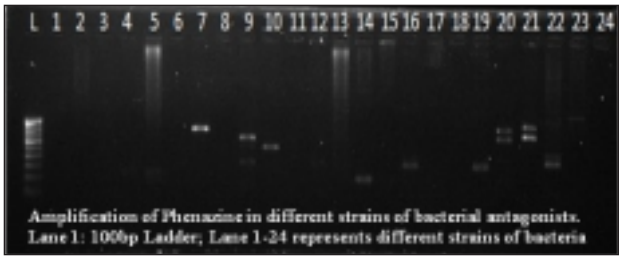


Amplification of chitinase in different strains of bacterial antagonists. Lane 1:100bp Ladder; Lane 1-24 represents different strains of bacteria

- Amplification of bacillomycine, and phenazine: similarly 19 and 11 isolates were found positive for bacillomycine and phenazine, respectively



Amplification of Bacillomycine in different strains of bacterial antagonists. Lane 1: 100bp Ladder; Lane 1-24 represents different strains of bacteria



Amplification of Phenazine in different strains of bacterial antagonists. Lane 1: 100bp Ladder; Lane 1-24 represents different strains of bacteria

Project 3 : Isolation, characterization and conservation of bacteriophages associated with some important phytopathogenic bacteria and their evaluation for use in agriculture

PI : Dr. Renu
Co-PI : Dr. Dipak T. Nagrale

Rationale:

Bacterial pathogens that infect important agricultural plants (phytopathogens) can reduce plant growth and the subsequent crop yield. Currently, phytopathogens are controlled through management programmes, which can include the application of antibiotics and copper sprays. However, the emergence of resistant bacteria and the desire to reduce usage of toxic products that accumulate in the environment mean there is a need to develop alternative control agents. An attractive option is the use of specific bacteriophages (phages), viruses that specifically kill bacteria, specific to the target bacteria, non toxic to workers and non targeted bacteria, providing a more targeted approach. There is resurgence in the use of bacteriophages for controlling plant diseases exhibiting great potential. *Xanthomonas campestris* pv. *campestris* infects a large numbers of cruciferous plants including cole crops, oilseeds and flowers and causes black rot disease which significantly reduce the yield of crops all over the world. Black rot disease management is generally by proper cultural practices, usage of chemical control methods and disease free planting material and usage of resistant varieties. However, due to emergence of copper resistsnce bacterial strains and environmental issues alternative control measures which are safer, more specific and environment-friendly of pathogen controlling agents are required. Bacteriophages provide highly specific control opportunities for bacterial diseases by specifically infecting and destroying the disease-causing bacteria.

The aim of the proposed research project is to collect, characterize and evaluate phages of phtyopathogenic bacteria, *Xanthomonas campestris pv. campestris*(Xcc) a causal organism of black rot of crucifers for their possible role in disease management programme.

Objectives:

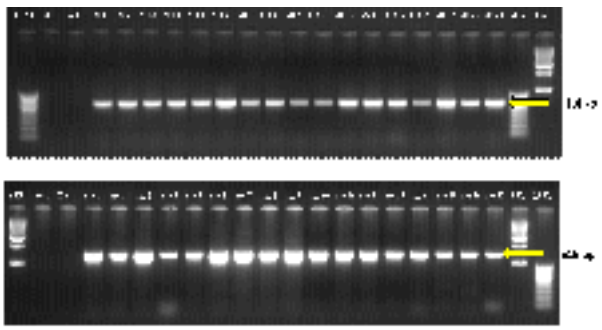
- Isolation and characterization of pathogenic bacteria of important crops
- Collection and isolation of bacteriophage from bacterial infected fields.
- Characterization of bacteriophages.
- Screening for evaluation of selected phages for disease control potentiality.

Salient achievement:

- During year 2012-13 more surveys and collection of black rot diseased samples was done.Six more isolates of Xcc from Lucknow, Himanchal Pradesh, Nauni, Solan, Mau and Jaunpur were collected from black rot infected cauliflower and raddish plants.
- In total isolation of bacterial isolates from seventeen black rot diseased samples collected from different cruciferous hosts and geographical regions of India was done.
- Presumptive Xcc isolates were tested for Xcc-determinative characteristics, including viscosity of bacterial suspension, Gram reaction, nitrate reduction, Kovacs' oxidase reaction, starch hydrolysis, catalase reaction and hypersensitive reaction in pepper plants.
- The *in planta* pathogenicity test and host range of *Xcc* strains was carried out by the leaf-clipping inoculation method on susceptible cabbage, Cauliflower (*Brassica oleracea* var. *botrytis*) and mustard.
- *Hrp* gene based diagnostic PCR with primer pair DLH 120 forward 5'-CCGTAGCACTTAGTGCAATG-3' and DLH 125 reverse: 5'-GCATTTCCATCGGTCACGATTG-3' and DLH 109 (5'-ATGTCGCTCAACACGCTTTC-3') and DLH 112 (5'-GTTTTGCGTGTAGCCCTTTGC-3') was carried out (Berg et al., 2005).
- All isolates were Gram-negative and showed typical *X. campestris* features, such as oxidative utilization of glucose, starch hydrolysis, viscosity and hypersensitive reaction on pepper.
- Host range studies showed that isolates Xcc 7 and Xcc 11 produced symptoms in all three hosts tested.

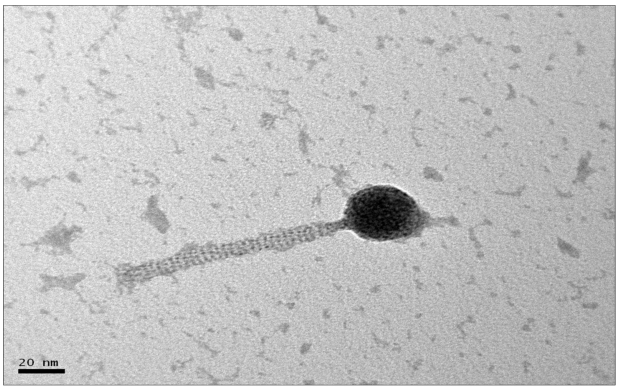
Symptoms of Xcc 7 isolate on Mustard and Caulliflower

S. No.	Isolate	Host		
		Cauliflower Kataki 1	Cabbage GoldenAcre	Mustard Pusa bold
1.	XCC1	+	-	_
2.	XCC2	+	-	_
3.	XCC3	_	+	_
4.	XCC4	+	_	_
5.	XCC5	+	_	-
6.	XCC6	+	_	_
7.	XCC7	+	+	+
8.	XCC8	+	+	_
9.	XCC9	+	+	-
10.	XCC10	+	-	_
11.	XCC11	+	+	+
12.	XCC14	+	_	_
13	XCC17	+	+	_
14	XCC18	-	-	+
15	XCC19	-	--	+
16	XCC21	+	-	-
17	XCC22	+	+	-



- Selective primers DLH 120 forward and DLH 125 reverse amplified 3' end of hrpF gene with predicted PCR product size 619 bp ; and DLH 109 and DLH 112 yielded an expected, single fragment of 1.4kb amplifying the 5' repeat sequence within hrpF gene, for all isolates. In contrast, this PCR product was not obtained for DNA of the *X. oryzae* pv. *oryzae* isolate and *Xathomonas axanopodis* isolate from rice and citrus respectively

- Six Xcc strains coolected and characterized under the period reported were submitted to NAIMCC at NBAIM (Accession no. NBAIMCCB-01358-63).
- Refinement in the technique of soft agar overlay method for isolation of phage from infected plant tissues and soil by enrichment with the indicator bacterial hosts was done. Soil samples from diseased fields and black rot diseased plant samples were collected for Xcc specific phage isolation.
- A total of 7 putative phage solutions were prepared from the soil and plant samples from black rot infected fields. Samples were enriched with overnight cultures of host bacterial strain (cell density, 10^8 CFU ml^{-1}) and incubated for 48 h. The presence of phages was assayed by the soft agar overlay method, as described by Adams (1959). Single plaques were picked, and three to four successive reselection steps were performed for each plaque type isolated on the appropriate indicator lawn.
- The host range of each phage isolate was tested against 12 *Xcc* strains using a plaque assay.
- A virulent phage Xc9SH3 was found to lyse all tested strains of Xcc. The plaque type produced by this phage is lytic, clear and transparent. Transmission electron microscopic observations of phage Xc9SH3 are presented in figure. The phage exhibited long and noncontractile tail and isometric head belonging to Siphoviridae (dsDNA viruses) family of bacteriophages. Based on multiple electron photomicrographs, the average size range of the phage was 100 nm in length and 20 nm in width.



Electron micrograph (300 000X magnification) showing the physicalc structure of a single bacteriophage particle of Xc9SH3 Black bar , 20 nm.

Conclusion

The present study focuses on collection of black rot diseased samples from different hosts plants like cauliflower, cabbage and rai and from different locations in India; isolation of *Xanthomonas campestris pv campestris* (Xcc) and their characterization by polyphasic approaches via morphologically, biochemically, carbon source utilization pattern as well as at molecular level in addition to *in planta* pathogenicity test and host range. Pathogenecity tests with all isolates on their respective susceptible hosts were able to produce typical V-shaped lesions. Host range studies revealed isolated Xcc 7 and Xcc 11 to be able to infect all cruciferous hosts tested. *Hrp* gene based diagnostic PCR with primer pair DLH120F & DLH125R and DLH109 & DLH112 was clearly able to differentiate and diagnose Xcc strains. For isolation of Xcc specific bacteriophages the isolated strains will serve as host source. Protocol for isolation of bacteriophage by enrichment method has been standardized. Isolation of 7 putative phage solutions revealed a virulent phage Xc9SH3 which was found to lyse all tested strains of Xcc. TEM studies revealed the morphology of Xc9SH3 phage. Further isolation of lytic phages of Xcc and their characterization will provide insight whether they can be asset for controlling growth of host bacteria.

Project 4 : Project: Exploration, preservation and evaluation of endophytic actinomycetes from Indo-Gangetic plain

PI : Anurag Chaurasia
Co-PI : Dhananjaya Pratap Singh

Rational

Endophytes are the microorganisms that dwell within robust plant tissue by having a symbiotic association. They are ubiquitously associated with almost all plants studied till date. Hallmann & coworkers defined any microorganisms 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. Endophytic population is greatly affected by the climatic conditions and location where the host plant grows. They produce a wide range of compounds useful to plants for growth, protection to environmental conditions and sustainability, in favour of a good dwelling place within the hosts. 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. A large amount of bioactive compounds produced by them are not only useful for plants but are also of economical importance to humans. 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. 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 with PGPR and biocontrol potential from various crops grown in this region which will be further used for enhancing agricultural productivity and human welfare.

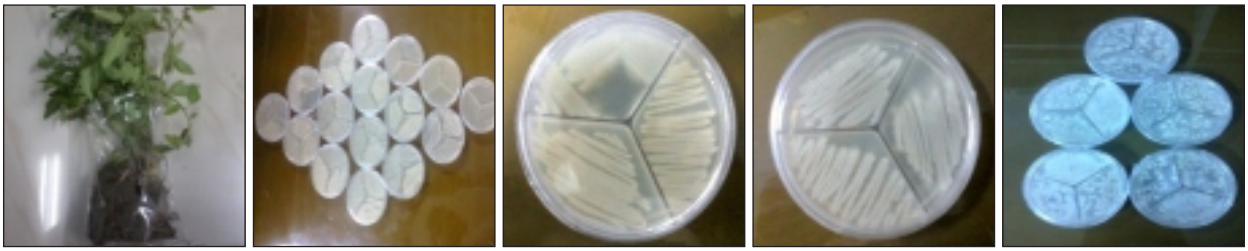
Objectives

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

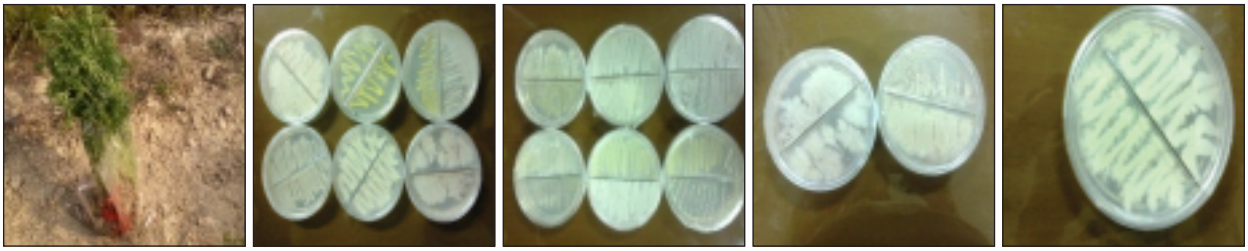
Significant Achievements

Endophytic actinomycetes were isolated from the root, stem and leaves of the healthy Tomato (***Lycopersicon esculentum*****),** Potato (*Solanum tuberosum)*, Lentil (*Lens culinaris)*, and Mustard (*Brassica campestris*) plants using standardized surface sterilization protocols and sterility check as control. Mercuric chloride with varying concentration (0.2% - root, 0.1% - leaves, 1.0% - stems, for 15 minutes) was used for surface sterilization of various plant parts. Actinomycetes isolation agar & starch casein agar media with nystatin as antifungal agent were used for

Endophytic actinomycetes isolates from *Brassica campestris* root



Endophytic actinomycetes isolates from ***Lycopersicon esculentum*** **root.**



Endophytic actinomycetes isolates from *Lens culinaris* **root**

Endophytic actinomycetes isolates from *Solanum tuberosum* root

the endophytes isolation experiment. Endophytic actinomycetes diversity was more in root compared with leaf & stem of the plants. Potato tubers were also reported to have the endophytic actinomycetes. Disease infected plant parts were used to isolate disease causative fungal isolates using PDA media. Endophytic actinomycetes were evaluated for their biocontrol potential against disease causative fungus using dual plate technique. Isolates were also evaluated for various physiological (temperature, salt) and biochemical (Ammonia, HCN, IAA, Siderophore, Phosphate Solubilization) parameters. Few isolates showed promising biocontrol & PGPR traits. We conclude that certain plants remain healthy in disease infected agricultural farms because of the presence of these specific endophytic actinomycetes. Isolates were preserved in the slant & 20% glycerol. Genomic DNA was isolated using conventional heat shock & enzymatic methods.

Project 5 : Metagenomic approaches for exploring the biodiversity of antibiotic producing agriculturally important microorganisms (AIMs)

PI : Udai B. Singh
Co-PI : Dhananjaya P. Singh

Rationales:

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. 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. 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. 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. 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. 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.

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:

- During the year 2012- 13, rhizospheric and non-rhizospheric soil samples were collected from Rice- wheat growing areas of Ballia. Physico-chemical properties of these soil samples were tested using HiMedia soil analysis kit and with the help of M.C. Manna, Division of Soil Biology, Indian Institute of Soil Science, Bhopal, M.P.
- A total of 51 different morphotypes belonging to bacteria and actinomycetes were isolated using different media. Further characterization of antibiotic producing AIMs were done using duel plate technique for preliminary screening. A number of actinomycetes are found to be good secondary metabolite producer which are of antimicrobial in nature. All the isolates were screened for the antimicrobial potential against potent fungal and bacterial phytopathogens.
- A total of 51 actinobacteria exhibiting distinct colony characteristic were isolated, purified and subjected to further molecular characterization. PCR amplification followed by restriction analysis

of 16S rDNA gene clustered the isolates into 12 groups (Fig. 1a and 1b).

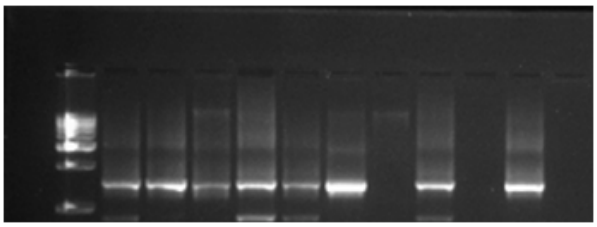


Fig. 1a. Specific amplification of 16S rDNA fragment (1.5kb) of actinobacteria isolated from wheat rhizosphere

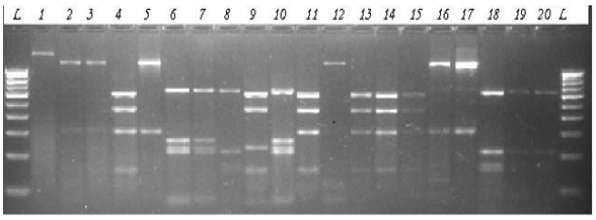


Fig. 1b. Taq 1 digestion of 16S rDNA PCR product of aerobic actinobacteria isolated from wheat rhizosphere

- A total of 5 isolates were chosen as representative based on PCR amplification of 16S rDNA and RFLP clustering using three different restriction endonucleases i.e. Taq 1, Hae III alu 1. Further phylogenetic analysis of 5 representatives was carried out for species level identification. Blast search revealed that these strains were closely matched with *Pseudomonas fluorescens.* A neighbor joining tree was constructed to verify the result (Fig. 2).

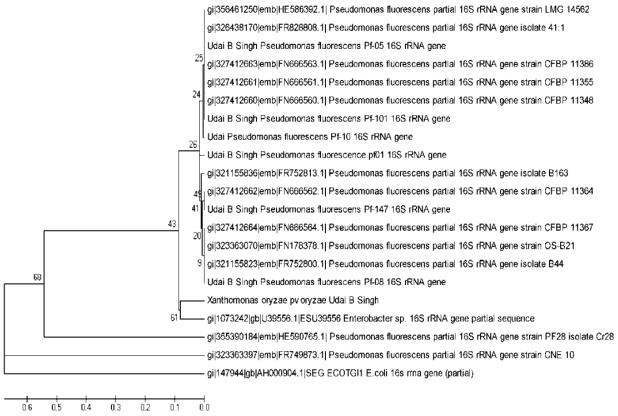


Fig. 2. Phylogenetic relationship of the potent *Pseudomonas fluorescens* strains with the known species of *Psedomonas* based on nucleotide sequence of 16S rDNA.

