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Annual Report 2007-08 Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)

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Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)

Nodal Centre of AMAAS

NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT MICROORGANISMS
राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो

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AMAAS

ICAR Networking Project on "Application of Microorganisms in Agriculture and Allied Sectors"

Annual Report 2007-08

वार्षिक प्रतिवेदन 2007-08

भाकृअनुप
ICAR

भारतीय कृषि अनुसंधान परिषद
Indian Council of Agricultural Research

Nodal Centre of AMAAS

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NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT MICROORGANISMS

Understanding and conserving our national heritage of agriculturally important microorganisms

First Review Meeting of the AMAAS Project held at NBAIM, Mau

Published by

Dilip K. Arora
National Coordinator AMAAS and
Director, NBAIM, Mau

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Preface

Microorganisms present in the soil play an important role in nutrient solubilisation, mobilization and recycling. They have very wide potential as they control soil-borne pathogens, stimulate plant growth, increase nutrients availability and accelerate decomposition of organic materials, and are anticipated to increase crop production as well as maintain sound environments for crop production. The health of the soil is chiefly determined by its resident microbial flora. 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. There is currently a gap of nearly 10 million tones of nutrients between what crops take out and what is added through fertilizers and manures. Thus there is an urgent need to recycle all available organics in a more efficient way and improve and expand biofertilizer usage.

Agriculture residue can be put to use in different ways by using microorganism that use it as substrate: development of enriched compost, vermicompost; production of bioethanol and certain enzymes like phytases, proteases, lipases and amylases. Therefore there is a need to develop cost-effective, ecofriendly and appropriate technology to maximize economic value of nutrients contained in agrowastes for sustainable agriculture.

Although research has been focused on most areas and soil types in the country, lesser attention has been paid to extreme environments like arid, saline and acid soils. Microorganisms present in the rhizosphere are reported to alleviate the salinity stress by different mechanisms. Thus it is necessary to develop a consortium of microorganisms that can help the plant to survive and yield more even under saline conditions. Thus emphasis has been given to management of abiotic stress through microbial inoculation.

The PGPR related research programme in India is unorganized in several centers on several crops. There is tremendous scope for working on a network mode in India as the crops are varied and with diverse climate and soil factors. Therefore, it was felt necessary to consolidate the research efforts on PGPR for reduced use of chemical fertilizers and plant protection chemicals. Apart from applied research, the basic mechanisms involved in rhizosphere competence, interaction among the microbial communities and cropping systems needs to be understood.

Despite the many achievements of modern agriculture certain cultural practices have actually enhanced the destructive potential of disease. Almost 30% of the yield in agriculture is lost because of combined effects of biotic and abiotic stresses with pathogenic fungi alone responsible for a reduction of about 12% plant disease control, therefore, now has become heavily dependent on pesticides to combat the wide variety of fungal diseases that threaten agricultural crops. Microorganisms can be exploited to overcome abiotic and biotic stress.

The field of microbial genomics has moved away from the primary initial focus on pathogens genomes to include the sequencing of diverse prokaryotes that occupy a range of environmental niches, and which are responsible for an array of environmental processes. Every genome that has been sequenced to date has provided new insight into biological processes, activities, and potential of these species that was not evident before the availability of the genome sequence.

The ongoing ICAR network project on 'Application of microorganisms in Agriculture and allied sectors' seek to initiate and strengthen the R&D efforts on various microbe based technologies that can be utilized to increase crop production, utilize agrowaste, manage abiotic stress, biocontrol of important insect pests and post harvest technology. It also seeks to strengthen research in the area of microbial diversity, identification and genomics. It seeks to strengthen infrastructure, research capacity and human resources of ICAR institutions with respect to various microbe-based applications. The project is operative in 67 centres throughout the country and was having total budget outlay of Rs 1600.09 lakhs in the first year of its implementation. There are six thematic areas: Microbial

diversity and identification, Nutrient management, Plant growth promoting rhizobacteria (PGPR), Antagonists, Biocontrol agents and Disease management, Microbial Management of Agrowaste, Bioremediation and Microbes in Post Harvest and Processing, Microbial management of abiotic stress, Microbial genomics and Human resource development. The second annual progress report from April 2007 to March 2008 is a compilation of significant achievements during this period. The progress report for each center is compiled theme wise. I thank all the Principal Investigators of the project for their contribution to bring out the progress report in time. I would like to thank Dr. Mangala Rai (Secretary, DARE and Director General, ICAR), Dr. P. L. Gautam, DDG (CS) and Dr T. P Rajendran, ADG (PP) for their valuable guidance in giving meaningful shape to this network project. The funding provided by ICAR is duly acknowledged.

Prof. D. K. Arora
Director, NBAIM

Executive Summary

Microorganisms represent by far the richest repertoire of molecular and chemical diversity in biological world. They underlie basic ecosystem processes such as the biogeochemical cycles and food chains, as well as maintain vital and often elegant relationships between themselves and higher organisms. Microbes provide the fundamental underpinning of all ecosystems. The enormous functional diversity across the country needs to be deciphered and utilized to interweave microbes in agriculture and allied sectors. Hence it is necessary to explore, preserve, conserve and utilize the unique microbial flora of our country for fulfilling the emerging food and nutritional needs, clean environment and improved soil health for sustainable production. Several agriculturally important microorganisms are used as biofertilizers, biocontrol agents, plant growth promoters, bioremediators and decomposers.The recognition that microbial genetic resources as well as plant genetic resources comprises a valuable resource for developing countries resulted in the establishment of the Convention on Biological Diversity in Rio in1992. At the national level, the technology developed will constitute an important tool exploration and assessment of microbial biodiversity.

Microorganisms, being the pioneer colonizers on this planet, have come to stay as a cosmopolitan conglomerate of highly compatible organisms. They abound in habits with extremes of temperature, pH and water and salt stresses. The recognition of 'deep hot biosphere' with unique microbial-animal assemblages and nutrient dynamics, speaks of versatility and importance of microbes in sustaining the life. Bestowed with remarkable inherent physiological and functional diversity 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. Not with standing the existing knowledge of microbes and microbial processes, we are still at the base of microbial diversity, which needs to be explored, investigated and exploited. Judicious and gainful utilization of this nature's treasures however, depends on the mankind. Till now very limited and isolated efforts were made to tapping of microbial diversity, identification, evaluation and preserving them for different applications. The present project aims at coordinated efforts leading to exhaustive surveys, isolations, and identification, molecular diversity, bioprospecting and conserving the microbial diversity from various niches for varied applications.

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 ha sled to serious nutrient imbalances, impaired soil health and declining factor productivity. There is currently a gap of nearly 10 million tones of nutrients between what is taken out by crops and what is added through fertilizers and manures. Thus there is an urgent need to recycle all available organics in a more efficient way and improve and expand biofertilizer usage. These are the only feasible and low cost and eco-friendly way of improving nutrient supply and improving soil health in the short and medium run. In many cases nutrients are locked in unavailable forms in soils. Mobilizing such reserves through microbes is an urgent imperative. Hence it is now strongly realized that integrated plant nutrient supply systems involving a combination of biofertilizers is the only alternative to improve nutrient use efficiency, sustain crop production and improve soil health. This requires us to strengthen microbiological alternatives as nutrient sources. Neglecting biofertilization will therefore be dangerous.