- 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. Metagenomic DNA was isolated and amplify 2,4-DAPG, Type I PKS and Type II PKS gene. Construction of metagenomic DNA library and Cloning of these gene fragments into suitable vector and result revealed that there is no such gene(s) encoding the antibiotic present.
- Application of *Pseudomonas fluorescens* strain Pf-08 and Pf-10 as seed treatment increase germination percentage and vigour in comparison to control.

Project 6 : Deciphering microbe-mediated mechanisms of Induced Systemic Resistance (ISR) and plant growth promotion in rice and tomato

PI : Udai B. Singh
Co-PI : Dr Renu, Dr. Dhananjaya P. Singh

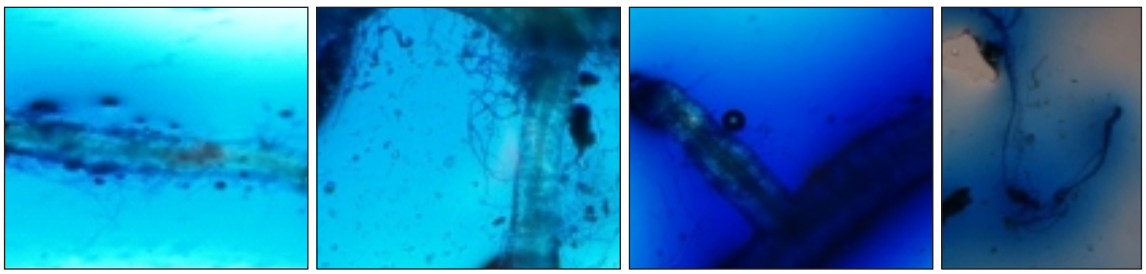
Rationale:

Objectives

- Screening and characterization of existing and new strains against important phytopathogens of rice and tomato.
- Development of eco-and farmer friendly bioformulation of selected biocontrol agents
- Deciphering mechanisms of induced systemic resistance and plant growth under pathogenic stress conditions.
- Development of suitable delivery system for judicious application in agriculture.

Significant achievements:

- Selected isolates of *Pseudomonas fluorescens* and *Trichoderma harzianum* and *T. viride* against *Rhizoctonia solani* were tested *in vitro* and selected most potential isolates for further utilization.
- Mycorrhizal plants are often more resistant to diseases, such as those caused by microbial soil-borne pathogens. AM fungi and their associated interactions with plants reduce the damage caused by plant pathogens. With the increasing cost of pesticides and the environmental and public health hazards associated with pesticides and pathogens resistant to chemical pesticides, AM fungi may provide a more suitable and environmentally acceptable alternative for sustainable agriculture and forestry.
- At present in our laboratory an attempt is being made to exploit and enhance native AMF activities leading to improved mineral requisition and induced systemic resistance to crop plants i.e. tomato and rice through manipulating on-farm produced (following farmer's friendly protocols) native AMF inoculum and readly available strains (*Glomus mosseae* and *Glomus fasciculatum*).
- Two strains of arbuscular mycorrhizal fungi *Glomus mosseae and G. fasciculatum* obtained from Dr DJ Bhagyaraj and mass multiplied in three crop species i.e. barley, oat and sorghum for further utilization and characterization on large scale.
- Better colonization on oat roots in comparison to barley and sorghum in green house condition. Root colonization of *Glomus mosseae* and *G. fasciculatum* in the roots of barley, oat and sorghum were examined and found that *Glomus mosseae* and *G. fasciculatum* prefer barley root for better colonization (Fig. 1).



. 1. Barley root colonization by Arbuscular Mycorrhizal Fungus *Glomous mosseae*

Project 7 : Exploration of pathogenecity gene(s) of *Magnaporthe grisea* in hot spot regions of India

PI : Dr. Prem L. Kashyap
Co-PI : Dr. Sudheer Kumar

Rationale:

Blast disease caused by *Magnaporthe grisea* is the most important fungal disease of rice. The fungus causes up to 15% annual yield loss, which is enough rice to feed about 60 million people. *M. grisea* is a polycyclic pathogen capable of several disease cycles in congenial weather within a single crop growing season, which may explain why the pathogen is so destructive to rice. Deployment of disease resistant varieties is the most practical and economical way of rice blast management. However, none of the existing rice cultivars possesses durable blast resistance because of the highly variable nature of the pathogen in various parts of India. Therefore, understanding the molecular basis of virulence of *M. grisea* enables the development of better strategies for the control of fungal crop disease. Extensive studies have been performed to identify and characterize the genes that participate in these developmental changes and pathogenicity in *M. grisea*. Several pathogenicity genes that include *ABC1*, (encodes for a member of the ATP binding cassette superfamily), *PDE* (encodes a P-type ATPase), *PLS1* (encodes a tetraspanin-like protein), *CYP1* (cyclophillin gene), *MST12* (a homologue of the yeast gene *STE12*) have been identified. A significant body of evidences showed that several signal transduction pathways including cAMP pathways regulate appressorium formation, penetration and invasive growth processes in *M. grisea*. Hydrophobin, secreted by the germ tube preconditions the adhesion of the germ tube tip, or hook, to the plant surface, and the corresponding gene *MPG1* has been shown to be necessary for development of the mature appressorium. In spite of these efforts, several other researchers also documented various pathogenecity gene (s) viz. *TPS1*, *CHM1*, *EMP1*, *MST7*, *MgPEX6*, *RAC1*, *COS1* and *MgATG5* in the blast fungus that is highly expressed during infectious growth and appears to be required for appressorium formation and full symptom development. 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 generated from this research project will help in proper identification of novel target sites to restrict the menace of the disease.

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:

- Thirteen different primer sets for genes associated with *M. grisea* development and colonization were designed using Primer 3 software. Primer annealing temperatures were evaluated by gradient-PCR using *M. grisea* 17 genomic DNA as template. Primer annealing temperatures were evaluated by gradient-PCR. All the primer pairs designed for the pathogen virulence gene amplified a single product with the expected size ranged from 200 to 440 bp (Fig 1).

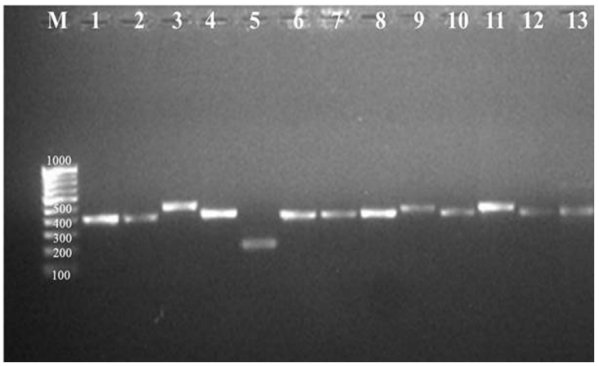


Fig 1: PCR based amplification of thirteen different virulence genes in *M. grisea* 17. 1-13 well represents the amplification of *CHM1*, *TRE1*, *MAC1*, *MPLC1*, *Osm1*, *ICL1*, *ABC1*, *NTH1*, *PDE1*, *CYP1*, *CAM*, *THNRF* and *ALS1*, respectively

- To understand the molecular mechanism of rice blast disease, an experiment was conducted under glass house to test the hypothesis that magnitude and time of expression of genes associated with fungus development and colonization. Real time PCR and plant bioassay tests were used to monitor

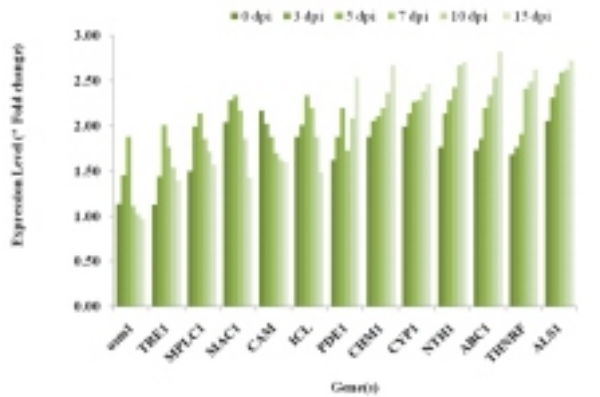


Fig 2: Expression pattern of virulence genes of *M. grisea* 17 inoculation at different days post inoculation in susceptible genotype (Karuna).* Fold change indicate the expression level difference between the inoculated plants at different days and control plants harvesting (0 dpi.), after normalization with β-tubulin.

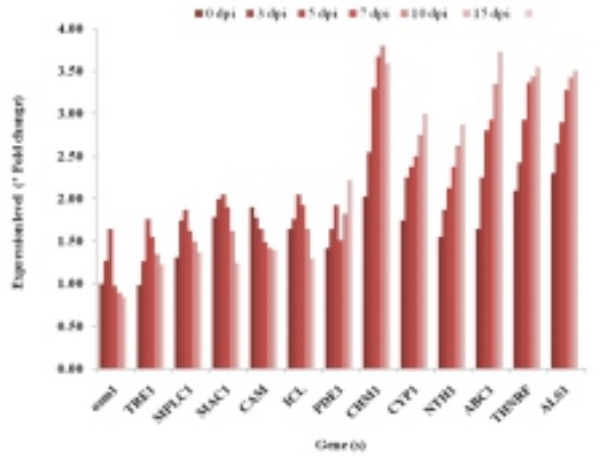


Fig 3: Expression pattern of virulence genes of *M. grisea* 17 inoculation at different days post inoculation in resistant genotype (Zenith).* Fold change indicate the expression level difference between the inoculated plants at different days and control plants harvesting (0 dpi.), after normalization with β-tubulin.

the expression of genes at different time points (0, 3, 5, 7, 10 and 15 days post inoculation) after pathogen challenged inoculation in susceptible (Karuna) and resistant (Zenith) genotypes. The data on the disease incidence and severity was recorded at regular intervals. In order to choose the best housekeeping genes, geNorm analysis was used to compare three different reference genes *viz.* β- tubulin, GAPDH and actin. β- tubulin was found most stable gene and hence used as a internal reference control. Data analysis revealed that all the thirteen genes were expressed at higher magnitude in susceptible genotype (Fig 2) relative to resistance genotype (Fig 3). The level of expression of *PDE1*, *CYP1*, *CAM*, *THNRF* and *ALS1* gene was several folds higher than *Osm1*, *TRE1*, *MPLC1*, *MAC1*, *ICL1*, *ABC and NTH1* genes, respectively at 15 dpi. The appearance of the disease symptoms was positively correlated with the expression of the virulence genes.

Conclusion:

The present investigation revealed that thirteen primer sets (*CHM1*, *MPLC1*, *LpMOD*, *ABC1*, *CAMGEN*, *CALMOD*, *OSM1*, *TRE1*, *UEP1*, *MPS*1, *MAC1*, *TPAGEN* and MAGNA) encoding virulence genes were associated with fungus development and colonization that determine the rice blast disease spectrum. Real time PCR and plant bioassay tests conducted under artificial epiphytotic conditions demonstrated that the magnitude and level of expression of these pathogenecity genes determine the disease development in resistant and susceptible genotypes. In nutshell, these findings clearly revealed that there might be a great possibility of regulation of rice blast disease spectrum by these genes under natural conditions.

Project 8 : Isolation and characterization of bacterial communities and their metabolites in rhizospheric rice ecosystem.

PI : Dr Lalan Sharma
Co-PI : Dr Dipak T. Nagrale

Rationale:

Rhizosphere is considered the soil volume surrounding the root-tissue. It is well established that microbial life only occupies a minor volume of soil being localized in hot spots such as the rhizosphere soil (Nannipieri *et al.,* 2003), 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 (Sorensen, 1997; & Brimecombe *et al.,* 2001). Both beneficial and detrimental interactions occur between microorganisms of rhizosphere soil and plants. Root exudation is generally confined to apical root zones. However, root architecture, and thus exudation can change depending on the nutritional status of plants. 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. Therefore, research is to be proposed that there are some metabolic interaction in rhizosphere that may influence plat growth and productivity.

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

Salient Achievement:

- As project aims, rice fields were surveyed and collected the rhizospheric soil samples from different regions (Gorakhpur, Lucknow, Kanpur, Varanasi, Meerut and Mau) of Indogangetic plains U.P. 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, Burk,s medium, NFB medium, Malate Medium and YEMA medium). A total of 143 rhizobacterial isolates have been isolated from rhizospheric soil samples that were visually characterized for their different morphotypes. Rhizospheric soil is determined for their physiochemical properties as pH (ranges 7.0-8.4), EC-value (ranges 1.3-1.9) and organic carbon in soil (ranges medium to medium high). The bacterial morphotypes have been tested for the HCN production, Siderophore production and Phosphate solubilization. In another assay, these rhizobacteria were tested for HPLC analysis with standards phenolic compounds chromatogram (retention time, area and height). After results of HPLC analysis for the prominent secondary metabolites producing rhizobacteria, an experiment was planned in gnotobiotic conditions. In which rice seeds were surface sterilized with 2% chlorex solution and then treated prominent rhizobacterial isolates (prepared in 1% cellulose carboxy methylase (CMC) solution. Seed germination percentage, root length and shoot length was measured. Rice growth and development was influenced with rhizospheric bacterial isolates.

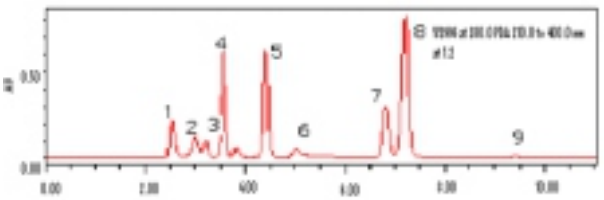


Fig. Chromatograph of phenolic compounds (retention time) prepared through Waters, HPLC (1- Gallic acid (2.53 minutes), 2- Rutin (2.99 minutes), 3- Gentisic acid (3.02 minutes), 4- Caffeic acid (3.22 minutes), 5- Chlorogenic acid (3.56 minutes), 6- Ferulic acid (4.32 minutes), and 7- Quercetin (4.98 minutes).

Rice seed vigour treated with rhizobacterial isolates after 5 days of experiment conducted

Project 9 : Diversity analysis of archaea from different ecological niches and their characterization

PI : Dr. Dipak T. Nagrale, Scientist
CO-PI : Dr. Renu, Senior Scientist

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. Hypersaline habitats are common throughout the world, but extremely hypersaline habitats are rare and having unique niche. Most such environments are in hot, dry areas of the world. Salt lakes, saltern ponds and hypersaline niche can vary considerably in ionic composition. The predominant ions in a hyper saline lake depend to a major extent on the surrounding topography, geology and general climatic conditions. Many hypersaline environments have originated by evaporation of sea water e.g. Great Salt Lake Sambar lake, India . Their salt composition is similar to that of sea water. However, sodium and chloride are the dominating ions, and the pH is near neutral to slightly alkaline. Due to the evaporation changes occur in the ionic composition as there is the precipitation of gypsum ($CaSO_4.2H_2O$) and other minerals due to their increased solubility. Various salts of chlorides saturated brines as like found in saltern crystallizer ponds oftenly displays a bright colouration due to the large numbers of pigmented microorganisms living in these niches as they releases extracellular enzymes and/or metabolites. These hypersaline niche like crystallizer ponds, salt lakes in which the concentration of divalent cations is more than that of monovalent cations with relatively low pH(6.0-6.5).and in which the pH is relatively low (around 6.0).These extremophile microorganisms has adapted to environments combine high salt concentrations with very high pH values. Alkaline salt lakes are known to found in Africa, India, China and other parts of the world with pH values more than11 and higher and salt. 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 approaches.
- Community analysis of archaea from different ecological niche.
- Development of molecular diagnostic tools of some agriculturally important archaea.

Significant achievement:

- Total 17 haloarchaea were isolated, out of which 11 were from Bhayander salt pan hypersaline sample and 6 from Meera road salt pan hypersaline sample. All the distinct pinpoint colonies obtained on haloarchaea agar showed different shades of

dark orange and blood red colours indicating the presence of haloarchaea. These isolates were characterized by morphological, biochemical and molecular characterization. The isolates B1(1), B1(2), B4(4), B4(7), B4(9), B5(2), B5(3), M1(1) and M3(1) were short rods whereas isolates B3(4), B3(5), B3(6), B(7), M2(1), M2(2), M4(1) and M4(2) were long rods in shape and size. 16S rRNA gene from all the haloarchaeal isolates was amplified by PCR method using archaea specific primer. The amplicon of size 1500 bp was obtained for all the isolates.

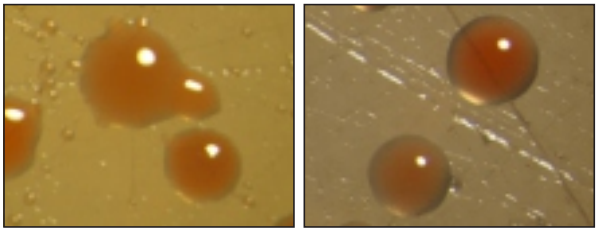


Fig 1. Haloarchaeal morphotypes on haloarchaea agar media (25% NaCl) at 37^{0}C isolated from hypersaline niche of Mumbai suburban area.