Plant Growth Promoting Rhizobacteria (PGPR) is one of the components of soil and could be effectively utilized for sustainable agricultural production by enhanced growth and disease suppression. The PGPR related research programme in India is unorganized in several centers on several crops. There is tremendous scope for working on a network mode in India as the crops are varied and with diverse climate and soil factors. There is a need to consolidate the research efforts on PGPR for reduced use of

chemical fertilizers and plant protection chemicals. Apart from applied research, the basic mechanisms involved in rhizosphere competence, interaction among the microbial communities and cropping systems needs to be understood.

Biological control agents (BCAs), particularly fungal and bacterial agents, offered considerable potential (generally unexploited) for insect, disease and weed control. There are a number of biological agents commercially available for use in crop protection, most notably products based upon *Bacillus thuringiensi*s and considerable progress has been made in the development of fungal BCAs in the last decade. Several agents are currently being used in niche markets and many others are at various stages of commercialization.

Agricultural wastes are the left over material generated as part of any agricultural and allied activities. In India about 355.67 – 507.836 m ton of crop residues are generated every year. Three fourth of total residues are produced by three crops viz. rice, wheat and oilseeds. Apart from crop residues, process based residues contribute 146.932 m ton, forestry based residue (22 m ton) and agro based wood residues 0.017 m ton. All these crop residues are the potential source of organic manure for use in agriculture. On an average, these crop residues contain 0.5% N, 0.2% P_2O_5 and 1.5% K_2O. Assuming that 50% of crop residues are utilized as cattle feed and fuel, the nutrient potential of the remaining residues will be 3.541 million tons of NPK per annum, which accounts for 25% of total NPK consumption in India.

Microorganisms are endowed with the ability to degrade and/or detoxify chemical substances such as petroleum products, xenobiotics including PAHs and PCBs, pesticides, heavy metals and are thus utilized in Bioremediation and cleaning environmental pollution. Their ability to withstand biotic and abiotic stresses successfully as compared to plants has made them an excellent source for various genes that can be used to develop transgenic crop plants.

Microorganisms have been implicated in alleviating the effects of abiotic stress by different mechanisms. Predominant are alteration in the availability of nutrients so as to maintain Na:K ratio in the plant, production of antioxidants to prevent injury to the plant because of salt stress, and production of growth promoting substances. In addition, bacterial exopolysaccharides have been implicated in providing protection from environmental stresses and host defenses. AM fungi plays an important role in mitigating drought stress and improves the uptakes of water under drought stress.

There are numerous examples where microbial genes have been used for development of transgenics against biotic and abiotic stress. By means of the advances of the latest functional genomics to further discover our microbial gene resources and reveal their molecular mechanism for biocontrol is not only the most significant needs for sustainable agriculture and economy development but also the critical and basic science problem to be solved for the development of biopesticide. During the last decade many resistance genes whose products are involved in recognizing the invading pathogens have been identified and cloned. Many of the antifungal compounds synthesized by plants which combat fungal infections have been identified. The field of microbial genomics has moved away from the primary initial focus on pathogens genomes to include the sequencing of diverse prokaryotes that occupy a range of environmental niches, and which are responsible for an array of environmental processes. Every genome that has been sequenced to date has provided new insight into biological processes, activities, and potential of these species that was not evident before the availability of the genome sequence. Most significantly, the tremendous success of genome sequencing has allowed us to pursue other avenues where we can now derive genomic information from the multitudes of uncultivable prokaryotic species and complex microbial populations that exist in nature. The identification of new genes from indigenous microbes will help in development of transgenic crops tolerant to abiotic and biotic stress

The ICAR network project on 'Application of microorganisms in Agriculture and allied sectors' encompasses 61 centres with a major objective to strengthen the research efforts in the area of agricultural microbiology and to develop technologies that can have far reaching effect on Indian agriculture.

The major objectives of the Network project are:

- Deciphering the structural and functional diversity of agriculturally important microorganisms and to develop "microbial map" of the country.
- Improving nutrient use efficiency through microbial interventions for sustainable crop production and maintenance of soil health.
- Characterization of plant growth promoting rhizobacteria and to develop bioconsortium for enhanced growth and yield of important crop plants.
- Formulation of microbe or microbe-based preparations for biocontrol of phytopathogens, insect pests and weeds.
- Development of microbe-based technologies for agrowaste management and biodegradation for sustainable crop production.
- Harnessing microbial activities for bioremediation of

organic and inorganic environmental pollutants.

- Management of abiotic stresses using microorganisms.
- Development of microbe mediated processes for product development and value addition in agriculture.
- Diagnostic kits for the identification of important plant pathogens, fish and animal pathogens.
- Post harvest technology for the important fish species of India.
- Diversity of ruminanat microorganisms and their utilization.
- Unraveling microbial genomics for its utilization in agriculture and industry.
- Human resource development in microbe conservation and utilization.

The network project have 6 components:

1. Microbial diversity and identification
2. Nutrient management, PGPR and Biocontrol
3. Agrowaste management, Bioremediation and Microbes in Post Harvest & Processing
4. Microbial management of abiotic stress
5. Microbial genomics
6. Human resource development

AMAAS : An Introduction

Theme 1 : Microbial Diversity and Identification

General Objectives of the theme "Microbial Diversity and Identification"

1. Microbial diversity analysis from various ecoregions/exotic environments.
2. Identification of microorganisms using conventional and molecular techniques.
3. Application of some important microbes in agriculture and allied sectors for enhancing food productivity.

Sub-Thematic Areas in the Microbial Diversity and Identification Component

1. Analysis of microbial diversity in terrestrial ecosystem.
2. Diversity in aquatic ecosystem.
3. Diversity in fermented dairy products.
4. Diagnostic kits for plant pathogens and soil microbes.

Sub Theme I: Microbial diversity in terrestrial ecosystem

Objectives

1. To Isolate and characterize microorganisms from different agro-ecological regions of India.
2. Identification of microorganisms based on conventional and molecular techniques.
3. Development of microbial map of the country.
4. To study the functional diversity of the microorganisms.

Sub Theme II: Microbial diversity in aquatic ecosystem

Objectives

1. To study the culturable microbial diversity of aquatic animals from different aquaculture systems.
2. To screen, characterize, identify microorganisms from diverse aquatic environments such as seas, high altitude lakes and other water bodies with properties such as salinity tolerance, cold tolerance, decomposition of resistant organic material, as a source *of* novel genes and compounds.
3. To isolate, characterize and document microbes for bioremediation of pesticides, heavy metal contamination and organic load in aquatic environment.

Sub Theme III: Microbial diversity of dairy products

Objectives

1. Microbial diversity in Indian fermented dairy foods (Dahi, lassi, Shrikhand, Misthi, Dahi and Cheese).
2. Molecular typing of new isolates/ available NCDC cultures.
3. Screening of microorganisms for novel probiotic/functional properties and their application

Sub Theme IV: Diagnostic kits for plant pathogens and soil microbes.

Objectives

1. To isolate, characterize, evaluate plant pathogens (*Macrophomina*, *Fusarium*) soil microbes (*Bacillus* and *Pseudomonas)*.
2. To study the genetic diversity of microbes obtained from different crops.
3. To develop rapid diagnostic kits for plant pathogens, and soil microbes.