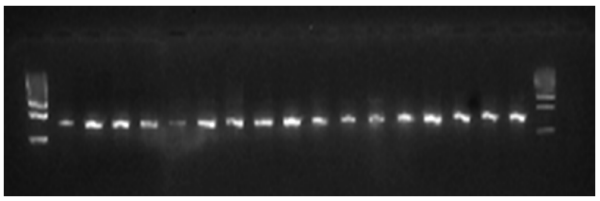


Fig 2. Amplification of 16S rRNA gene from all the 17 haloarchaeal isolates by PCR programme using archaea specific primers.

National Agricultural Innovation Project

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

Consortium Leader : Dr. A. K. Sharma
Consortium PI : Dr. Sudheer Kuamr
Coordinating Scientists : Dr. Alok K Srivastava

Rationale:

Salinity is one of the major abiotic stress factors that prevent plants from taking up nutrients, water and exposing them to drought stress. It has an adverse effect on plant growth and ultimately results in lesser production. It also affects the quality and nutritional value of agricultural production. Evolving efficient, low cost, easily adaptable methods for management of salinity stress is a major challenge. Most of the technologies for salinity stress management such as chemical remediation and application of synthetic fertilizers give negative effects on soil, environment and non-target microbes, whereas, development of salinity tolerant varieties, resource management practices, etc. are labour and cost-intensive.

Due to above mentioned negative effects of salt, saline environments have been largely ignored. Recent studies indicate that bacteria which flourish in these environments may retain the potential to express various types of activity under extreme conditions and can help crops to cope with saline stress. For the salt stress alleviation, use of plant growth-promoting bacteria from saline environments may be an economical and environmental friendly approach to ameliorate adverse effects of salt stress and sustainable crop production. *Bacillus* and related genera, have evolved highly sophisticated regulatory networks for protection against sudden unfavourable environmental changes, including nutrient starvation, high salinity, changes in temperature, humidity and oxidative stress. Common physiological traits important to their survival include production of a multilayered cell wall structure, formation of stress resistant endospores and secretion of peptide antibiotics, peptide signal molecules and can osmoregulate by synthesizing specific compatible organic osmolytes. *Bacillus* has been known for enhance biomass, nitrogen and phosphorous uptake and crop yield by producing phytohormone such as indole acetic acid, siderophores HCN ammonia and solubilizing phosphate. These compounds are natural and beneficial to promote plant growth by direct and indirect mechanisms. Identification of culturable microbial communities from saline soil and their characterization will not only strengthen database of salt loving of these group of microbes for basic studies, but their potential can also be utilized in agriculture by mitigate salt stress if they posses plant growth promoting attributes or have ability to produce salt tolerant important enzymes.

Objectives:

- Diversity analysis and identification of *Bacillus* and other predominant genera from extreme conditions of salinity.
- 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:

- The *Bacillus* and other predominant genera were isolated from extreme conditions of salinity and characterised through biochemical and molecular methods.

- Gas chromatographic analysis of whole cell fatty acid methyl ester (FAME) was performed for identification of unique fatty acid composition of 37 ARDRA *Bacillus* strains isolated from saline soil of eastern U. P.
- The different *Bacillus* species showed difference in their whole-cell fatty acids composition. Each species has a unique fatty acid profile, making it a "microbial fingerprint." The major fatty acids observed in all the *Bacillus* strains were C14:0, C14:0 iso, C15:0 iso, C15:0 anteiso, C16:0 iso, C16:0, C17:0 iso, C17:0 anteiso, C18:0, C18:0 iso, C16:1 w7c alcohol, C17:1 w11c and Sum in Feature 4 etc.

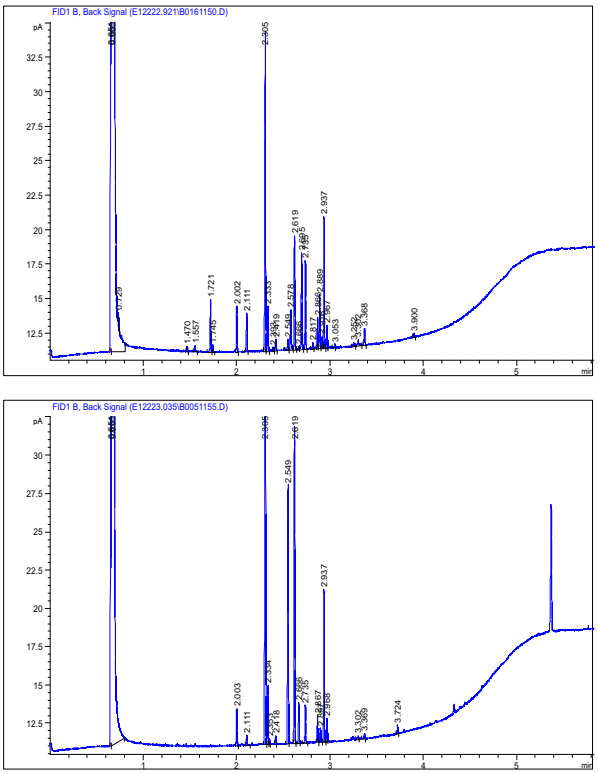


Fig. Chromatograph showing the peaks of the FAME analysis

- The output profile was organized into a chromatogram and a report generated by comparing against an inbuilt Sherlock TSBA Library version 6.0 (MIDI Inc., DE, USA) indicate that all the isolates were belongs to genus *Bacillus* which support the Molecular Identification analysis.
- Cluster analysis, based on the FAME profiles of representative bacilli, revealed 2 major groups at 75 euclideans: Cluster I included 26 isolates, whereas Cluster II included 11 strains.

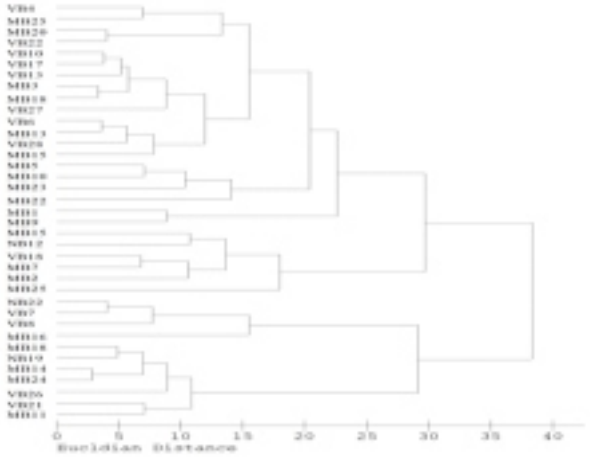


Fig. Dendrogram of *Bacillus* sp. based on whole-cell fatty acid composition by GC-FAME constructed using MIDI Sherlock analysis software, ver. 6.0.

- On the basis of 16s rDNA sequencing 72 predominant genera were identified as *B. licheniformis, B. niabensis, B. aryabhattai, B,. subtilis, B. thioparans, B. flexusm, B. marisflavi, B. endophyticus, B, cereus, B. Pumilus, B. thuriengiensis, Lycinibacillus xylanilyticus, Pseudomonas stutzeri, P. sp, Staphylococcus, Enterobacter cloacae, Micrococcus sp, Cellulosimicrobium funki, Ochrobacterium sp., Acinetobacter sp*. etc. 16S rRNA gene sequences of predominant genera were submitted to NCBI gene bank.
- The best five bacterial isolates possessing salt tolerance as well PGP activities were characterized based on the fatty acid methyl ester analysis (FAME) and 16S rRNA gene sequences as *B. Subtilis, B. subtilis, P. fluorescence, P. putida* and *Pseudomonas* sp.

S. No.	Strain	Nearest Match	Max. Identity	FAME identity	SI
1	BC39	*Bacillus subtilis* strain KISR-1 1	99%	*B. subtilis*	.805
2	RC13	*Pseudomonas putida strain IMBG 294*	99%	*P. putida*	.870
3	RC25	*Bacillus subtilis* strain CRB115	99%	*B. subtilis*	.750
4	KC30	*Pseudomonas fluorescence strain ost5*	99%	*P. fluorescence*	.690
5	KC31	*Pseudomonas* sp. DG1a	99%	*Pseudomonas* sp.	.757

- These cultures were evalulated individually as well as in different for growth promotion and salt stress elliviation under pot and field conditions.
- There was two best consortiums were observed

which significant enhance root length, shoot length, dry weight and total yield in both salt susceptible and tolerant cultivars of chickpea.

- Anti-oxidant activity viz., catalase, peroxidise, SOD and APX in chickpea plants were analysed. It was interesting to note that, inoculation of salt tolerant isolates gave significant reduction in the activity of antioxidant enzymes when compared to uninoculated chickpea plants.
- A second year field trial with the same bacterial consortium were further applied and different growth as well as biochemical parameters were taken for assessing best bacterial consortium. A significant growth promotion has been recorded on bacterial fortification of chickpea seeds over the control.

Fig: Effect of bacterial inoculants on growth and yield of chickpea cultivars at pot and field level

Allele mining and bioprospecting of gene for abiotic stress tolerance

CCPI : Alok K. Srivastava

Rationale:

In order to understand the genic variability between thermophilic and mesophilic fungi, frequency and distribution of microsatellites was studied in transcript sequences of two thermophilic (*M. thermophila and T. terrestris*) and two of its closest mesophilic neighbors (*C. globusum* and *N. crassa*). The genome sequences of all chromosomes of thermophilic fungi *M. thermophila* (NC_016472- 78) and *T. terrestris* (NC_16457-62) were downloaded from National Centre for Biotechnology Information (ncbi.nlm.nih. gov) whereas the annotated transcripts were downloaded from Department of Energy Joint genome Institute. The frequency of the repeat motifs in the genome as well as transcript sequences were identified using WebSat online software which is accessible through internet requiring no programme instillation. This programme searches both perfect and compound SSRs and also designs primers using its Primer 3 in-build operation. To compare the total SSR count between all four species more accurately, we have taken the total length of each set of sequences analyzed as a reference. Thus, total relative abundance (SSR/Mb) and total relative density (bp/Mb) were calculated

Overall Objectives of Sub-project

- 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 achievement:

Genome-wide distribution of SSRs

- For the analysis of SSRs in thermophilic fungi, the whole genome sequences of recently sequenced thermophilic fungi *M. thermophila* (38.7 Mb) and *T. terrestris* (36.9 Mb) were downloaded and analyzed for perfect and compound SSR over 12 base pair long. A total of 15943 SSRs were found in *M. thermophila* whereas, *T. terrestris* harbors 11868 SSRs. Chromosome one of both the organism contained maximum number of SSRs, which is followed by chromosome 2, whereas, Chromosome 6 harbors least number of SSRs in both the organisms (Table 1and 2).

Table 1: Number and distribution of SSRs in chromosomes of *Myceliophthora thermophila*

Chromosome No.	Size (Mb)	No. of SSRs	Perfect	Compound	Total length of SSR (bp)	Relative abundance	Relative density
Ch1	10.9	4965	4434	531	90698	455.5	8320.9
Ch2	5.4	2432	2151	281	45064	450	8345.1
Ch3	5.06	2034	1666	368	40832	401.9	8069.5
Ch4	4.7	1799	1539	260	33773	382.7	7185.7
Ch5	4.3	1564	1410	154	27415	363	6375.5
Ch6	4.1	1462	1335	127	24722	356.5	6029.7
Ch7	4.1	1687	1405	282	33320	411.4	8126.8
	38.5	15943	13940	2003	295824	403	7493

Table 2: Number and distribution of SSRs in the chromosomes of *Thielavia terrestris*

Chromosome No.	Size (Mb)	No. of SSRs	Perfect	Compound	Total length of SSR (bp)	Relative abundance	Relative density
Ch1	10.10	3212	2844	368	55761	318.01	5520.8
Ch2	9.4	3177	2847	330	56912	337.9	6054.4
Ch3	4.7	1358	1220	138	22796	288.9	4850.2
Ch4	4.5	1487	1222	265	27863	330.4	6191.7
Ch5	4.3	1324	1129	195	23791	307.9	5532.7
Ch6	3.5	1310	1075	235	25485	374.2	7281.4
	36.5	11868	10337	1531	212608	326	5905

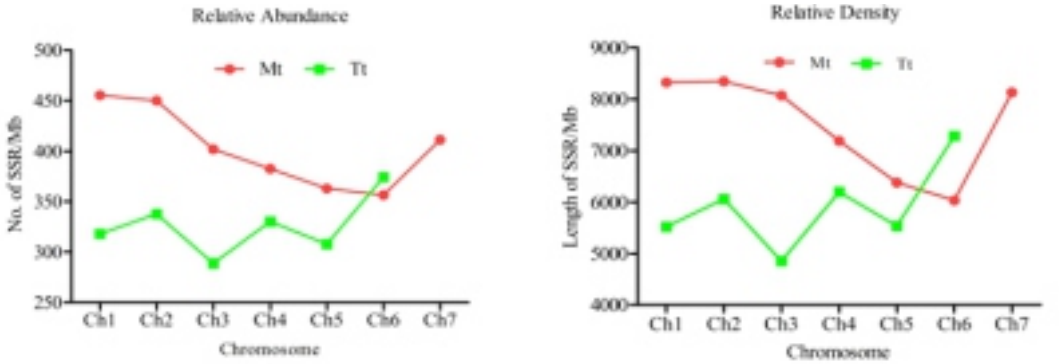


Figure 1: Relative abundance (RA) and relative densities (RD) of SSRs in the chromosomes of thermophilic fungi *Myceliophthora thermophila and Thielavia terrestris*

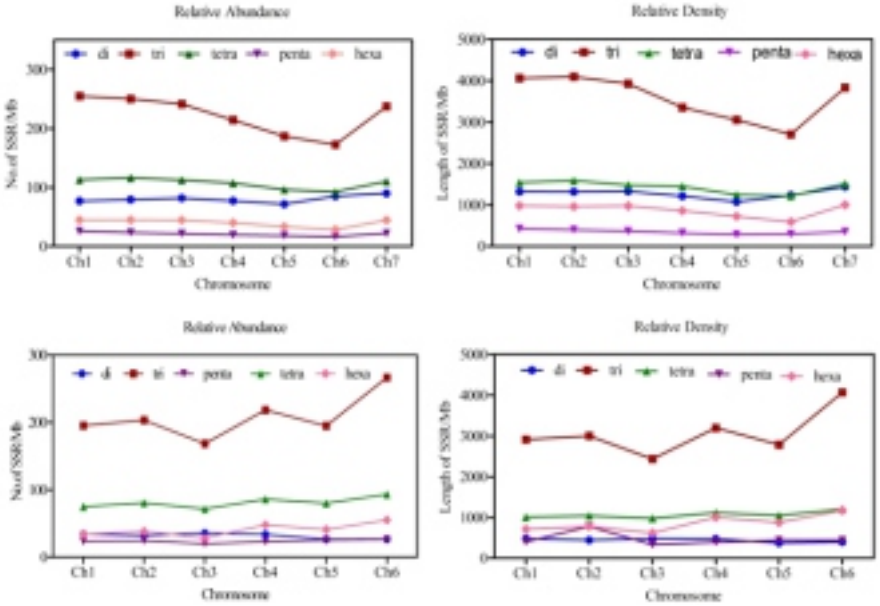


Figure 2: Comparison of relative abundance (RA) and relative densities (RD) of different SSR types present on different chromosome in the whole genome sequences of thermophilic fungi

Whole genome comparison of SSR: To compare the SSR abundance and density with mesophilic fungi, we surveyed SSRs in two closest neighbor of thermophilic counterpart. We observed that mesophilic fungi *Neurospora crass* harbor maximum number of SSR (18681) whereas *C. globusum* showed 9584 SSRs

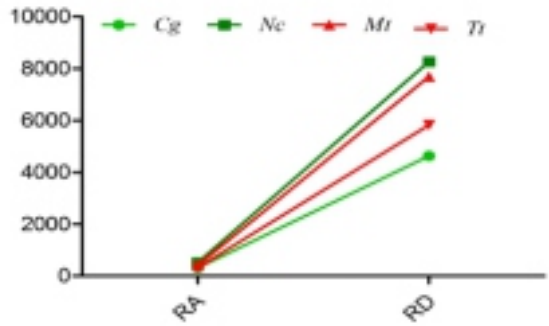


Figure 3: Comparison of relative abundance (RA) and relative densities (RD) in the whole genome sequences of mesophilic and thermophilic fungi

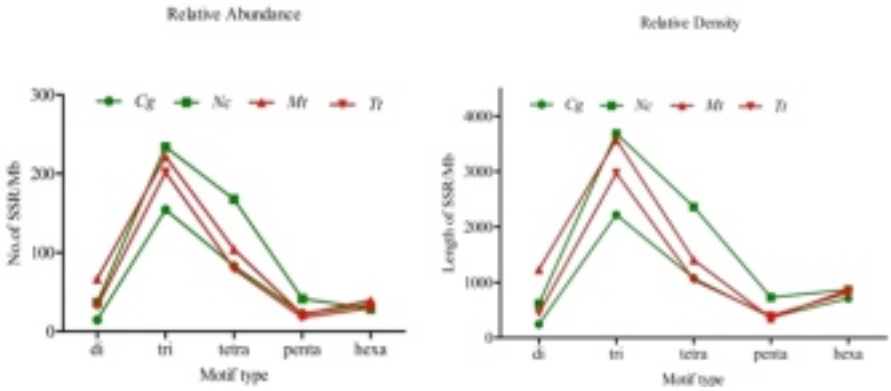


Figure 4: Comparison of relative abundance (RA) and relative densities (RD) of different SSR types present in the whole genome sequences of mesophilic and thermophilic fungi

SSR in transcript sequences

Maximum number of SSRs (6198) was identified in *M. thermophila* followed by *T. terrestris* (6073), *N. crassa* (5090) and *C. globusum* (4246). It was found that relative abundance of SSRs in *M. thermophila* (433.4) was maximum when compared to *T. terrestris* (368.1), *N. crassa* (341.6) and *C. globusum* (258.9).

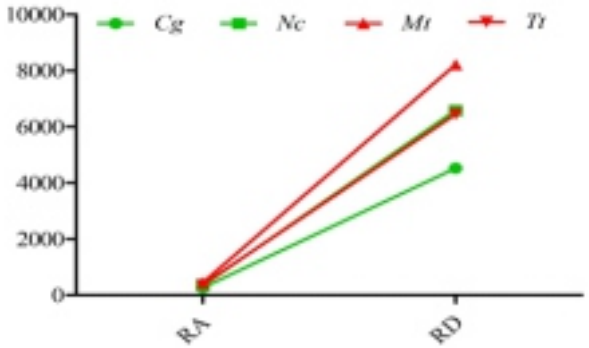


Figure 5: Comparison of relative abundance (RA) and relative densities (RD) in the transcript sequences of mesophilic and thermophilic fungi

All three sequence sets contained SSRs that were mainly trinucleotide repeats (75.6%), while the dinucleotide repeats represented less than 4.6%. Hexanucleotide repeats constituted the second most frequent motif (14.03%) which was followed by tetranucleotide (7.12%) and pentanucleotide (1.2%) repeat motifs. However, the percentage of di and pentanucleotide repeat was higher in *Fom*.

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

CCPI : Alok K. Srivastava

Rationale:

Soil microorganisms significantly contribute to the maintenance of the matter and energy turnover in terrestrial environment. Each soil has a characteristic pattern of enzymes because all biochemical actions are dependent on or related to their presence. Soil enzyme assays are process level indicators and are presented as a means of determining the potential of a soil to degrade or to transform substrates. Soil enzyme activities are influenced by management practices because they are also related to microbial biomass which is sensitive to different treatments.

Dehydrogenases are generally present in every upper layer of soils. Soil microflora is responsible for the decomposition and conversion of organic substances, aggregation stability and the carbon, nitrogen, sulphur and phosphorus cycles. Dehydrogenase as respiratory chain enzymes, play the major role in the energy production of organisms. They oxidize organic compounds by transferring two hydrogen atoms. Dehydrogenases are essential components of the enzyme systems of microorganisms. Dehydrogenase activity can therefore be used as an indicator of biological redox systems and as measure of microbial activity in soil. However, soil dehydrogenases come from activity of plants and soil animals, as well. Concentration of soil dehydrogenase depends on conditions and intensity of biological conversion of organic compounds. Addition of suitable chemical (triphenyltetrazolium chloride) enhances bioavability of endogenous soil organic compounds to microflora. At the same time chloride is converged by hydrolytic reaction to formazan which can be extracted by organic solvents (methanol, acetone).Addition of organic substrate, e.g. compost, induces maximum DHA.

Large proportions of the nitrogen in many soils are organically bound and the mineralization of these portions is of agricultural importance. Organic nitrogen compounds in soil can constitute huge amount percent of total nitrogen and the assimilation of this nitrogen by plants and microorganisms is preceded by soil enzymes. Several enzymes are involved in the decomposition of organic nitrogen compounds.

Urease enzyme influence the optimum use of urea fertilization, Nitrogen volatilization nitrogen leaching and environmental pollution related to N. Urease is ubiquitous cell free exoenzyme in natures that is produced by plants and microorganisms. It's is stablized by absorption on clay mineral. In contrast to phosphate and protease it does not sensitive to air drying. Urease activity is used in many cases as soil fertility. The quantification is based on the incubation of soil sample with urea. Either decrease in urea concentration, increase in ammonia production or production CO_2 production.

Objectives

Significant achievements:

Microbial Population assessment

- The rhizospheric soil of Bhagalpur and Nagpur has the highest and lowest distribution of Phosphate solubiizig microorganisms under Ekchari and Singhpura soil series.
- Cfu of *Azotobacter* was highest in Fatehpur soil series and minimum population was noticed in Nagpur, Singhpura soil series.
- In all soil horizons microbial population decreases with increase of depth.
- Regarding soil urease activity 3-4 times decrease was noticed in bottom most profiles with the compared to surface soil.
- Cfu/gram data was converted to $\log_{10}$cfu/g for knowing the uniformity of distribution of microbial population.
- Microbial density was intensified between and

bottom profiles in sem arid sub-ecological region where as under humid and sub-humid microbial density were maximum at surface soil and lowering against depth gradient.

- Microbial populations sharply influence the enzyme activity.
- Bacterial population was dominant across all BM followed by Actinomycetes and least were fungal population.
- Microbial distribution in sub-humid was maximum under both high and low management for surface soil whereas at deepest horizons under low management humid hub ecological and sub-humid region high management has lowest microbial density.
- **Soil dehydrogenase activity**
- Soil dehydrogenase activity which is indicator microbial metabolism and microbial viability has direct influence of increasing depth.
- Regarding soil dehydrogenase activity 2-2.5 times decrease was noticed in bottom most profiles with the compared to surface soil.
- For the surface soil under high management practice, soil dehydrogenase activities were highest in sub humid ecological regions.
- Soil urease activity has sharp declination against depth gradient.
- Regarding soil urease activity 2-2.6 times decrease was noticed in bottom most profiles with the compared to surface soil.
- At high management of surface soil urease activity was highest under humid ecological regions.
- For the low management of surface soil similarly humid region has highest urease activity.
- In bottom soil profiles under high management , it hard to interpret the urease activity since the very close mean was observed but sub-humid condition has highest urease activity .
- Conclusion can be drawn regarding urease kinetic from the soil urease activity, that urease enzyme concentration across indo-gangetic plain was similarly distributed.
- Usually high Km for urease enzyme has been reported which can be seen in the results, thus urease enzyme has low affinity for urea substrate.

Variation in microbial population

- Total microbial population reduces significantly below the depth of 50 cm and drastically below 100 cm cutting across three different bioclimatic systems in the IGP. The highest population was observed in subhumid bioclimate followed by semi-arid and humid systems in high management, while in low level of management the order is subhumid > humid > semi-arid.

Variation in urease activity

- Urease activity follows the trend of humid > semi-arid > subhumid bioclimatic systems in both high and low management conditions. The activity of urease reduces marginally from 30 to 50 cm depth and then appreciably below 50 cm.

Variation in dehydrogenase activity

- Dehydrogenase activity (DHN) reduces with soil depth. The highest DHN was observed in sub-humid, followed by semi-arid and humid bioclimatic systems in high management, while in low management the order is sub-humid > humid > semi-arid. Dehydrogenase activity was found to decrease more than 50 per cent below 100 cm soil depth in all the bioclimatic systems.

Outreach Programmes

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

Conservation, characterization and documentation of different species of *Fusarium*

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

Rational

Fusarium are soil inhabiting plant-pathogenic fungi, widely distributed from tropical to temperate regions and causes vascular wilt, root rot and other disease of economically important crop. *Fusarium* infects an extraordinary range of host plants such as rice, bean, soybean, pigeon pea, chickpea and other important crops. While most species are common at tropical and subtropical areas, some inhabit in soils of cold climates also. *Fusarium* reproduces through asexual means and produces microconidia, macroconidia, and chlamydospores. The conventional way to characterize and identification of Fusarium is based on morphological traits, but are not necessarily easily applied to different at species level. The molecular techniques can able to give the clear picture of species / races present and its host range. The use of resistant varieties is the most economical and effective way to manage the disease. Therefore, knowledge of the genetic variation within and among populations is an important component to understand the population biology of *Fusarium* for developing strategies to enhance the durability of resistance. Virulence tests are commonly used to detect the pathogen variations. However, these tests are subjected to availability of host selection pressure, tedious, inconclusive and preclude nonpathogenic strains. To circumvent these problems, DNA based molecular markers have been used in diversity analysis, virulence evaluation and genetic structure of pathogen races.

The network project is aimed to develop a repository of *Fusarium* and a wide collection of *Fusarium* will be generated with related information like place of origin, pathogenicity, and DNA fingerprinting. This information may be helpful for designing the management strategy.

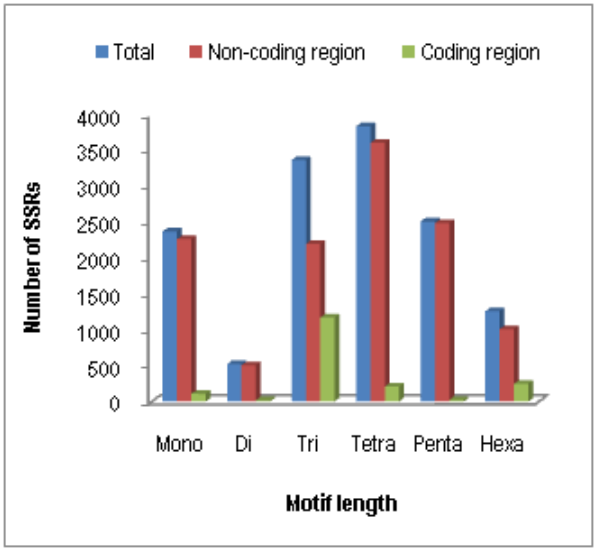
Objectives:

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

Significant Achievements:

Computational Mining and Genome Wide Distribution of Microsatellite in *Fusarium oxysporum* f. sp. *lycopersici*

- *Fusarium oxysporum* f. sp. *lycopersici*, is an important pathogen of tomato. The use of resistant varieties is the most economical and effective way to manage the disease. However, new races of pathogen have been emerged that overcome resistance in currently growing tomato cultivars Therefore, knowledge of the genetic variation within and among populations is an important component to understand the population biology of *F. oxysporum* f. sp. *lycopersici* for developing strategies to enhance the durability of resistance. Recent availability of genome sequence information of *F. oxysporum* f. sp. *lycopersici* has provided the opportunity to study the genome wide distributional pattern of SSRs motifs. The
- comprehensive study was planed on mining and analysis of microsatellite dynamics in *F. oxysporum* f. sp. *lycopersici* using bioinformatics approaches



- Total genome sequence data (59.9 Mb) of *F. oxysporum f. sp. lycopersici* was assembled into 423 scaffolds and used to explore mono-, di-, tri-, tetra-, penta- and hexa-nucleotide motifs with a repeat of ≥6 times. A total 13864 SSRs were identified from whole genome data of *F. oxysporum* f. sp. *lycopersici*. The relative abundance and density of SSRs were 231.45 SSR/Mb and 2643.73bp/Mb, respectively

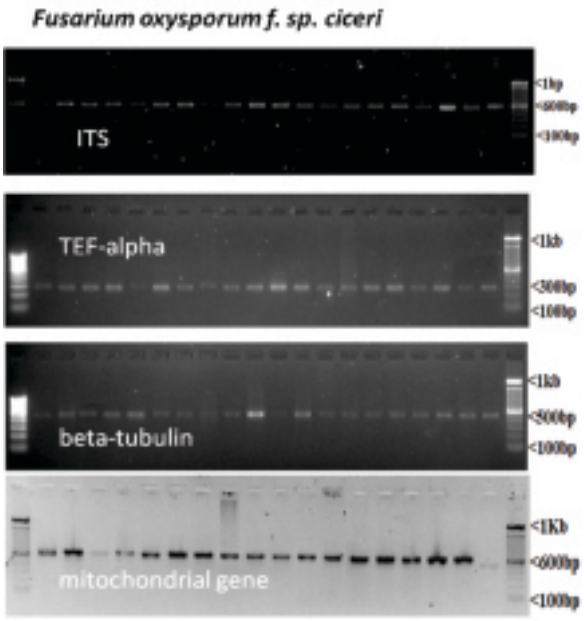
Total size covered by examined sequences (Mb)	59.9
Total number of SSR identified	13864
Perfect SSR	13608
Compound SSR	256
Imperfect SSR	139
Total relative abundance (SSR/Mb)	231.45
Total relative density (bp/Mb)	2643.73

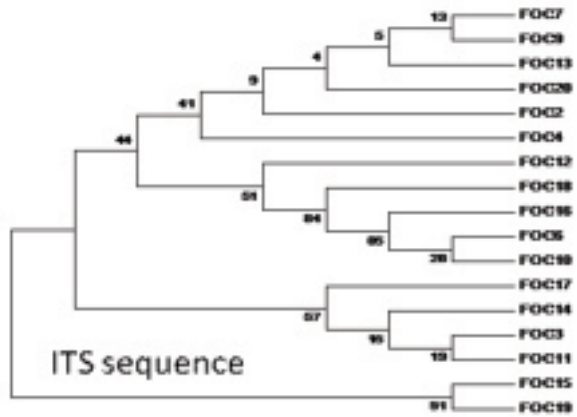
Characterization of Fusarium isolates through multilocus sequence typing

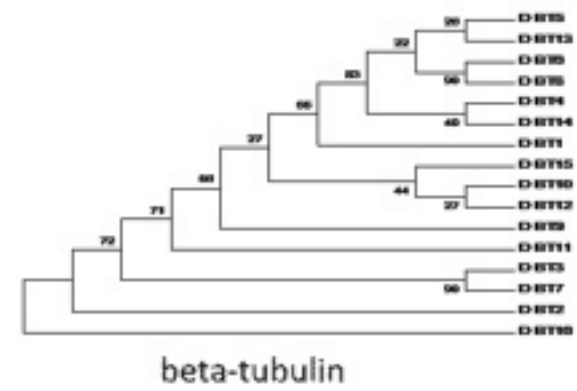
The multilocus sequence typing of different *Fusarium* species were initiated using various conserved and house keeping genes like ITS, *Beta-tubulin*, Transcription elongation factor alpha and mitochondrial gene. Culture receive from different centre were used for MLST which includes *Fusarium oxysporum f. sp. ciceri, Fusarium udum, Fusarium solani* and *Fusarium oxysporum f. sp. lycopersici*.

Fusarium oxysporum f. sp. ciceri:

For multilocus sequence typing the conserved and house keeping genes viz. ITS, *Beta-tubulin*, Transcription elongation factor alpha and mitochondrial gene were amplified in varous isolates of *Fusarium oxysporum f. sp. ciceri*. Twenty isolates were use for amplification of above mentioned genes. All resulted in the amplification of desired size of amplicom.







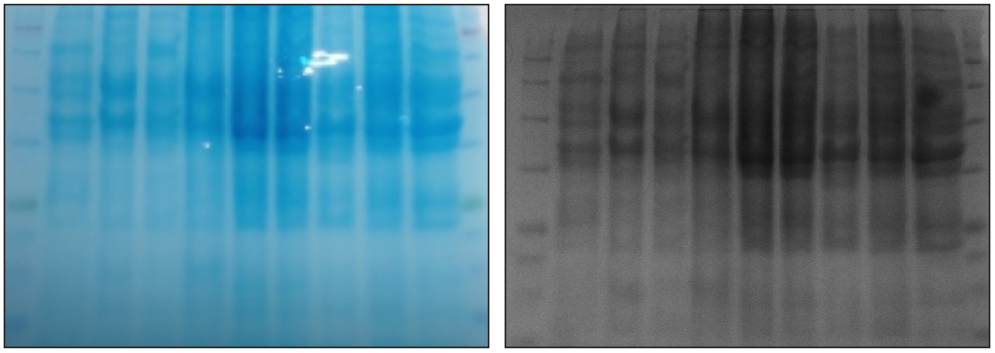


Figure: Total protein profiling of *F. udum*

Protein profiling of different isolates of *Fusarium* spp:

To study the variability through protein profiling, total protein was extractioned. Nine sample of *Fusarium udum* was run on SDS-PAGE. Range of maker was used for the quantification of total protein and range of marker is ~11kDa to ~245kDa. In *F. udum* protein range was started from the ~25kDa to ~180kDa.

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

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

PI : Sudheer Kumar
Co-PIs : Alok Kumar Srivastava

Rational

Leaf spot diseases are very common and destructive to a wide range of host especially under cool and moist weather. The majority of the leaf spots are caused by fungi. Leaf spot diseases, caused by *Alternaria, Colletotrichum* and *Cercospora*, have emerged as a major production constraint in many field, vegetable, fruit, spice and plantation crops. The losses caused by leaf spot diseases ranged from 10-90 per cent depending upon the crop and severity of disease. Major contributory factors for emergence and spread of above pathogens include evolution of new pathogenic races, fungicidal resistance in pathogens, changes in cropping systems, introduction of host susceptible genes and climatic changes. Many of these pathogens occur either singly or in combinations in causing leaf spots and often existing management strategies failed. The host resistance is very effective and economical method to manage the diseases, whereas, source of resistance again leaf spot pathogens are limited and/or possess low level of resistance. For resistance breeding the knowledge of pathogenic variability is crucial factor. A wide range of collection is required to know the full spectrum of variability present in pathogen population and a blend of conventional and molecular methods are more effective to elucidate the variability.

The leaf spots are diagnostic symptoms but are often mistaken as they vary with the host variety, crop growth stage, agroclimatic practices and prevailing weather conditions. Correct diagnosis is very crucial for the timely effective management and epidemiological studies. Current detection methods, based on culture and morphological identification of the fungus are time consuming, laborious, and not always reliable. Therefore, there is an urgent need to exploit various molecular diagnostic techniques and kits for its early and rapid detection.