Theme 2 : Nutrient Management, PGPR and Biocontrol

General Objectives of the Sub Theme: Nutrient Management

1. To study the culturable microbial diversity of soils from different agro-ecological sub-regions, production systems and land use practices, including in stressed ecosystems.
2. To characterize the isolated microorganisms for their nutrient mobilization (N, P, Micronutrients).
3. To evaluate the establishment of strains, particularly in mixed cropping systems and select strains for multiple crops and geographical locations.
4. To standardize methods for mass multiplication and identify appropriate delivery systems and improve the formulations, quality, shelf life of the above bio-agents with superior delivery systems.
5. To carry out multi-location testing for evaluation of the promising formulations.
6. To make multiple-repositories of isolated strains of microorganisms.

General Objectives of the Sub Theme: Plant Growth Promoting Rhizobacteria:

1. To isolate, characterize, evaluate and utilize rhizobacteria and their primary and secondary metabolites specific for growth promotion and pathogen suppression.
2. To assess the ecological plasticity of strains particularly in mixed cropping systems and identification of strains for multiple crops and geographical locations.

3. To study the rhizobacteria mediated induced systemic resistance in crop and adopting this for crop management.
4. To study the mechanism of rhizobacteria induced growth promotion in crop plants.
5. To develop bioconsortium of geographically, phenotypically and genotypically distinct rhizobacterial strains and standardize methods for mass multiplication and develop appropriate delivery systems.

General Objectives of the Sub Theme: Antagonists, Biocontrol Agent and Disease Management

1. To isolate and characterize antagonistic organisms from diverse ago-climatic/cropping systems in India for pest and disease management.
2. To screen potential isolates against major soil/ seed/air borne plant pathogens, nematodes and insect pests of important crops.
3. To identify strains with broad host range or specific to a group of plant pathogens and insect pests and develop improved strains by molecular interventions.
4. To study different mechanisms like biochemical and molecular interaction between promising antagonists against pathogens/pests.
5. To develop formulations suitable for various delivery systems and their evaluation against target pests.
6. To establish mass production facility for identified bioagents.

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

General Objectives of sub theme: Microbial Management of Agro waste

1. Isolation, identification and characterization of microorganisms from various selected agro, industrial and urban wastes.
2. Development of microbial consortia for rapid degradation and effective utilization of selected waste.
3. Production of value added products like bio-fuels, enzymes and mushroom using selected agro, urban and industrial wastes.
4. To assess the impact of organic waste application in agriculture on shifts in soil microbial community structure and functions in relation to soil physiochemical properties.

General Objectives of sub theme: Bioremediation

1. Develop an understanding of the structural and functional diversity analysis of microbial communities and their dynamics in response to normal environmental variation and novel anthropogenic stresses.
2. Determine the biochemical mechanisms, including enzymatic pathways, involved in aerobic and anaerobic degradation of pollutants.
3. Expand understanding of microbial genetics as a basis for enhancing the capabilities of microorganisms to degrade pollutants.
4. Conduct microcosm/mesocosm studies of new bioremediation techniques to determine in a cost-effective manner whether they are likely to work in the field, and establish dedicated sites where long-term field research on bioremediation technologies can be conducted.
5. Develop, test, and evaluate innovative biotechnologies, such as biosensors, for monitoring bioremediation *in situ*; models for the biological processes at work in bioremediation and reliable, uniform methods for assessing the efficacy of bioremediation technologies; establish a culture collection for bioremediation purposes.

General Objectives of sub theme: Microbes in Post Harvest & Processing

1. Development of fermented products from fruits, vegetables and cereals.
2. Value addition of pulses, millets and horticultural produces through microbial fermentation.
3. Biopreservation of vegetables for extension of shelf life and control of spoilage in processed products.
4. Assessment of microbial contamination and safety of agricultural produce.

Theme 4 : Microbial Management of Abiotic Stress

General Objectives of the theme: Microbial management of abiotic stress

1. Isolation of microorganisms from rhizotic zones of cereal crops (wheat and millets) grown under stress conditions of salt, drought and extreme temperatures.
2. Selection of bacteria capable of growing under stress conditions of salt, drought and extreme temperatures.
3. Evaluation of the selected organisms in the rhizosphere of wheat and millets (phytotron studies).
4. Biochemical characterization of selected microorganisms
5. Development of consortium of microorganisms that can alleviate the effect of drought, salinity and extreme temperature.
6. Field evaluation of consortium of microorganisms for

improvement of wheat, rice and millets under stress conditions.

Theme 5 : Microbial Genomics

Sub themes in Microbial Genomics

1. Structural Genomics
2. Functional Genomics

Sub theme: Structural Genomics:

- Genome analysis of the nitrogen-fixing symbiotic bacterium *Mesorhizobium ciceri*

Overall Objective of structural genomics

- Complete genome sequencing of *Mesorhizobium ciceri*.

Overall Objective of functional genomics

- Isolation of genes and their alleles for abiotic and biotic stress tolerance from isolates of *Pseudomonas flourescens*, *Anabaena* and through metagenomes.
- Sequence determination of the isolated genes.
- Functional validation of selected alleles in microbes and model plants

Project Wise Significant Achievements for the Year 2007-08

Theme: Microbial Diversity and Identification

Mapping, assessment of the geographical distribution and *in vitro* conservation of agriculturally important microorganisms of the Western Ghats of India

PI : A. R. Alagawadi
Co PIs : P.U. Krishnaraj, K.S. Jagadeesh & R. Vasudeva
Department of Agril. Microbiology, UAS, Dharwad – 580 005

Objectives:

- Isolation, enumeration, characterization, and inventorization of AIMs (N_2 fixers, P-solubilizers, VAM, PGPRs, fluorescent pseudomonads, chitin decomposers, cellulose and lignin degraders) along the Western Ghats and identification of potential isolates.
- Assessing the geographical distribution and developing thematic maps for the above groups of organisms.
- Assessing the functional potentials of each group of organisms for use in agriculture and the molecular diversity of a key selected species
- Setting up the culture bank of the potential isolates under each group and deposit with the NBAIM.
- Setting up the Western Ghats region specific data base on the population and diversity of AIMs.