The project is aimed for the conservation and characterization of the leaf spot pathogens of *Alternaria, Colletotrichum* and *Cercospora* species under network mode, data digitization and characterization.

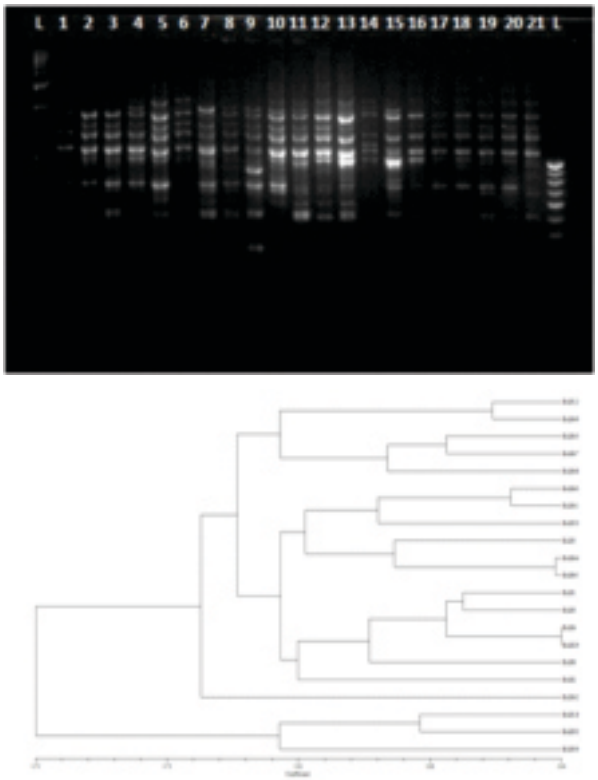
Objectives:

- 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:

Molecular Characterization

- To study the genetic variability in different leaf spot pathogens, characterization of different *Alternaria* species was under taken through various approached *viz.* random amplified polymorphic DNA (RAPD), Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR and simple sequence repeats (SSR) markers. Molecular variability among the twenty one cultures of *Alternaria brassicae* from different parts of India was determined based on the random amplified polymorphic DNA (RAPD) and ERIC PCR.
- RAPD-PCR generated very distinct fingerprinting pattern showing considerable variability among the different isolates of Alternaria. The number of amplified bands was variable depending on the primers or the isolates used. Out of ten primers, only five (RFu-C1, RFu-C4, RFu-C7, RFu-C9, RFu-C10) produced distinct and reproducible fingerprint pattern. All the isolates produced different number of fragments. The dendrogram constructed showed that all the *Alternaria brassicae* form three major clusters and shows difference at isolate level
- Fig : RAPD of 21 isolates of *Alternaria brassicae* and UPGMA dendrogram showing genetic relationships based on Jaccard's similarity coefficients .



Molecular variability among the twenty two cultures of *Alternaria brassicae* from different parts of India was also determined by ERIC PCR and dendrogram constructed showed that all the isolates grouped in two major clusters. The results show that RAPD has more discriminate ability then the ERIC.

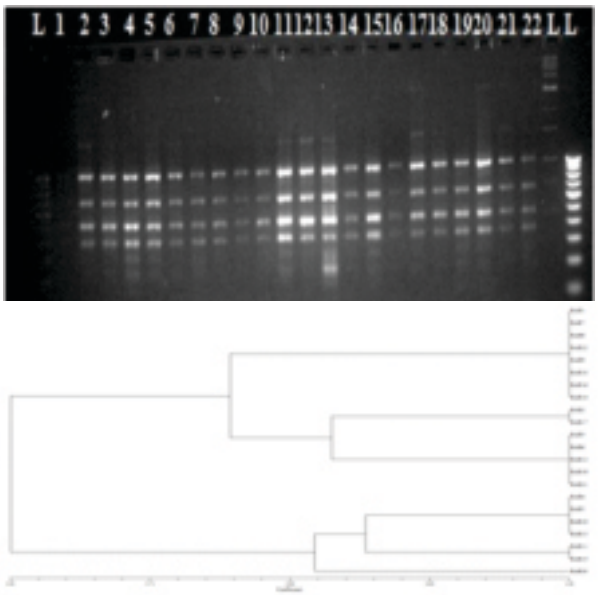


Fig : ERIC PCR of 22 isolates of *Alternaria brassicae* and UPGMA dendogram showing genetic relationships among based on Jaccard's similarity coefficients.

Multi locus sequence typing (MLST) of *Alternaria* species:

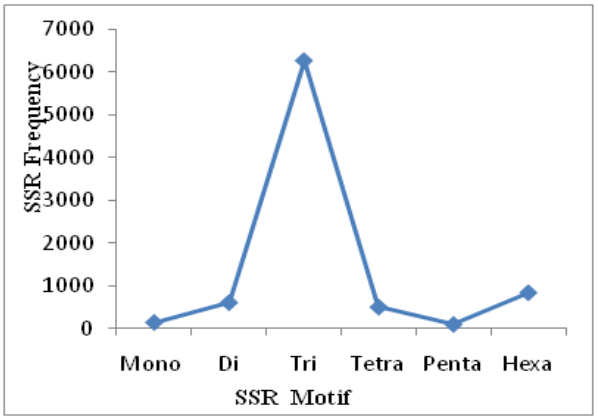
The identification and phylogenetic analysis on the basis of ITS sequence does not able to differentiate the closely related species of *Alternaria*. Multilocus sequence typing is a better alternate for correct identity and study the evolutionary relationship. The work has been initiated in this direction using *Alternaria brassicae* (15), *A. brassicicola* (10), *A. porri* (09) and *A. sesame* (09) with ITS, Beta tubulin, Histone-3, EF-alpha gene specific primers. The genomic DNA was extracted, purified and amplified from forty three cultures of *Alternaria* species received from different geographical and biological origins. The amplification of histone 3 gene with primer H3-1a (ACTAAGCAGACCGCCCGCAGG) and H3-1b (GCGGGCGAGCTGGATGTCCTT) yielded a single amplicon of expected size of 450 bp, for Beta tubulin primer pair BT-2A (GGTAACCAAATCGGTGCTGCTTTC) and BT-2B (ACCCTCAGTGTAGTGACCCTTGGC) yielded 540 bp amplicon, and EF-alpha gene amplified with primer pair EF1-78F (CATCGAGAAGTTCGAGAAGG) and EF1-986R (TACTTGAAGGAACCCTTACC) resulted 350 bp amplicom with all the isolates of *Alternaria* species. All the amplified PCR products were purified and sequencing is under way.

Computational analysis of simple repetitive sequences in *Alaternaria brassicicola* expressed sequence transcripts

Simple sequence repeats are the strong molecular marker to study the genetic variability in eukaryotes. Widely used in plants but less exploited for fungi. Genomic length of *A. brassicicola* is about 31.9 Mb size. Annotated gene transcripts of *A. brassicicola,* 14.25 Mb sequenced data are available on http://genomeportal.jgi-psf.org were retrieved to search for mono-, di-, tri-, tetra-, penta- and hexa-nucleotide motifs with a repeat of ≥6 times. The retrieved sequences were analyzed for repeat patterns using WebSat (SSR finder program). The generated data was further used for screening of SSR containing sequences by Simple Sequence Repeat Identification Tool (SSRIT).

A total number of 8457 SSRs were identified from the transcript database of *A. brasscicola*. Among all motif, tri-nucleotide motif (6261, 74.15%) was most abundant SSR and AAG motif was highly repeated.

Next to tri-nucleotides, hexa-nucleotides (10.97%) were dominant followed by di-nucleotides (8.16%). Tetra-, mono- and penta-nucleotide repeats were gradually the least frequent repeats accounting 6.0%, 1.7% and 1.26% of SSRs.



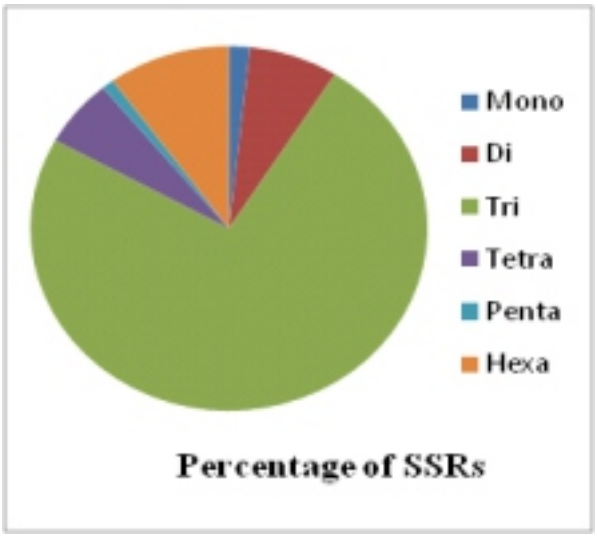
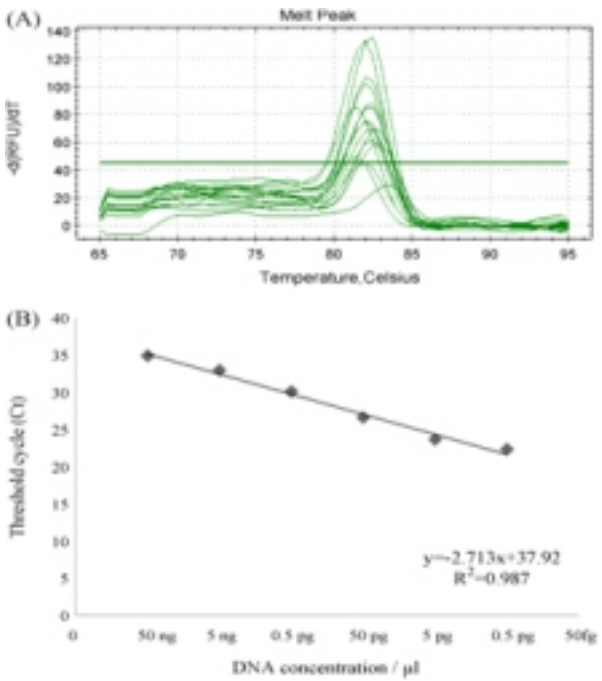


Fig: 2. Distribution of frequency and percentage of SSRs in *A. brassiscola*

Rapid detection and quantification of *Alternaria solani* in tomato

In continuation of the earlier work on design of specific primer for the detection of *A. solani*. Real time quantitative assay was developed for the detection and quantification of *Alternaria solani* in tomato. The system is based on primers targeting beta-tubulin gene of *Alternaria solani* and amplified specific band of 289 bp. We obtain good correlation (R2 = 0.987) between the Ct-value and *Alternaria solani* DNA concentration. Lowest detection limit of the assay is 0.5 pg. The developed system was used to analyze the occurrence of *Alternaria solani* on artificially inoculated tomato plants.



Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)

Project: Complete Genome Sequencing of *Mesorhizobium ciceri* Ca181

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

Rationale:

Mesorhizobium ciceri Ca181 is a be0neficial microbe for farmers. It is very specific for host and form nodule in the roots of chickpea and shows good competitiveness among other chickpea rhizobium strain and also very efficient in the fixation of nitrogen in different agro-climatic regions and soil types. Rhizobium strains are very sensitive to soil environmental abiotic factors which include desiccation, water stresses, high salt, pH and temperature stresses and affects their efficiency of nitrogen fixation and finally productivity of legumes. Microorganisms are involved in a range of processes that affect the transformation of soil P from phosphate and are thus very important part of soil P cycle. Complete genome sequence gives us a whole genomic blue print of *Mesorhizobium ciceri* Ca181. Results will dissect the process of nitrogen fixation, host specificity and competitiveness at molecular level so as to enable us manipulate genes involved for increasing crop productivity. The analysis of SSRs will help to study the population genetics of Rhizobia.

Screening of random mutants for important genes like genes involved in water stresses tolerance and phosphate solubilization will increase our understanding about these genes and processes. Using the knowledge of different gene sequences and their function, it may be possible to use them for increasing crop yield and development of transgenic with enhanced biological nitrogen fixation ability and enhanced phosphate solubilization under abiotic stresses.

Objective

- Complete genome sequencing of *Mesorhizobium ciceri* Ca181.

Significant Achievements

Sequencing of the genome was done by next generation sequencing (454-pyrosequencing, Solexa-illumina) and Sanger technology. A total of 20 large contigs/ Scaffolds are formed after assembly. There are still 19 gaps are remain to be filled to achieve the circular chromosome. Fosmids are mapped and pseudo-molecule has been made.

Host Specificity Test:A field experiment was set up for the selection of best varieties (cultivars) of chickpea with *M. ciceri* strain Ca181.

- 34 different varieties of chickpea were used in the experiment with 5 strains of chickpea Rhizobia. All the tests were performed in replication.
- The experiment was designed in RBD method and showing was done in Paired Row design.
- All the 34 varieties (PDG3, JGK1, JG130, PUSA372, PUSA 1053, DCP 923, W-4, SAKI9570, GCP105, RSG98, ICCB02, PANTG 186, VAIBHAV, WGG10, RSG 888, PBG 5, IGG 1, BGD72, GPF2, IG332, JAKI 9218, IC3808, PUSA 391, VIJAY, PUSA 362, ICCV10, Vishal, PUSA 1003, KPG 59, IG218, GNG663, Sadabahar) were sown for multiplication of seeds in separate plots.

Fig.1: Chickpea plants in the Field

Fig.2: Chickpea Grown Field

Fig.3: Distribution of root nodules of Chickpea plant

Fig. 4: Root Nodules

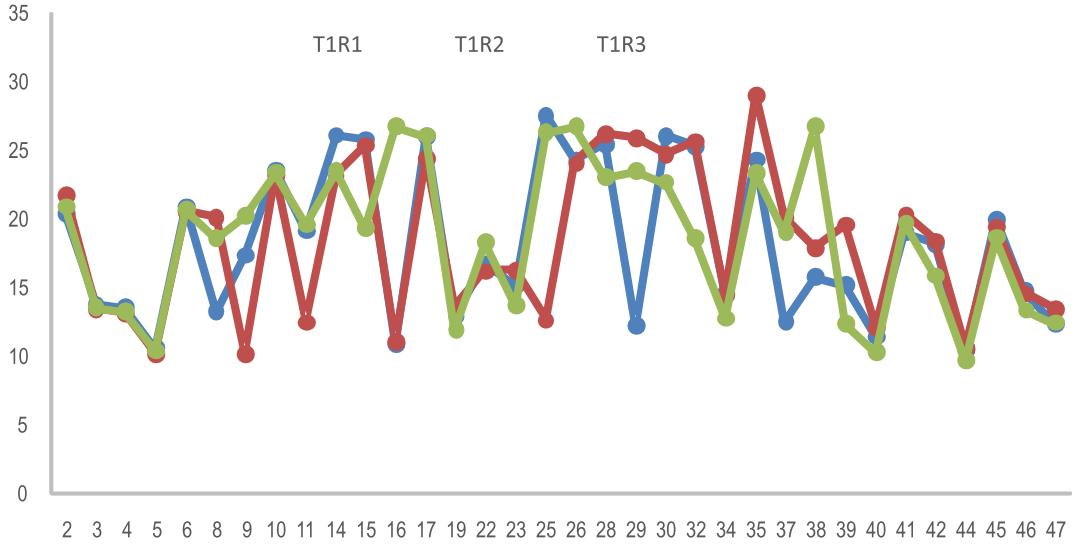


Fig. 5 Weight of 100 chickpea seed in three replicates

Competitiveness Test:

ERIC and REP PCR profiles are developed for experimental strains including *M. ciceri* Ca181, for identification of strains during competitiveness test among the different strains of chickpea rhizobium.

Project: Diversity analysis of microorganisms in extreme environment.

PI : Dr. Sudheer Kumar
Co-PIs : Dr. Alok Srivastava, Dr. P.L. Kashyap

Rational

Microbes are widely distributed everywhere, even in extreme environments such as arctic, glaciers, deep-sea vents, hot springs, contaminated soil, waste-water discharge, etc. India, considered as a diversity hot spot in the world because of it climatic conditions ranges from extreme cold (blow 0°C) to very hot (above 100°C) , from very acidic to very alkaline site, or high saline concentration, high altitude etc. Large amount of diversity is expected in this region because of the undistributed nature. Microbial diversity screening is an active area of research as it provides inexhaustible data, which can be used for the purpose of developing products for industries, agricultural, chemical processing and pharmaceuticals. Very few and isolated efforts were made to tapping of microbial diversity and identification of extreme environment. Microbes thriving under extreme conditions evolved the mechanism to overcome the stress imposed due to extremity. Thus extreme environments can be considered as ideal model systems for studying ecology properties of microorganisms, their physiology, adaptive properties and many other characteristics related to the microbial communities and specific microbial cells. The study of microbial communities and their interaction with environment are key aspect to understand the role and function of microorganism in nature ant its exploitation in agriculture.