Significant Achievements:

- The samples collected from different locations in western Ghats during the year 2007-08 (fig 1) yielded 592 isolates consisting of 109 *Azotobacter*, 53 *Azospirillum* (all from endorhizosphere), 153 P-solubilizers (146 PSB and 7 PSF), 40 fluorescent pseudomonads, and 41 PPFMs (all form leaf samples) and 122 lignin degraders.
- Morphological, biochemical and physiological characterization of 198 *Azotobacter* isolates, 36 *Azospirillum*, 28 *Beijerinkia*, 170 phosphate solubilizing bacteria and 84 fluorescent pseudomonads has been completed and the identification of these isolates is in progress.
- The nitrogen fixing ability of the 109 *Azotobacter* isolates ranged from 2.97 to 25.79 mg/g of carbon

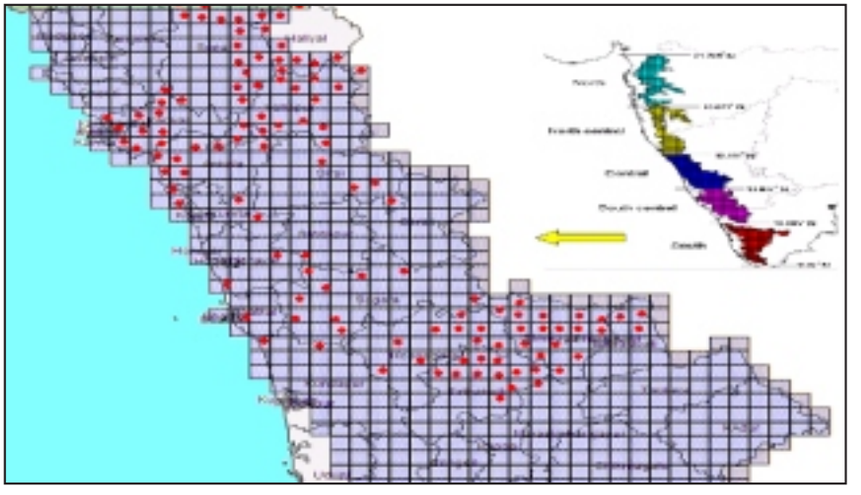

Fig 1. Grids/sites of sampling in the Central Western Ghats 2006-08

source utilized. While 36 *Azospirillum* isolates fixed N_2 in the range of 7.21 to 11.27 mg/g of carbon source used, 28 *Beijerinckia* isolates fixed N_2 in the range of 1.05 to 7.77 mg/g carbon source utilized.

- All 36 *Azospirillum* isolates, 44 isolates of fluorescent pseudomonads were subjected to quantitative analysis of IAA and GA. The amounts of IAA and GA produced by the *Azospirillum* isolates were in the range of 480 to 1011 µg/L and 44 to 143 µg/L broth, respectively. The isolates of fluorescent pseudomonads produced IAA in the range of 30 – 782 µg/L and that of GA in the range of 55 – 260 µg/L.
- One hundred and forty four PSB isolates, 7 PSF isolates and 84 isolates of fluorescent pseudomonads were examined for their ability to solubilize TCP. While PSB isolates showed TCP solubilization in the range of 6.24 – 16.07%, PSF isolates recorded 15.67 – 32.10% TCP solubilization. Fifty two out of 84 fluorescent pseudomonads were found to solubilize TCP which was in the range of 9.05 – 84.87%.
- The biocontrol potential of 30 fluorescent pseudomonads against five fungal plant pathogens viz., *Alternaria carthami, Fusarium oxysporum* f. sp. carthami, *Sclerotium rolfsii* , *Rhizoctonia bataticola* and *Pyricularia oryzae*; and three bacterial pathogens viz., *Xanthomonas axanopodis*, *X. compestris* and *Ralstonia solanacearum* was also tested under in vitro conditions (fig 2). Out of 30 isolates 8 were inhibitory to all the 8 plant pathogens tested whereas two isolates each were inhibitory to 7, 6 and 5 pathogens; 10 were inhibitory to 4 pathogens and six were able to control three pathogens.
- The other functional groups of organisms isolated from the study area include pink pigmented facultative methylotrophs (PPFMs), cellulose degraders and lignin degraders which are being assessed for their functional properties.

Conclusion:

Samples of soils, roots, leaf samples, decaying wood samples, mushrooms, leaf litter samples and termite mound samples were collected from 48 grids in the study area and used for isolation of AIMs. Morphological, biochemical and physiological characterization of *Azotobacter*, *Azospirillum, Beijerinkia,* phosphate solubilizing bacteria and fluorescent pseudomonads has been completed. The nitrogen fixing ability of *Azotobacter, Azospirillum* and *Beijerinckia* isolates ranged from 2.97 to 25.79 mg 7.21 to 11.27 mg and 1.05 to 7.77 mg/g carbon source utilized respectively. The amounts of IAA and GA produced by the *Azospirillum* isolates ranged from 480 to 1011 µg/L and 44 to 143 µg/L broth, respectively and fluorescent pseudomonads produced IAA in the range of 30 - 782 µg/L and that of GA in the range of 55 - 260 µg/L. PSB isolates showed TCP solubilization in the range of 6.24 – 16.07% and PSF isolates recorded 15.67 – 32.10% TCP solubilization. 52 out of 84 fluorescent pseudomonads were found to solubilize TCP in the range of 9.05 - 84.87%. Eight out of 30 fluorescent pseudomonads were found to inhibit all the eight plant pathogenic fungi and bacteria (*Alternaria carthami*, *Fusarium oxysporum f. sp. carthami*, *Sclerotium rolfsii*, *Rhizoctonia bataticola* and *Pyricularia oryzae*, *Xanthomonas axanopodis*, *X. compestris, Ralstonia solanacearum*) under *in vitro* conditions.

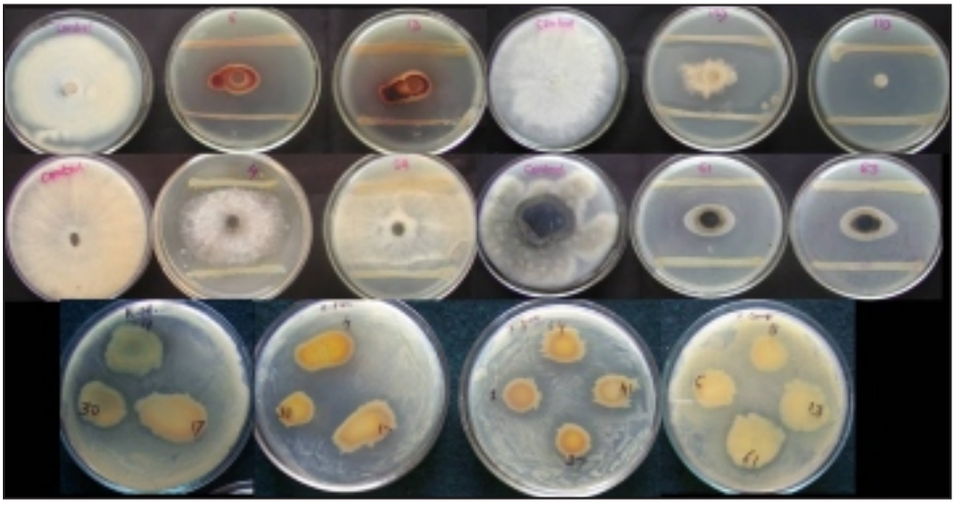

Fig 2. Biocontrol ability of fluorescent pseudomonads of Western Ghats against plant pathogens

Diversity analysis of microbes in extreme conditions

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National Bureau of Agriculturally Important Microorganisms, Mau, Uttar Pradesh

Objectives:

- Microbial diversity analysis in extreme climates.
- Identification of osmolyte production by extremophilic bacteria.
- To look for the production of enzymes (amylase, cellulase, CMCase, xylanases and protease) in thermophilic bacteria.
- Molecular characterization of extremophilic bacteria.

Significant Achievements:

- Soil and water samples were collected from extreme environments: Manikaran thermal springs, Vashisht sulphur springs, Kerala acidic Soils, Sambhar salt lake (Rajasthan) and mangroves of Sunderbans.
- From Rajgir thermal springs, earlier we reported the isolation of diversity of bacteria. A total of 29 isolates were characterized through 16S rDNA and 16-23S rDNA –RFLP analysis with three restriction endonucleases. Combined dendrogram revealed that most of the isolates were different at molecular level (fig 2). However sequencing of the 16S rDNA identified the isolates as belonging to 5 different genera: *Pseudomonas aeruginosa* (5), *Acinetobacter junii* (1), *Acinetobacter baumannii* (7), *Acientobacter Caloaceticus* (2) and *Aquitalea maqnusonii* (3). The sequences were submitted to NCBI GenBank and have the Accession numbers : EU661693 to EU 661712.

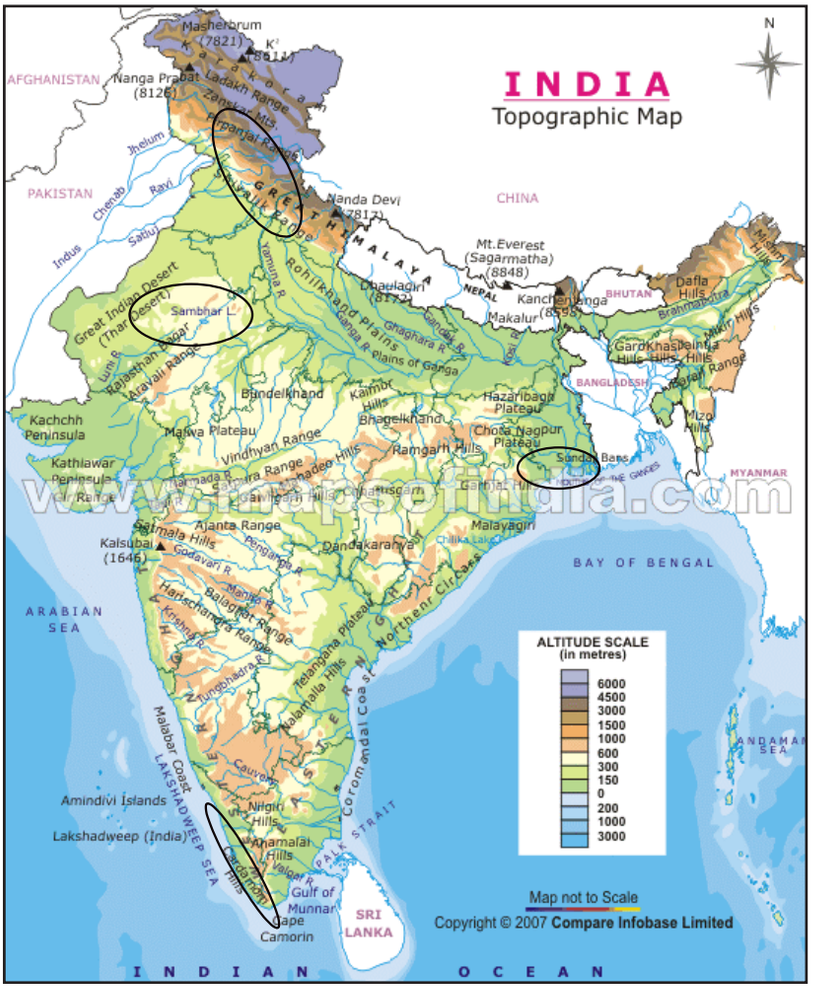

Fig 1. Sampling sites shown on map of India

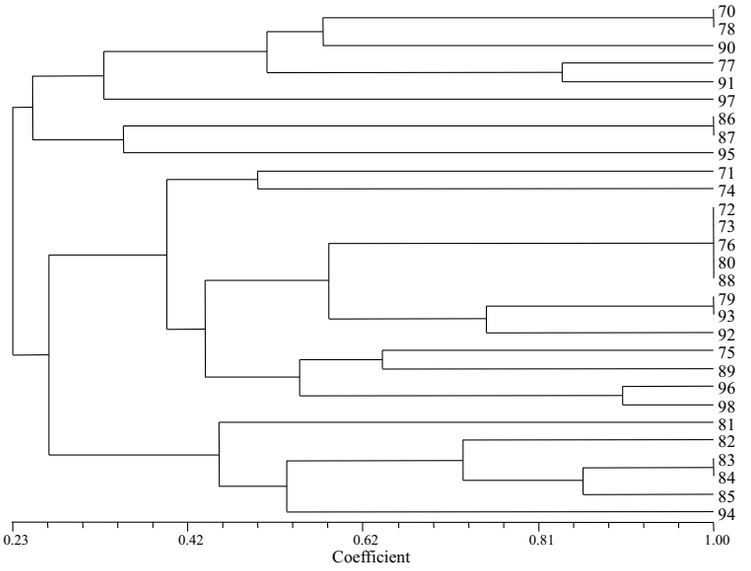

Fig 2: Dendrogram showing similarity among *Bacillus* isolates obtained from acidic soils of Kerala based on 16SrDNA –RFLP with restriction endonuclease (RE) *Alu* 1, *Msp*I and *Hae* III; and 16-23S rDNA RFLP analysis wth RE *Alu* 1, *Mbo*I and *Hae* III.

- A total of 31 isolates were obtained from soil samples collected from Kerala. All the isolates were able to grow at a pH of 4.0 and only one isolate could grow at a pH of 3.0.
- Isolation of *Bacillus* species was carried out from the soil samples collected from different districts of Kerala. The population of *Bacillus* isolated at different temperatures of incubation is shown in table 1. In general the population count was more in samples incubated at 37^{0}C. A total of 41 different morphotypes were selected for studies on functional attributes and molecular diversity.
- Of the 41 isolates obtained, 28 and 22 were positive for xylanase and cellulase activity. Of the biocontrol attributes, 7 isolates were positive for siderophore production, 17 could produce ammonia whereas 18 could produce HCN. As regards to the plant growth promoting attributes, 18 showed P-solubilization on Pikovskaya's medium whereas 23 were positive for IAA production.

Table 1: Total *Bacillus* count in soil samples collected from Kerala

Sampling area	Sample type	cfu x 10^4 g^{-1} soil			Morphotypes selected
Kerela region		30ºC	37ºC	45ºC	3
Alun Kaljar	Soil	300	20	0.3	5
Aroor	Soil	5	0.5	10	7
Cherathole	Soil	0.7	2	1.5	6
Turavoore	Soil	3	10	25	3
Allappy	Soil	0	25	0	2
Allappy	Soil	3.5	3.0	0	7
Allappy	Soil	10	5	0	8

PCR amplification of 16S rDNA gene followed by RFLP analysis with Alu1 showed the presence of wide range of diversity among the *Bacillus* isolates (fig 3). Among the 41 isolates, 10 diffferent clusters were formed with similarity % ranging from 3 to 100 % (fig 4). The clustering of the isolates was irrespective of their geographical location