Objectives:

Significant Achievements:

Diversity analysis of extremophilic microorganisms from Jharia coal mine Thermophilic Microorganism were isolated and characterized from rhizospheric and non rhizospheric soil samples collected from Jharia coalmine field Dhanbad

- Population of the fungi was found $1.3x10^2$, while bacteria from 36-40 $x10^3$ cfu/g and actinomycetes from 1-16 $x10^2$ cfu/g.
- On the basis of colony morphology, substrate mycelium, pigmentation and microscopic examination, a total 21, 39 and 26 different morphtypes of bacteria, fungi and actinomycetes, respectively, were isolated from different sampling sites of Jharia coal mines field.
- All the thermophilic isolates of bacteria fungi and actinomycetes were screened for temperature tolerance.
- Plant growth promoting activity of all the fungal isolates revealed that 46.15% produced siderophore, 61.53% Ammonia, 84.61% HCN and no morphospecies could solubilized phosphate in pikovaskays agar medium.
- Biochemical tests and production of extracellular metabolites by bacterial isolates such as Starch hydrolysis (5%), Gelatin liquefaction(12%), Casein hydrolysis(14%), Hydrogen sulfide (7%), Urease (14%), Citrate utilization(7%), siderophore production(18%), phosphate solubilising(13%) and cellulase production(10%)
- All the thermophilic isolates of fungi, bacteria and actinomycetes exhibiting distinct colony characteristics were isolated, purified and subjected for further molecular characterization. PCR amplification followed by restriction analysis of 16S rDNA/ITS gene of all the isolates was done and purified PCR product has been sent for sequencing. (Sequencing result is awaited).

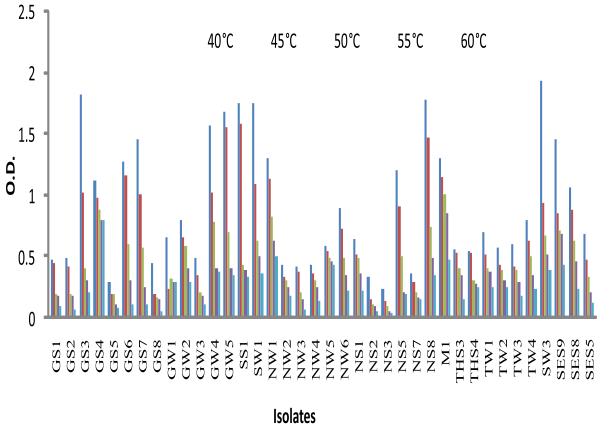
Microbial diversity analysis of tatta pani hot spring

Tatta pani hot water spring is situated in Himachal Pradesh and till date very limited and isolated efforts were made to tapping of microbial diversity and identification of extreme aquatic environment. Large amount of diversity is expected in this region because of the undistributed nature.

- Aerobic thermophilic microorganism were isolated and characterized from water and sediment samples collected from tatta pani hot spring shimla, H.P.
- Population of the fungi and bacteria in the sediments and water samples were found 4x 10^1cfu/ml and 2.1x10^3cfu/ml, respectively.
- 38 bacterial and 21 fungal morphotype selected, all could grow at temperature 60 °C and 45°C, respectively. 5 bacterial and 4 fungal isolates could grow at 65°C and 55°C, respectively.
- Plant growth promoting activity of the fungal isolates revealed that a total of 16.15% produced siderophore, 43.53% Ammonia, 27.62% HCN and 12.7% could solubilized phosphate in agar medium.
- Production of extracellular metabolites by bacterial isolates revealed that a total of siderophore production (22%), phosphatase (15%) cellulose (18%) amylase (19%), Gelatin liquefaction (12%), Protease (14%).
- PCR amplification followed by restriction analysis of 16S rDNA/ITS gene of all the isolates was done and purified PCR product has been sent for sequencing. (Sequencing result is awaited)

Microbial diversity analysis of Goa mangrove

Mangrove is a highly productive marine ecosystem, where halophilic microbes actively participate in bio-mineralization and biotransformation of minerals.



Screening of bacterial isolates at Different Temperature

Microorganisms (bacteria, archaea and fungi etc.) which survive in high salinities are known as halophilic extremophiles, and have the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salt.

- Thirty four isolates of halotolerant fungi were isolated from Chorao mangrove (15°27' to 15°38'N, 73°42' to75°50'E), Goa, India.
- Morphological and physiological charactersaition of all the isolates revaled that the all the isolates were able to tolerant up to 30% NaCl and showing luxuriant growth in the pH range 6.4-8.5.
- The collected isolates were characterised in terems of colony colour, texture, spore size and pigemention.
- PCR amplification of ITS gene of all the isolates was done. The fungal isaoltes were identified as: *Emericella nidulans, Cladosporium sp., Penicillium griseofulvum, Aspergillus sp., Eurotium amstelodami, Penicillium citrinum, Aspergillus versicolor, Aspergillus tubingensis, Emericella striata, Emericella nidulans, Eurotium cristatum* and *Penicilliopsis clavariiformis* etc.
- A rare salt tolerant fungus isolate *Penicilliopsis clavariiformis* AP, was isolated from Goa mangrove and to best of our knowledge, this halotolerant fungus was not recorded previously from India. The isolate was characterized by means of polyphasic taxonomy. Optimal growth was determined to occur at 24°C and able to tolerate up to 10 % (w/v) NaCl. Scanning electron micrographs showed that the fungus has unique features such as biverticillate penicilli, bearing masses of oval to ellipsoidal conidia.

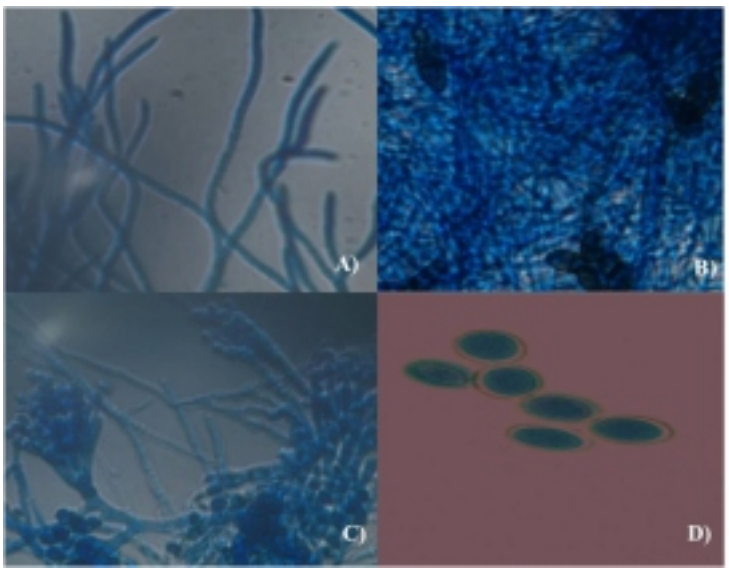


Fig: Microscopic examination of *Penicilliopsis clavariiformis* AP. A) Sterile mycelia;B) Conidiogenus structures;C) Ascus.;and D) Conidia

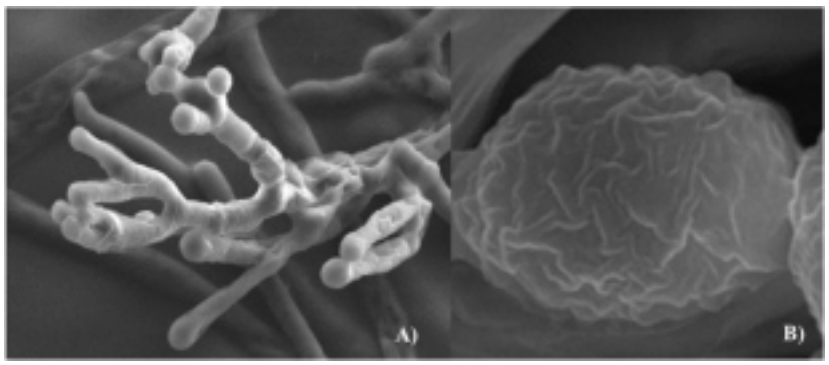


Fig: SEM micrographs of *Penicilliopsis clavariiformis* AP showing biverticillate penicillium (A) and conidia (B).

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

PI : Dr. Sudheer Kumar
Co-PI : Dr. Alok Srivastava, Dr. P.L. Kashyap

Rational

There are different management practices to prevent, mitigate or control plant diseases. Apart from use of resistant varieties, good agronomic and horticultural practices, farmers often rely heavily on chemical pesticides. However, the environmental pollution caused by excessive and misuse of agrochemicals changes in people's attitudes towards the minimization of their use in agriculture. Consequently, the efforts towards developing alternative inputs to synthetic chemicals are emphasized for controlling plant diseases, among these alternatives most important is biological controls.

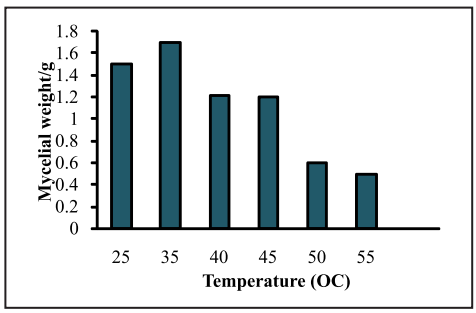
Integrated diseases management model, includes a number of management practices along with the use of BCAs introduced as inoculants having a low impact on the environment and non-target organisms. A number of BCAs are identified effective against one or more plant pathogens but practical implementation on a commercial scale has been constrained by a number of factors viz. cost, convenience, efficacy, and reliability of biological controls are important considerations. Among various BCAs, *Trichoderma* species is most common and widely exploited for diseases management. Massa multiplication of *Trichoderma* spp propagules can be produce in liquid or solid fermentation. Solid state fermentation is more suitable for mass production of *Trichoderma* propagules. To achieve maximum yielding at low cost, the material like crop residue, livestock waste, industrial waste and any organic material can be used. The condition can be optimized for maximum yield. The propagules may be formulated with substrate materials and stored after addition of some spreaders and stickers. Therefore, the project is aimed to identify the low cost sbustrate and optimization of growth conditions.

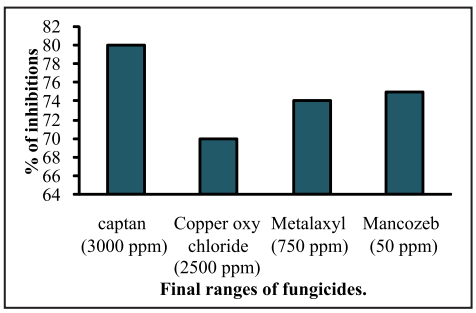
Objectives:

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

Significant Achievements:

- A number of bacterial and fungal strains isolated from the rhizosphere of wheat, mustard, potato and chickpea crops were screening under *in vitro* conditions against chickpea pathogens *Fusarium oxysporum* f. sp. *ciceri*, causes wilt, *F. solani* causing black root rot and *Macrophomina phaseolina* causing charcoal rot. Among these, bacterial isolates (B-7, A-90, B-11) and fungal isolate (T-8) found promising and selected for pot and field experiment against wilt pathogen in different combinations. Out these, a combination of B-7 + T-8 + B-11 showed a significant increase in plant growth and reduced disease incidence.
- The growth parameter were optimised for the fungal isolate (T8) that gave good results in *in vitro* as well as pot and field experiment. The parameters optimised were temperature, pH and fungicide resistance by using poisoned food technique. The pH range 2 to 14 with the increment of 2 was evaluated and maximum sporulation was observed at pH 6. Similarly the 35°C temperature was recorded optimum. The fungal isolate (T8) also tested for it tolerance to different systemic and non systemic fungicides viz. Metalaxyl, Mancozeb, Captan and Copperoxycloride.





Different substrates viz. tea waste, pearlmellet seeds, rice husk, wheat bran, saw dust, soya cake and compost with some moistening agents were used optimized for mass production. Tea waste and pearlmellet seeds with 2% sucrose solution as moistening agent were recorded suitable for mass production.

Substrate	Rate of sporulation	Reading after 10 days	Reading after 20 days	Reading after 30 days
Tea waste	+++	15.25 ×104	20×104	22.75 ×104
Pearlmellet seeds	+++	8.9×104	17.25 ×104	22.25 ×104
Rice husk	+++	11×104	16×104	11.25 ×104
Wheat bran	+++	3×104	8.5×104	15.12 ×104
Saw dust	-	-	-	-
Soya cake	-	-	-	-
Compost	-	-	-	-

Fig. Substrates after 30 days of incubation

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

PI : Dr. Alok Srivastava

Rationale:

Agricultural productivity is affected by several factors, among which environmental stresses is the most limiting one. Apart from biotic stress caused by plant pathogens, there are a number of abiotic stresses such as extremes in salinity, temperature, drought, heavy metals and radiation which all have detrimental effects on plant growth and yield. Drought and Salinity exerts negative impact on critical ecological balance and affects the plant growth and development adversely and in the agroecosyste. Metabolic imbalances caused by ion toxicity, osmotic stress and nutritional deficiency under saline conditions may also lead to oxidative stress. Plant growth-promoting rhizobacteria (PGPR) are known to participate in many important ecosystem processes, such as the biological control of plant pathogens, nutrient cycling, seedling growth and abiotic stress tolerance. PGPR strains possess the enzyme ACC deaminase and this enzyme can cleave the plant ethylene precursor ACC, and thereby lower the level of ethylene in stressed plants. Plants that are treated with ACC deaminase-containing PGPR are comparately more resistant to the deleterious effects of stress ethylene that is synthesized as a consequence of stressful conditions such as, heavy metals, presence of phytopathogens, drought and high salt. In each of these cases, the ACC deaminase-containing PGPR markedly lowered the level of ACC in the stressed plants, thereby limiting the amount of stress ethylene synthesis and hence the damage to the plant. These bacteria are beneficial to plant growth, as in the natural environment, plants are often subjected to ethylene producing stresses. More specifically, the *Pseudomonas* spp. and *Bacillus* spp have received particular attention throughout the world because of their catabolic versatility, excellent root colonizing ability and their capacity to produce a wide range of enzymes and metabolites that favour the plants withstand both under biotic and abiotic stress conditions. Understanding the complexity of microbial adaptations into stressed rhizosphere environment and the effect of these microorganisms on biological, chemical, and physical properties of rhizosphere soil and the plants remains a significant challenge. The project therefore addresses the application of microbial consortium for the alleviation of salt and drought stress in wheat crop.

Objectives

- 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.

Significant achievements:

Thirty four morphotypes isolated from Sambhar salt lake, Rajasthan were screened for different salt concentration (15 to 30%) in different media HB, YEMA, VNYM, MB, TYESM, and NA.

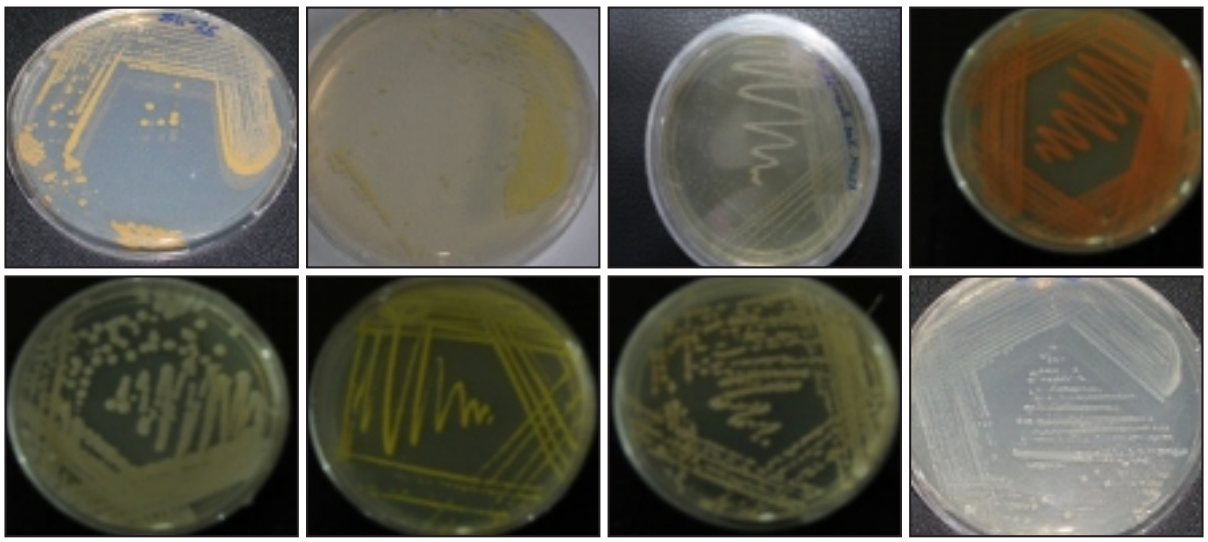
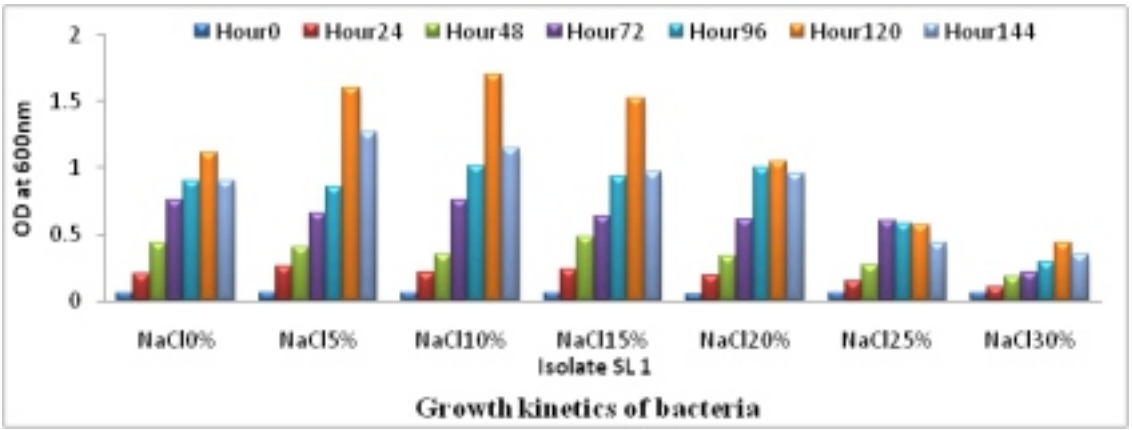
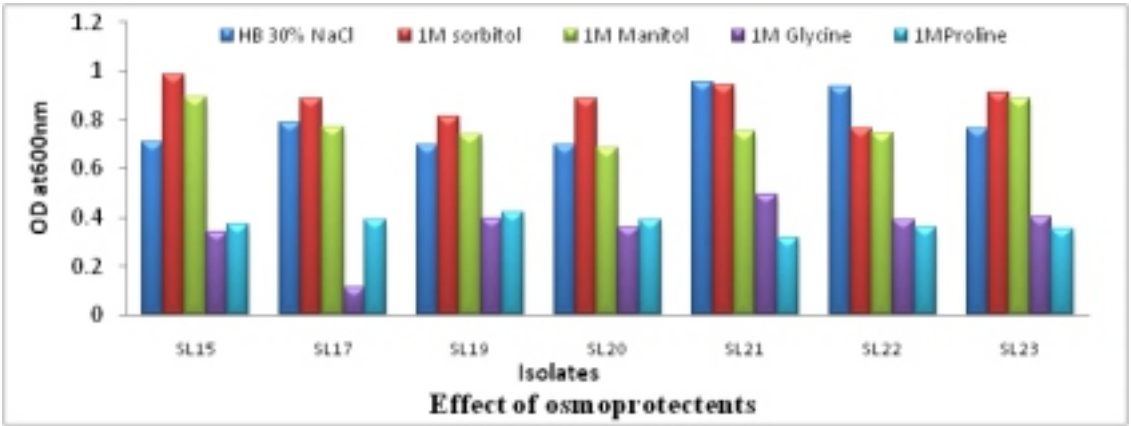


Fig: Morphotypes of Halophilic bacteria isolated from sambher salt lake at different medium.