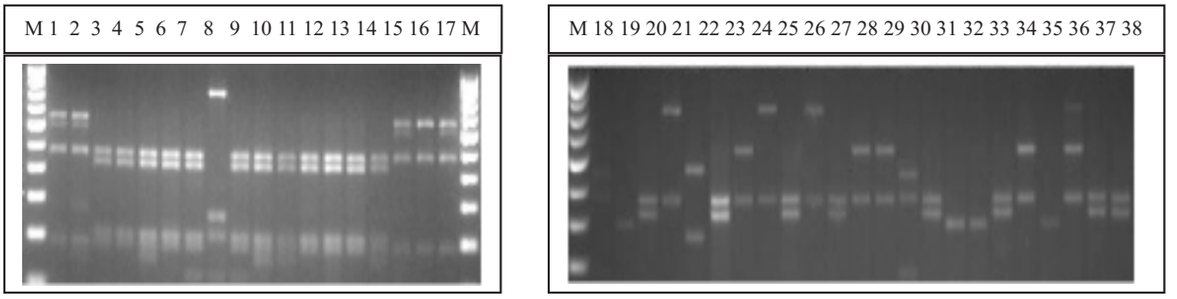

Fig 3: Restriction patterns of PCR amplified fragment of 16S rDNA of *Bacillus* isolates digested with *Alu*1, M: Molecular Marker 100 bp ladder, 1-38 *Bacillus* isolates

- Twenty isolates of *Bacillus* were obtained from the soil samples collected from Leh and Laddakh. Of the 20 only one isolate appeared on plates incubated at 45^{0}C and 3 isolates on plates incubated at 37^{0}C. All other isolates were obtained at 30^{0}C.
- From Sambhar salt lake, 29 isolates were obtained. Except for one sample, none of the sample showed any growth on Jensen's N-free medium indicating the absence of N-fixing bacteria at high salt concentration (fig 5). All isolates were screened for salt tolerance and 7 isolates showed growth upto 10% NaCl level. One of the isolate was found to grow even at 25% NaCl concentration.
- Employing five different media for the isolation, from Manikaran and Vashisht thermal springs, 77 bacteria were obtained. Out of 77 cultures 60 cultures could grow at temperature of 45, 30 at 55 °C and 12 cultures at 65 °C. These 12 cultures were further screened up to a temperature of 90 °C. Nine cultures retained more than 50% growth even when exposed to 90 °C for 10 min (Table 2).

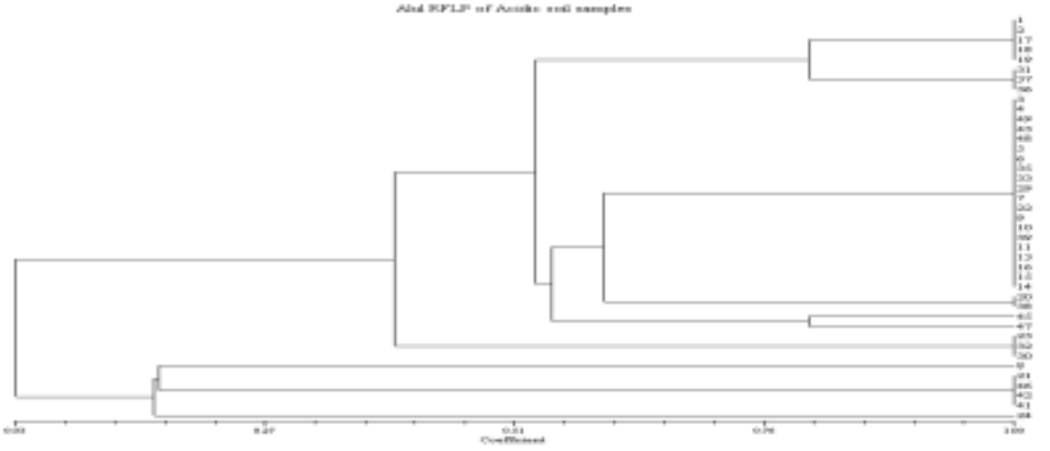

Fig 4. Dendrogram showing similarity among *Bacillus* isolates obtained from acidic soils of Kerala based on 16SrDNA –RFLP with restriction endonuclease *Alu* 1.

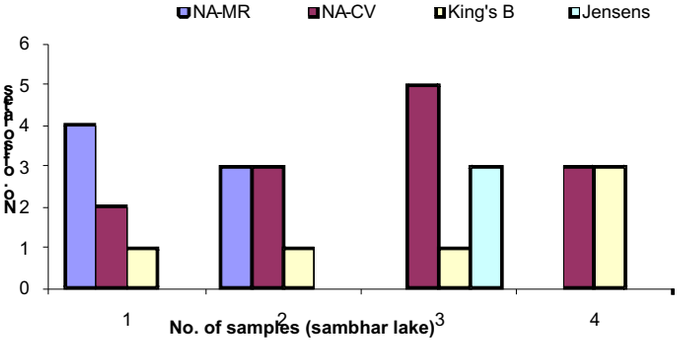

Fig 5: Number of isolates (different morphotypes) obtained from sambhar salt lake samples on different media.

Table 2: Colony forming unit (cfu) ml^{-1} of temperature tolerant bacteria when incubated at different temperatures for 10 min.

Isolate No.	cfu ml^{-1} x 10^6				
	50 °C	60 °C	70 °C	80 °C	90 °C
11	51.33	49.00	47.33	45.66	30.00
15	55.00	50.66	49.66	47.66	-
19	50.66	45.33	44.00	27.66	-
21	46.00	43.66	41.66	25.66	-
28	193.33	186.00	168.33	165	95.00
30	135.00	133.66	127.66	126.33	83.66
37	150.00	114.00	111.00	99	43.00
37A	29500	29400	29400	28600	3700
47	147.33	143.00	127.66	123.33	57.66
48	94.33	94.00	85.66	84.33	31.00
56	142.00	139.00	130.00	83	20.00
50 A	118.33	105.83	103.00	102.33	39.00

- Out of 77 isolates from Manikaran and Vashisht, 13 isolates were positive for xylanase activity and 11 were positive for amylase activity. Eleven isolates that showed xylanase production at 65 °C were quantified and isolate RXA-9 showed the maximum xylanase activity (Table 3).

Table 3: Xylanase Production by potent thermophilic bacterial isolate.

Isolate No.	Temp.	Zone diameter(cm)	Xylanase Activity(IU/ml)
H-7	65 °C	2.7	102.5
H-8	65 °C	2.8	63.3
H-9	65 °C	2.3	79.4
H-11	65 °C	1.8	69.4
11	60 °C	1.4	88.1
37	60 °C	2.5	67.0
37A	60 °C	2.0	67.3
RXA-1	50 °C	1.8	145.5
RXA-2	50 °C	1.7	103.8
RXA-9	50 °C	3.0	410.0
RXA-10	50 °C	2.1	87.0

Conclusion:

Analysis of diversity from different extreme environments hot springs, cold deserts, acidic soils and salt lakes has led to the isolation of unique microorganisms that are tolerant to high temperature (90 °C), high salt concentration (25% NaCl), low pH (pH 3.0). The huge database of microorganisms generated from extreme environments will help in identification of new species and genera. The sequencing of 16S rRNA gene helped in the identification of bacteria isolated from thermal springs. These thermal springs are known to have healing properties but the microorganisms isolated from these springs were all opportunist human pathogens and are Gram –ve. The presence of enzyme activity (xylanase, cellulase and amylase) in these bacteria gives a wide database for selection of potent enzyme producers that can be industrially exploited.

Development of Diagnostic kit for the identification of *Bacillus* and fluorescent *Pseudomonas*

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National Bureau of Agriculturally Important Microorganisms, Mau, Uttar Pradesh

Objectives:

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

Significant Achievements:

- Survey of the fields in Punjab was carried out for collection of soil and plant samples from different cropping systems (fig 1).
- In the last annual report, it was reported that all the species of *Bacillus* yielded a fragment of 265 bp when 16S rDNA is digested with restriction endonuclease *Alu*I. None of the *bacillus* derived genera and *Bacillus* related genera showed the presence of this fragment.
- The presence of 265 bp fragment was linked to the identification of *Bacillus* as a genus. The sequence of this region was hypervariable and was found to contain information for the identification of species.
- Based on the results primers were designed to specifically amplify the hypervariable region of 265 bp fragment. Three primers were designed, two forward and one reverse with the following sequences.

  265F1 - 5' GTGCTACAATGGACAGAACAA 3'

  265F2 - 5' ATACGGCTACCTTGTTACGAC 3'

  256R1 - 5' GTGAGATGTTGGGTTAAGTC 3'
- These primers were used for amplification and a combination of 265F1 and 265R1 was found to yield a single amplicon of about 220 bp (fig 1).
- Sequencing of 220 bp product of some of the reference strains obtained from ATCC (*B. brevis* ATCC8246; *B. cereus* ATCC10876; *B. laterosporus* ATCC 64; *B. megaterium* ATCC 4525; *Geobacillus stearothermophilus* ATCC 7953, *Paenibacillus polymyxa* ATCC 43865), NCIM (*Bacillus firmus* 2264; *B. thruingensis* NC 5116; *B. circulans* NC 2107) and NBAIM (*B. subtilis*; NBAIM-696; *B. licheniformis; B. circulans*) was carried out and BLASTn search was done.
- The results of BLASTn search confirmed that sequencing of this small region could help in the identification of *Bacillus* upto species level. The sequences showed 99% homology and in some cases up to 100% homology with the sequences of the species for which the fragment is amplified.
- Multiple alignment of sequences revealed that this is the hypervariable region of the 16S rRNA gene.
- Based on the sequencing of small fragment of about 220 bp, the following isolates obtained from Indo Gangetic region were identified up to species level following BLAST search : *Bacillus subtilis, Bacillus pumilus, Bacillus thuringiensis* (showed similarity with *B. cereus, B. anthracis*), *Bacillus cereus, Bacillus aminovorans, Bacillus licheniformis* (showed similarity with *B. subtilis*), *Bacillus circulans, Bacillus coagulans* and *Bacillus stearothermophilus*.
- Some of the cultures were further confirmed for their identity by complete gene sequencing. The results confirmed that partial sequencing of 265 bp region can give the identity of the species.
- Accession numbers and identity of some of the sequences submitted to NCBI GenBank are EU430985 *Bacillus oleronius*, EU430986 *Paucisalibacillus globulus*, EU430987, *Bacillus stearothermophilus*, EU430989 *Bacillus circulans*, EU 439990 *Bacillus pumilus*, EU430993 *Bacillus fusiformis*.
- Fluorescent *Pseudomonas* was isolated from soil samples collected from different locations; 5 from Dehradun 29 from Rajgiri thermal springs, 9 from Sunderbans, 35 from Maunath Bhanjan, 41 from Kanpur – Allahabad and 78 from Lucknow area. These isolates were picked based on the colour of fluorescent pigment and colony morphology.

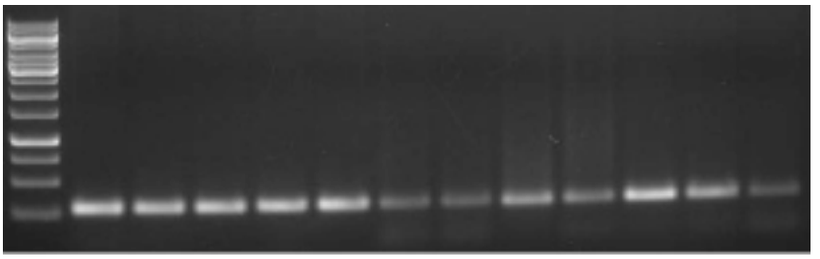

Fig 1. PCR Amplification of 220 bp fragement in some *Bacillus* strains using the designed primers 265F1 and 265R1.

Conclusion:
Based on the results of the present investigation we suggest a simple approach for identification of *Bacillus* sp. per se and to classify them into different species: PCR amplification of 16S rDNA; development of ARDRA with *Alu*I, look for the presence of 265 bp band to identify genus *Bacillus,* to carry out nested PCR using primer pair 265F1 and 265R1, sequencing of 265 bp fragment to predict species. This technique will help in the rapid and accurate identification of the *Bacillus* species and is economically cheap, as it does not require the sequencing of complete 16S rRNA gene.

Development of diagnostic kits for fungal pathogens *Macrophomina phaseolina* and *Fusarium*

P.I.: D. K. Arora
Co PI: A. K. Singh
National Bureau of Agriculturally Important Microorganisms, Mau, Uttar Pradesh

Objectives:

- Collection of strains, races and species of plant pathogens (*Macrophomina, Fusarium*).
- Biochemical profiling of microbes.
- Optimization of PCR techniques for identification.
- Designing of DNA probes or species specific primers.
- Validation of probes both *in vitro* and *in vivo*.

Significant Achievements:

- A phylogenetic approach based on comparison of stable 28s r DNA, a functional gene cellobiohydrolase-C (cbh-C) and a house keeping gene topoisomerase-II was employed to distinguish species belonging to three different clusters, that is *Fusarium oxysporum*, *Gibberella fujikori* and *Nectaria haematococci*.
- Twenty strains of *Fusarium* belonging to different species and forma specialis were used in the study.
- PCR amplification of Topoisomerase-II and Cellobiohydrolase-C gene gave an amplification product of 720 bp and 340 bp respectively.
- The sequences of 28S rDNA, Cellobiohydrolase-C and Topoisomerase-II were used for phylogenetic analysis. The sequences were submitted to NCBI and the details are gine in table 1.

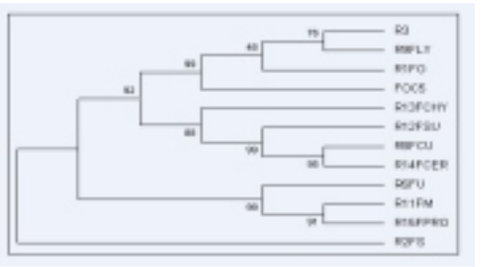

Fig. 1 Parsimonious dendrogram obtained with the rDNA data set. Bootstrap values, greater than 50%, obtained by 1000 replicates are indicated at respective nodes.

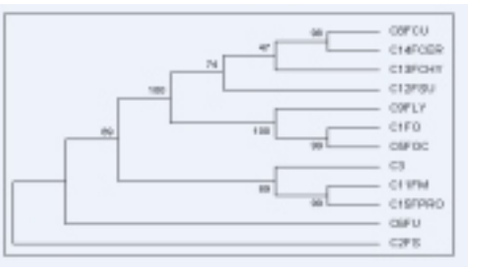

Fig 2. Parsimonious dendrogram obtained with the CBH-C data set. Bootstrap values, greater than 50%, obtained by 1000 replicates are indicated at respective nodes.