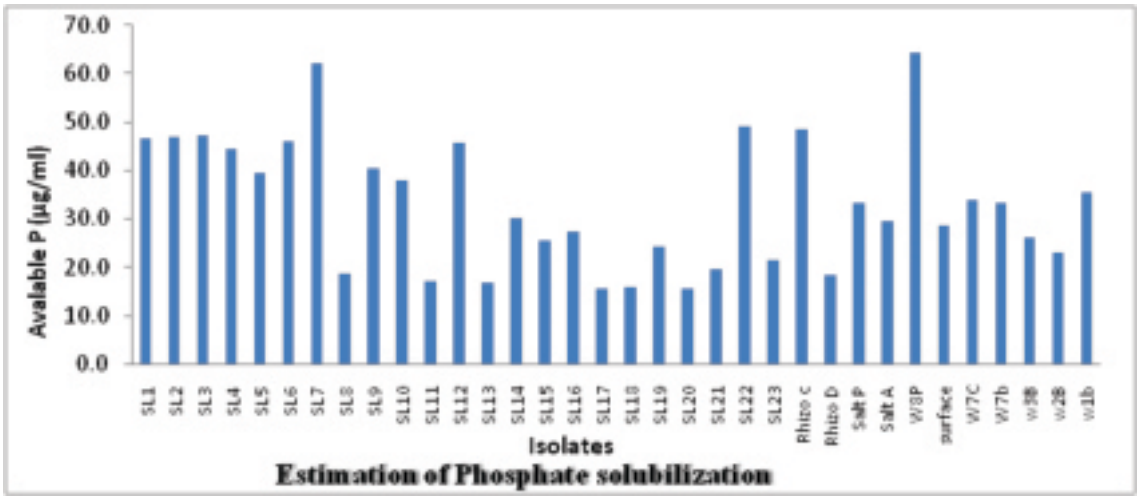
- The intrinsic tolerance of the isolates against salinity and alkalinity was evaluated by observing the growth on HA medium. Growth kinetics was studied under deferent level of salt.



- Effect of osmoprotectents viz: sorbitol, manitol, glycine betain and proline was evaluated on growth of selected isolates. The isolates SL15, SL19, and SL23 exhibited better growth as compared to control in the presence of osmoprotectent; sorbitol and manitol.



- The isolates showed maximum tolerance up to 30% NaCl are also having attributes to PGP traits (IAA, Ammonia, ACC deaminase, Siderophore and P-Solubilization). The Urease activity of 34 isolates was also evaluated and only 9 isolate were found as urease producer.
- Twenty seven out of 34 strains isolates showed phosphate solubilization activity. Maximum phosphate solublization was obtained by W7C (64.3µg PO4/ml), followed by SL 7 (62.2µg PO4/ml), SL 6 (46.01µg PO4/ml), SL 9 (40.09µg PO4/ml), SL 12 (45.9µg PO4/ml), and SL 15 (25.7µg PO4/ml) whereas, it was minimum in SL 13 (16.9µg PO4/ml), SL 18 (15.9µg PO_4/ml).



- The halotolerant isolates were assayed for IAA production, maximum IAA was produced by isolate SL15 (335.52 mg/ml protein), followed by SL1 (76.27mg/ml protein), SL6 (60.14mg/ml protein) and it was comparatively less in SL19 (38.57 mg/ml protein), SL20 (39.85 mg/ml protein) and SL22 (40.75 mg/ml protein).

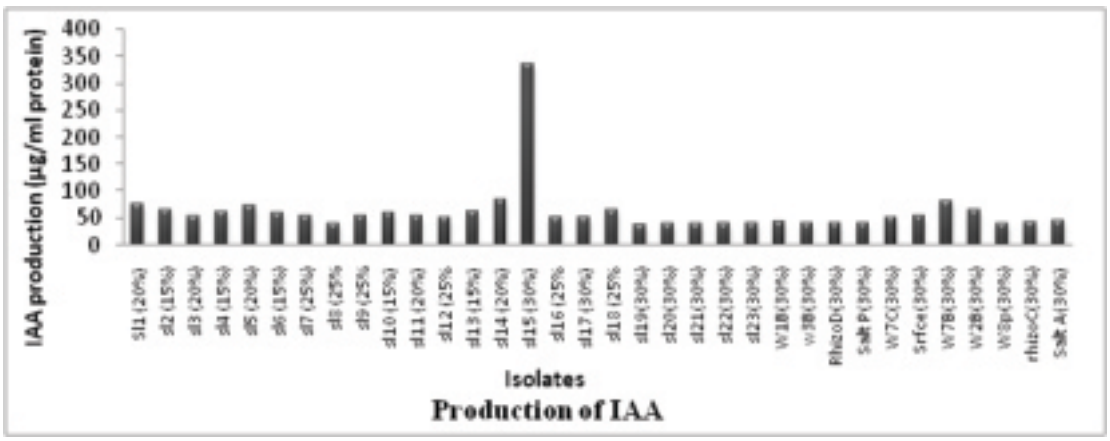


Fig: Production of IAA by bacterial isolates

Fig: Estimation of Siderophore

- Proline accumulation was recorded and it was maximum in isolate SL6 (18.03µm/ml), followed by SL 9 (17.46µm/ml), SL7 (11.49µm/ml), SL15 (9.43µm/ml), Salt A (12.13µm/ml), and W7C (9.45µm/ml) whereas the isolates W1b (1.77µm/ml), SL12 (3.35µm/ml), and SL 16 (4.43µm/ml) produced less proline.

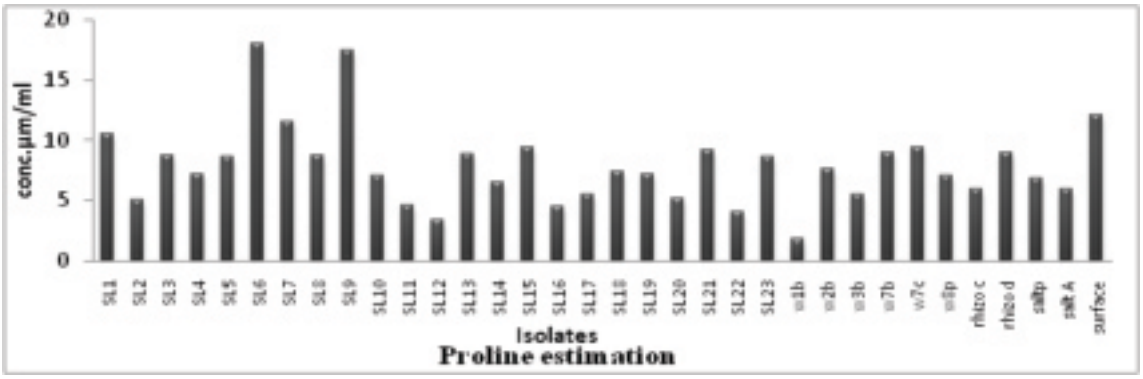
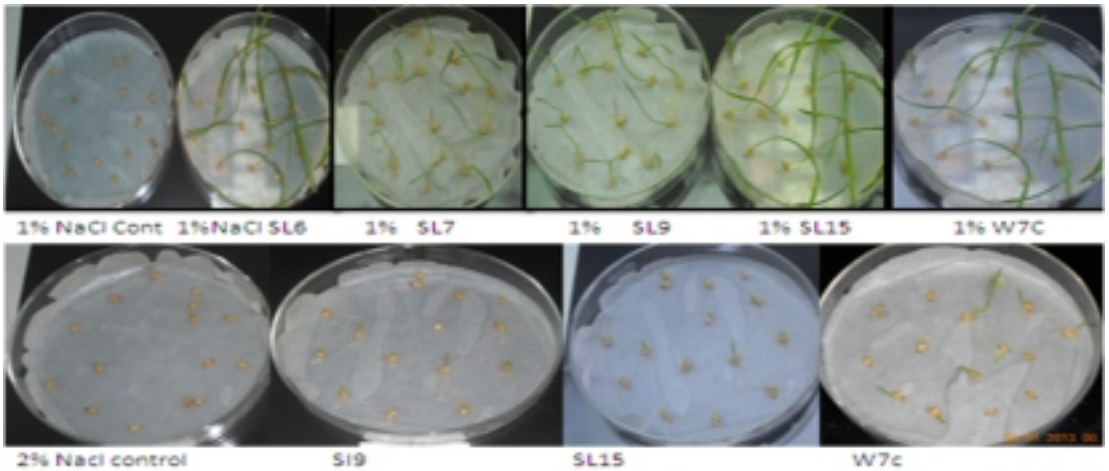
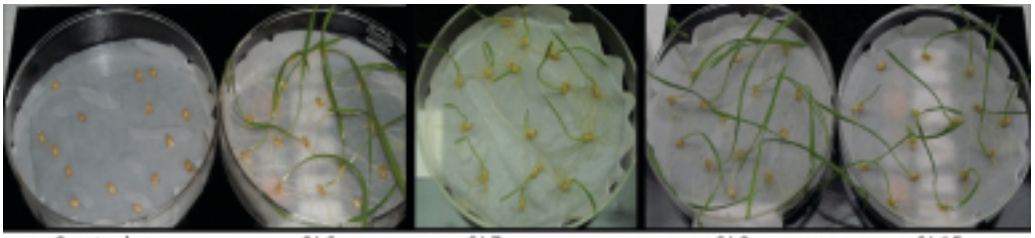


Fig: quantitative estimation of proline

Seed germination assay: Effect of the five potential isolates (SL6, SL7, SL9, SL15 and w7c) was evaluated on germination of wheat var: at different NaCl concentration *in vitro*. The results showed that more than 80% germination could be obtained with SL15, SL9 and w7C, they also have the positive attributes of multiple PGP traits. Presence of the osmotolerant proline provides insight for further exploration of these isolated in consortia mode to alleviate salt stress for growth of wheat crop.





Control SL6 SL7 SL9 SL15

Total percent of germination

Isolates	0.5% NaCl	1% NaCl	2% NaCl	3% NaCl
SL6	95%	80%	78%	Nil
SL7	90%	92%	76%	Nil
SL9	96%	85%	80%	Nil
SL15	100%	90%	85%	Nil
W7C	95%	82%	80%	10%

Project: Microbial Genomic Resource Repository (AMAAS)

PI : Arun Kumar Sharma
Co PIs : Alok Kumar Srivastava, Sudheer Kumar

Rationale

Microbial genetic materials (eg. genomes, plasmids, vectors, cDNAs) are very important 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, specializes collections that support small groups of researchers to the large international public 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 Microorganism (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 indicated 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, component cells from sequencing projects, promoter DNA-fragments with reporter genes, RFLP probes specific for different microbes and expression vectors.

Genetic material 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 passport data or MTA form. We can store your DNA samples at our secure, environmentally controlled facility for many years.

Objectives

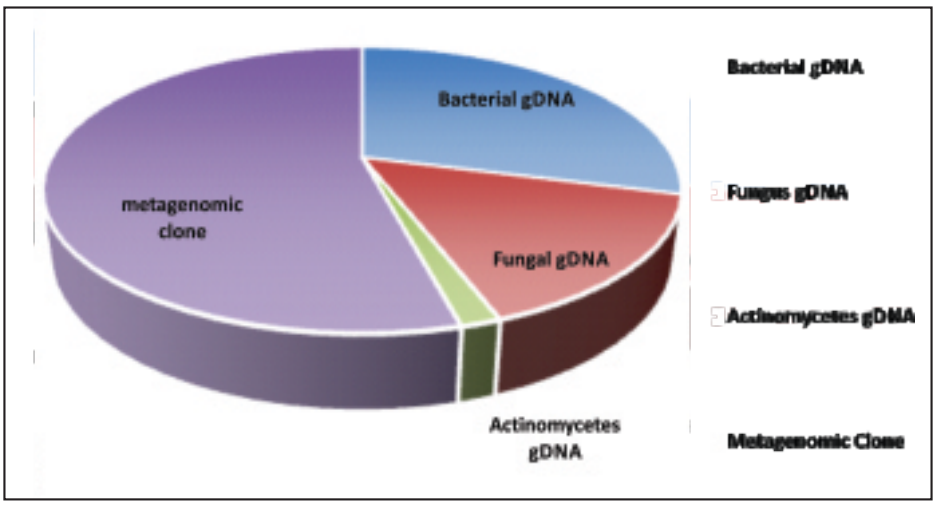
- Nationwide survey and collection of information about the genetic resources/DNA.
- Development of linkages between research institutes/ Universities and researchers.
- Technology and Protocols development for collection/ transportation of microbial samples.
- Collection of environmental samples from different agro-climatic regions and exploration of non-culturable microorganism.
- Development of databases/Information Bank for Microbial Genomics 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 achievements:

- Previous stored genetic material was checked and stored again.
- Microbial (Bacteria, Fungus and Actinomycetes) strains were received from NAIMCC and grown on respective culture media. All the strains had been processed for genomic DNA extraction.
- A total of 1088 Genomic DNA from bacteria, 62 Genomic DNA from Actinomycetes and 568 Genomic DNA from fungal isolates of different species were extracted and have been kept in vials with concentration of 100ng per microliter and stored at -20 ^{0}C.
- All the Genomic DNA were amplified for 16S rDNA for bacteria and Actinomycetes and ITS region for fungal samples.
- Beside this the representative strains of different species were taken and being amplified for *gln*D, gndD, *gap*, *nod*C, *nod*A, *nod*D, *ed*D, *rpo*B, *gac*A and *rpo*D gene for structural as well as functional characterization. All the amplified gene product was purified and stored in MGRR genome bank.
- Sequencing analysis of ribosomal genes is in process for identification of isolates.

Metagenomic Library of 16S rRNA gene from Extreme environment (Psychrophilic)

- Total metagenomic DNA was extracted with help of MoBio Genomic DNA extraction kit from glacial soil of Leh Laddakh, J& K, India. Metagenomic DNA was quantified and stored at -20^{0}C.
- Amplified 16S rDNA products were gel purified and prepared for cloning in T/A vector. Product was ligated in T/A vector and transformed in *E. coli* DH5-α strain. Transformants were screened on L.A plates amended with Kanamycin (75µg/ml) and X-Gal (20µg/ml). Transformants were picked and stored in LB freezing Buffer at -80^{0}C for further Processing. Total of 2016 (96x21) metagenomic clone were deposited in Genome Bank.



Inter- Institutional Project

Project: Developing technique for acceleration of decomposition process using thermophilic organisms.

PI : Asha Sahu
Co-PI : Udai B. Singh, HL Kuswaha, M.C.Manna, A. Subba Rao

Rationale:

Objectives

- Isolation and identification of thermophilic bacteria, fungi and actinomycetes.
- Evaluation of selected thermophilic decomposers in municipal solid waste and agricultural waste compost at different stages of decomposition.
- Develop appropriate machinery/protocol for scaled up decomposition process mediated by microbes and its possible application in agriculture.
- Evaluation of physico-chemical properties of compost and economics of composting.

Significant achievements:

- A total of 60 morphotypes of Bacteria and 20 morphotypes of actinomycetes and 10 morphotypes fungi were isolated by using different media namely Nutrient agar medium, Starch casein agar medium and PDA medium.
- Among different isolates 7 bacteria, 7 actinomycetes and 6 fungi as lingo-cellulytic thermophilic microbes.
- Morphological characterization was done with the help of light and scanning electron microscopy.
- 16S rDNA amplification was done for characterization of bacterial strains at molecular level and sent for sequencing of 16S rDNA gene.
- Lab-scale study of decomposition of various collected agro-waste was done by inoculating the screened organisms.

Human Resource Development

Microorganisms are highly diverse group of organisms, able to sustain very harsh conditions, and are key players in important ecological processes such as soil structure formation, decomposition of organic matter , recycling of essential and nutrients. Microorganisms are essential components of the Earth's biota and represent a large unexplored reservoir of genetic diversity. Understanding and exploitation of this unexplored genetic diversity for sustainable agriculture production needs the knowledge of number of conventional and molecular technologies. Molecular approaches such as genetic fingerprinting, metagenomics, metaproteomics, metatranscriptomics, and proteogenomics are vital for discovering and characterizing the vast microbial diversity and understanding their interactions with biotic and abiotic environmental factors.

NBAIM providers training on cutting age technologies in the area on microbial identification, diversity analysis through culture dependent and culture independent techniques, and microbial interactions for its exploitation in abiotic and biotic stress management. The bureau has made significant achievements to train the human resources in microbial identification, characterization, and its application in crop stress management. During 2012-13 the following training were organized by bureau:

- National training on Bioinformatics: Methods, Tasks and Applications in Microbial Research from December 04-15, 2012.

- Staphna Divas Samaroh and Kisan Sangosthi held on 1st June, 2012.

- Krishak Vaigyanik Sangosthi held on 08th November, 2012.

- Specialized training on Molecular Microbiology and Pathology from February 05-14, 2013.

- Patent Awareness Workshop held on 23rd February, 2013.

- National training on Polyphasic Microbial Identification: Methods and Application from March 05-14, 2013.

Publications

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- Manoj Kumar Solanki, Rajesh Kumar Singh, Supriya Srivastava, Sudheer Kumar, Prem Lal Kashyap, Alok K. Srivastava and Dilip K. Arora (2013) Isolation and characterization of siderophore producing antagonistic rhizobacteria against Rhizoctonia solani. J. Basic Microbiol. DOI 10.1002/jobm.201200564.
- Prem Lal Kashyap, Sudheer Kumar, Alok Kumar Srivastava, Arun Kumar Sharma (2013) Myconanotechnology in agriculture: a perspective. World Journal of Microbiology and Biotechnology, February 2013, Volume 29, Issue 2, pp 191-207
- Srivastva Rishi, Tripathi Binu Mani, Singh Rahul Kunwar, Srivastva Pratima, Kumari Priyanka, Srivastav Madhusudan, Srivastava Alok K, Kumar Sudheer, Kashyap Prem Lal, Sharma Rajesh, Tiwari Shree Prakash (2013) Profiling of Plant Growth Promoting Bacteria Associated with Jaunpuri Giant Raddish Rhizosphere. International Journal of Agriculture, Environment & Biotechnology 6(2):187-193. pp.187-193
- Binu Mani Tripathi, Priyanka Kumari, Kela P. Weber, Anil Kumar Saxena, Dilip Kumar Arora, Rajeev Kaushik (2013) Influence of Long Term Irrigation with Pulp and Paper Mill Effluent on the Bacterial Community Structure and Catabolic Function in Soil. Indian Journal of Microbiology, 10.1007/s12088-013-0398-8
- V. Keshri, Dhananjaya P. Singh, R. Prabha, A. Rai, A. K. Sharma (2013) Genome subtraction for the identification of potential antimicrobial targets in Xanthomonas oryzae pv. oryzae PXO99A pathogenic to rice. 3 Biotech. 10.1007/s13205-013-0131-7
- U.B. Singh, A. Sahu, N. Sahu, R.K. Singh, S. Renu, D.P. Singh, M.C. Manna, B.K. Sarma, H.B. Singh, K.P. Singh (2013) Arthrobotrys oligospora-mediated biological control of diseases of tomato (Lycopersicon esculentum Mill.) caused by Meloidogyne incognita and Rhizoctonia solani. Journal of Applied Microbiology. Volume 114, Issue 1, pages 196–208.
- Udai B. Singh, Asha Sahu, Nisha Sahu, Bhanu P. Singh, R.K. Singh, Renu, Dhananjaya P. Singh, R.K. Jaiswal, B.K. Sarma, H.B. Singh, M.C. Manna, A. Subba Rao, S. Rajendra Prasad (2013) Can endophytic Arthrobotrys oligospora modulate accumulation of defence related biomolecules and induced systemic resistance in tomato (Lycopersicon esculentum Mill.) against root knot disease caused by Meloidogyne incognita. Applied Soil Ecology 63, 45–56.
- Manoj Kumar Solanki, Amrita Shalini Robert, Rajesh Kumar Singh, Sudheer Kumar, Akhilesh Kumar Pandey, Alok K. Srivastava, Dilip K. Arora Characterization of Mycolytic Enzymes of Bacillus Strains and Their Bio-Protection Role Against Rhizoctonia solani in Tomato. Current Microbiology, 65 (3): 330-336.
- Rajesh Kumar Singh, D. Praveen Kumar, Manoj Kumar Solanki, Pratiksha Singh, Alok K. Srivastva, Sudheer Kumar, Prem L. Kashyap, Anil K. Saxena, Pradeep K. Singhal, Dilip K. Arora (2012) Optimization of media components for chitinase production by chickpea rhizosphere associated Lysinibacillus fusiformis B-CM18. J. Basic Microbiol. DOI: 10.1002/jobm.201100590
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potential to control Sclerotinia sclerotiorum through oxalate oxidase like protein. World Journal of Microbiology and Biotechnology, November 2012, Volume 28, Issue 11, pp 3197-3206

- S. Vardhan, A.K. Yadav, S. Kashyap, A. K. Panday, A.K. Srivastava, and D.K. Arora (2012) Isolation and Characterization of Psychrotolerant Paenibacillus Agaridevorans Strain S26 from Subglacial Himalayan Lake. International Journal of Pharma and Bio Sciences . Vol 3/Issue 2/ April – June 2012
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- Dhananjaya P. Singh, Ratna Prabha, Anil Rai and Dilip K. Arora (2012) Bioinformatics-assisted microbiological research: tasks, developments and upcoming challenges. American Journal of Bioinformatics. Volume 1, Issue 1, Pages 10-19.
- Mahesh S. Yandigeri, Kamlesh Kumar Meena, Divya Singh, Nityanand Malviya, Dhananjaya P. Singh, Manoj Kumar Solanki, Arvind Kumar Yadav, Dilip K. Arora (2012) Drought-tolerant endophytic actinobacteria promote growth of wheat (Triticum aestivum) under water stress conditions. Plant growth Regulation December 2012, Volume 68, Issue 3, pp 411-420.
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- Solanki MK, Kumar S., Panday AK., Srivastava S., Singh RK., Kashyap PL., Srivastava AK., Arora DK (2012). Diversity and antagonistic potential of bacillus spp. associated to the rhizosphere of tomato for the management of Rhizoctonia solani. Biocontrol Science and Technology, 22, 203-217.

Meeting of NBAIM

- QRT Meeting of NBAIM held at ICAR, Krishi Bhavan, New Delhi on 12th July, 2012 under Chairmanship of Deputy Director General (CS), ICAR
- QRT Meeting of NBAIM held at Directorate of Oil Seeds Research, Hyderabad on 5th October, 2012.
- QRT Meeting of NBAIM held at Central Institute of Brakishwater Aquaculture, Chennai on 15th December, 2012.
- QRT Meeting of NBAIM held at NBAIM, Mau on 28-29 August, 2012.
- Expert Consultation Meeting on Opening Avenues of Bioinformatics in Agricultural Research: Perspective and Challenges held at NBAIM, Mau on 26th November, 2012.
- QRT Meeting of NBAIM held at Conference Room, NASC Complex, New Delhi on 19th February, 2013.
- AMAAS review meeting held at Conference Room, NASC Complex, New Delhi on 20th February, 2013.
- Institute Management Committee Meeting of NBAIM held at Division of Biochemistry, IARI, New Delhi on 21st February, 2013.
- Research Advisory Committee Meeting of NBAIM held at NBAIM, Mau on 27th February, 2013.

Attended:

- Attended the 2nd meeting of National Advisory Board held on 13.08.2012 at NBFGR, Lucknow and discussed about Management of Genetic Resources of NBAIM, Mau.
- Visit NBAII, Hebbal, Bangalore for monitoring of AMMAS project coordinate by NBAIM, Mau on 10.07.2012
- Meeting of Regional Committee IV at A.N. Sinha Institute of Social Studies at Patna, Bihar from 21-22 September, 2012.
- QRT Meeting at DOR and COP 11 Exhibition on Biological Diversity from 30 September to 03 October, 2012.
- Deliver a lecture and have discussion with VC, SKUAST-J about AMAAS for XIIth Plan Period from 04-08 October, 2012.
- National conference on Managing threatening diseases on crop and a lead lacture was delivered at IISR, Lucknow from 02-04 November, 2012.
- Meeting of the QRT at CIBA, Chennai for presentations by the AMAAS Centres on 15th December, 2012.
- RFD Meeting at Delhi on 14th January, 2013.
- Global Consultation on Use and Management of Agrobiodiversity for Sustainable Food Security organized by NBPGR and Bioversity International from 12-14 February, 2013.
- QRT review meeting of NBAIM at NASC Committee Room, New Delhi on 19th February, 2013.
- AMAAS review meeting at NASC, Committee Room, New Delhi on 20th February, 2013.
- IMC meeting of NBAIM held at Division of Biochemistry, IARI, New Delhi on 21st February, 2013.
- National Workshop on "Foresight and Future Pathways of Agricultural Research through Youth in India" at NASC Complex, New Delhi from 01-02 March, 2013.
- Third NABMGR meeting at NBAGR, Karnal on 5th March, 2013.
- Director's conference at NASC Complex, New Delhi on 19-20 March, 2013.

Library Information and Documentation

Books	No.
Administration	121
Bacteriology	63
Biochemistry	143
Bioinformatics	62
Bioinstrumentation	01
Biotechnology	41
Environmental science	16
Integrated pest management	16
Microbiology	455
Molecular biology	203
Phycology	12
Plant pathology	101
Plant virus	03
Mycology	178
Genetics	43
Genomics	47
Intellectual property Right	70
Proteomics	09
Virology	38
Enzymology	06
Other	25
Biocontrol	06
Biostatistics	07
Biofertilizers	03
Soil biology	15
Yeast	05
Hindi book	62

Periodicals

Annual Review Microbiology [vol-47 to 56 (1993-2002), 58 to 65 (2004-2011)]

Annual Review Phytopathology [vol – 30 to 41 (1992-2003), 44 to 45 (2006-2007), 47 to 48 (2009-2010)]

Miscellaneous literature

Annual report of ICAR institutes
Complete solution for biotech Research
Advanced Biotech
Current content of life science
Catalogues
Dictionaries
ICAR News/ Bulletins
Laboratory Manuals

News Papers- Following daily newspapers are being received

Hindi: Dainik Jagran, Hindustan
English: The Times of India
Weekly: Employment News

Journal	No of Issues
Applied and Environmental Microbiology	24
Asian Journal of Microbiology, Biotechnology and Environmental Sciences	3
Biology and Fertility of Soils	21
Canadian Journal of Microbiology	43
Clinical Microbiology	4
Current contents	50
Current Science	135
FEMS Microbiology Reviews	1
Fungal Genetics and Biology	10
Indian Journal of Experimental Biology	24
Indian Journal of Microbiology	16
Indian Journal of Sugarcane Technology	21
Indian Phytopathology	74
Plant Pathology	23
Journal of Biosciences	24
Journal of Biotechnology	10
Journal of Eco-friendly Agriculture	5
The Indian Journal of Agricultural Science	3
Journal of Mycology and Plant Pathology	3
Journal of Scientific and Industrial Research	42
Indian Farming	2
Molecular Plant Microbe Interaction	29
Molecular Plant Pathology	13
Mycobiology	10
Mycologia	12
Mycological Research	18
Nature	120
Pestology	11
Plant Diseases	12
Soil Biology and Biochemistry	11
The Journal of the Indian Botanical Society	9
Eukaryotic Cell	11
Fungal Biology	1
Journal of Bacteriology	100
Microbiological Research	17
Science	46
Kheti	1
Indian Horticulture	1

RAC and IMC

Research Advisory Committee (RAC)

1. Dr. D.J. Bagyaraj, Chairman
2. Dr. H. Shekhar Shetty, Member
3. Dr. D.L.N. Rao, Member
4. Dr. Appa Rao Podile, Member
5. Dr. T.P. Rajendran, Assistant Director General (PP)
7. Dr. Arun Kumar Sharma, Director, NBAIM
8. Prof. Sudhir Meshram, VC, North Maharashtra University
9. Dr. Banwari Lal, Director Teri, New Delhi
10. Dr. Alok K. Srivastava, Member Secretary

Ninth Research Advisory Committee (RAC) Recommendations.

- RAC suggested to have some projects on field studies related to some important plant disease and its biological control.
- PAC suggested that the Cyanobacteria from salt affected region may be characterized and attempts may be made to isolate the strains from alkaline soil from nearby area and advised to analyze the soil samples properly to be sure about the salinity or alkalinity of the soils and the extent of salinity or alkalinity and suggested initiating research on mycorrhizal fungi and microbial control of post harvest diseases. Regarding research on endophytes.
- RAC suggested that the work related to endophytes should be done more carefully to establish the real endophytic association ship in case of actinomycetes and further suggested to look for secondary metabolites which may have potential role in imparting the medicinal traits of medicinal plants.
- Regarding microbial diversity analysis and to use out of the box approaches especially for the difficult to culture microbes. The field evaluation of biocontrol agents and to conduct trials on some identified crops in farmers' fields and that the accessions available in the culture collection may be evaluated for specific application in agriculture and post harvest loss management.
- The RAC suggested to start a network on rhizobia and photosynthetic bacteria.
- Assistant director General (Plant Protection) explained about the interrelationship and linkages between NBAIM, other institutions under NARS system, DBT, DST, CSIR, Universities etc., and stressed that under AMAAS, research program may be formulated with better representation and linkages with the researchers outside NARS system. He requested for the views of the RAC regarding the future road map of AMAAS national network project. He suggested NBAIM to review the assessment status of the products or application of microbes form the AMAAS network project.
- RAC suggested that new research area related to mass formulation of BCAs and identification services etc. may be started at NBAIM only after getting sufficient number of scientific manpower.
- A culture storage facility of duplicate set of microorganisms has been established at NBPGR, New Delhi on 1st of January, 2013. Second set of the cultures in lyophilized form and glycerol stock has been shifted. Further the accessions have reached to 4300 cultures.
- The RAC has taken note about the deployment of scientist as it is less than 50% of the sanctioned cadre strength and all the posts of Principal Scientists are lying vacant. The Director NBAIM and ADG (PP) informed that the ASRB recruitment process in progress for vacant pots.

IMC Members

1. Dr. Arun Kumar Sharma, Director, NBAIM
2. Dr. T.P. Rajendran, ADG (PP)
3. Dr. S.P.S. Ahlawat, Former VC, Vikram University, Muzaffarnagar
4. Dr. D.L.N. Raol, IISS, Bhopal
5. Dr. A.R. Alagawadi, UAS, Dharwad
6. Dr. R.D. Rai, IARI, New Delhi
7. Administrative Officer, Member Secretary

9th Institute Management Committee (IMC) Recommendations

- The proposal regarding establishment of Regional station may be reflected in the XII Plan EFC.
- Institute is to take appropriate action for repair and maintenance of UPS.
- No recommendation of the Committee on the agenda is recorded. It is not clear whether the item was discussed at all. As such no new work may be undertaken till the finalization/ clearance of XIIth Plan EFC. The PIM letter No. 5(3)/2013-PIM dated 20-04-2013 may be adhered to in this regard.
- Proposal may be included in the XIIth Plan EFC.
- Work under Plan may be carried out only after approval of XII Plan EFC. However, maintenance work under 'Non-Plan' within delegated powers of director may be carried out as per requirement / necessity.
- The instruction issued vide PIM letter No.5 (3)/2013-PIM dated 25-4-2013 may be adhered to.
- The agenda is repeated, as it has already been considered in agenda No. 3.1.Proposal is also ambiguous. However, a specific proposal is to be reflected in the XII Plan EFC along-with budget.
- Recommendation not approved, as the proposal for recognition of PVT. Hospital/ Nursing Homes is not supported by requisite documents. The Institute has to initiate action as per ICAR Guidelines issued **vide letter no. 3(23)82-Per.IV** dated 5-04-2013 copy enclosed)

NBAIM Personnel

Scientific staff

Dr. Arun Kumar Sharma - Director
Dr. Alok K. Srivastava – Senior Scientist
Dr. Sudheer Kumar – Senior Scientist
Dr. D. P. Singh – Senior Scientist
Dr. Renu – Senior Scientist
Mr.Anurag Chaurasia –Scientist
Mr.Udai Bhan Singh –Scientist
Dr. P. L. Kashyap –Scientist
Dr. Lalan Sharma –Scientist
Mr. Sanjay Goswami –Scientist
Dr. Dipak T. Nagrale –Scientist
Dr. Hillol Chakdar –Scientist

Technical staff

Manish Roy
Anchal Kumar Srivastava
Mahesh Yadav
Alok Upadhyay
Amit Rai
Ashutosh Rai
Shabana Khan

Administrative, finance and other supporting staff

Samar Nath Yadav
Shyamji Shukla
Rehan Asad Khan
Abhishek Kumar
Ashok Kumar
Siddarth Arora
Satish Pal
Amar Nath Singh Patel
Manoj Kumar
Bali Ram
Chetan Singh
Rekha Gupta
Ram Gopal
Ram Avadh Singh
Chandra Kishore
Anil Kumar Rana
Ashish Kumar
Ajay Vishwakarma
Subhash Kushwaha