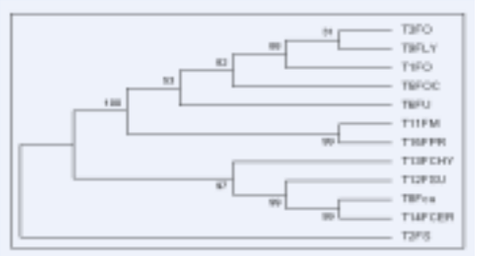

Fig 3. Parsimonious dendrogram obtained with the TOP-II data set. Bootstrap values, greater than 50%, obtained by 1000 replicates are indicated at respective nodes.

Fig 4 Parsimonious dendrogram obtained with the combine data set. Bootstrap values, greater than 50%, obtained by 1000 replicates are indicated at respective nodes.

- Homologous alignment of the nucleotide sequences was performed with Clustal X, and modified manually on the basis of conserved domains and columns containing more than 50% gaps were removed.
- The phylogenetic tree was constructed on the aligned datasets using parsimony method for the three targets and the combine dataset using the program MEGA 3.1 (Fig 1-4).
- Parsimony analysis revealed that of the three target genes used, cellobiohydrolase-C has the maximum percentage (61.38) of parsimony informative sites (218/354) followed by topoisomerase-II 22.87 (177/752) and rDNA cluster 8.86% (99/1140).
- The results clearly indicate the existence of higher variability in cellobiohydrolase-c and topoisomerase II genes between the species than the rDNA cluster.
- Employing the sequence data of rDNA genes alongwith other functional genes and housekeeping genes could unravel the phylogenetic position of different species and forma specialies of this otherwise large group of fungi belonging to genus *Fusarium*.
- Using the sequence data on fungal DNA topoisomerase, we are in process of developing species specific primers for *Fusarium udum* and other species of *Fusarium oxysporum* complex.

Table 1. Accession number and definition of sequences submitted to NCBI GenBank.

Accession No.	Definition
EU214547	*Fusarium oxysporum* NBAIM strain 1281 cellobiohydrolase-C gene
EU214548	*Fusarium solani* NBAIM strain 350 cellobiohydrolae-C gene
EU214549	*Fusarium oxysporum* f.sp.ciceris NBAIM strain 2791 cellobiohydrolase-c gene
EU214550	*Fusarium oxysporum* strain 4998 cellobiohydrolase-c gene
EU214551	*Fusarium udum* NBAIM strain 138 cellobiohydrolase-c gene
EU214552	*Fusarium culmorum* NBAIM strain 349 cellobiohydrolase-c gene
EU214553	*Fusarium oxysporum* f. sp. lycopersici NABIM strain 690 cellobiohydrolase-C gene
EU214554	*Fusarium moniliforme* NBAIM strain 110 cellobiohydrolase-C gene
EU214555	*Fusarium proliferatum* NBAIM strain 344 cellobiohydrolase-c gene
EU214556	*Fusarium cerealis* NBAIM strain 784 cellobiohydrolase-c gene
EU214557	*Fusarium chlamydosporum* NBAIM strain 236 cellobiohydrolase-c gene
EU214558	*Fusarium sambucinum* NBAIM strain 455 cellobiohydrolase-c gene
EU214559	*Fusarium solani* NBAIM strain 350 18s ribosomal RNA gene, partial sequence;
	internal transcribed spacer1, 5.8s ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28s ribosomal RNA gene, partial sequence.
EU214560	*Fusarium moniliforme* NBAIM strain 110 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal
	transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
EU214561	*Fusarium chlamydosporum* NBAIM strain 236 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
EU214562	*Fusarium culmorum* NBAIM strain349 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
EU214563	*Fusarium oxysporum* f. sp. ciceris NBAIM strain 2791, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
EU214564	*Fusarium oxysporum* f. sp. lycopersici NBAIM strain 690, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

EU214565	*Fusarium sambucinum* NBAIM strain 455, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
EU214566	*Fusarium udum* NBAIM strain 138, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
EU214567	*Fusarium oxysporum* NBAIM strain 1281, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
EU214568	*Fusarium oxysporum* NBAIM strain 4998, 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
EU214569	*Fusarium cerealis* NBAIM strain-784 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.
EU214570	*Fusarium oxysporum* NBAIM strain 4998 topoisomerase II gene, partial cds.
EU214571	*Fusarium solani* NBAIM strain 350 topoisomerase-II gene, partial cds.
EU214572	*Fusarium moniliforme* NBAIM strain 110 topoisomerase-II gene, partial cds.
EU214573	*Fusarium cerealis* NBAIM strain 784 topoisomerase-II gene, partial cds.
EU214574	*Fusarium chlamydosporum* NBAIM strain 236 topoisomerase-II gene, partial cds.
EU214575	*Fusarium culmorum* NBAIM strain 349 topoisomerase-II gene, partial cds.
EU214576	*Fusarium oxysporum* NBAIM strain 1281 topoisomerase-II gene, partial cds.
EU214577	*Fusarium oxysporum* f. sp. ciceris NBAIM strain 2791 topoisomerase-II gene, partial cds.
EU214578	*Fusarium oxysporum* f. sp. lycopersici NBAIM strain 690 topoisomerase-II gene, partial cds.
EU214579	*Fusarium sambucinum* NBAIM strain 455 topoisomerase-II gene, partial cds.
EU214580	*Fusarium udum* NBAIM strain 138 topoisomerase-II gene, partial cds.
EU214581	*Fusarium proliferatum* NBAIM strain 344 topoisomerase-II gene, partial cds.

Conclusion:

Employing the sequence data of rDNA genes alongwith other functional genes and housekeeping genes could unravel the phylogenetic position of different species and *forma specialies* of this otherwise large group of fungi belonging to genus *Fusarium.* The sequence data on fungal DNA topoisomerase showed variations among the species and could be used to develop species-specific probes. At present we are in process of developing species-specific primers for *Fusarium udum* and other species of *Fusarium oxysporum* complex.

Diversity of Actinomycetes from Indogangetic Plains

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Co PI: Mahesh Yandigeri
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Objectives:

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

Significant Achievements:

- Soil samples were collected from regions around Dehradun, Nainital, Mau, Assam, Saharanpur, Meerut, Baghpat and Muzaffarnagar falling under Indogangetic plains (fig 1). A total of 230 isolates were isolated using starch casein agar, glucose yeast extract malt extract agar, actinomycetes isolation agar, soil extract agar and ISP media. The isolates were purified and stored in both slants and glycerol stocks.
- The isolates were characterized morphologically using the parameters like colour of aerial mycelium, substrate mycelium and colour of soluble pigment produced (fig 2).
- The isolates were screened for temperature, salt tolerance and copper resistance. A total of 35 isolates were able to tolerate up to 55°C, 25 isolates were able to tolerate 8% NaCl concentration and 20 isolates were able to tolerate up to 80µg/L concentration of $CuSO_4$.
- Biochemical characterization done for plant growth promotion activities like ammonia and HCN production, biodegradation activities like filter paper assay, carboxy methyl cellulose assay, xylanase assay, cellulase assay, and production of enzymes like protease, amylase and antifungal activity (fig 3 and 4).
- Genomic DNA of 200 actinomycetes isolates was isolated and its 16Sr DNA was amplified using universal primers (fig 4).

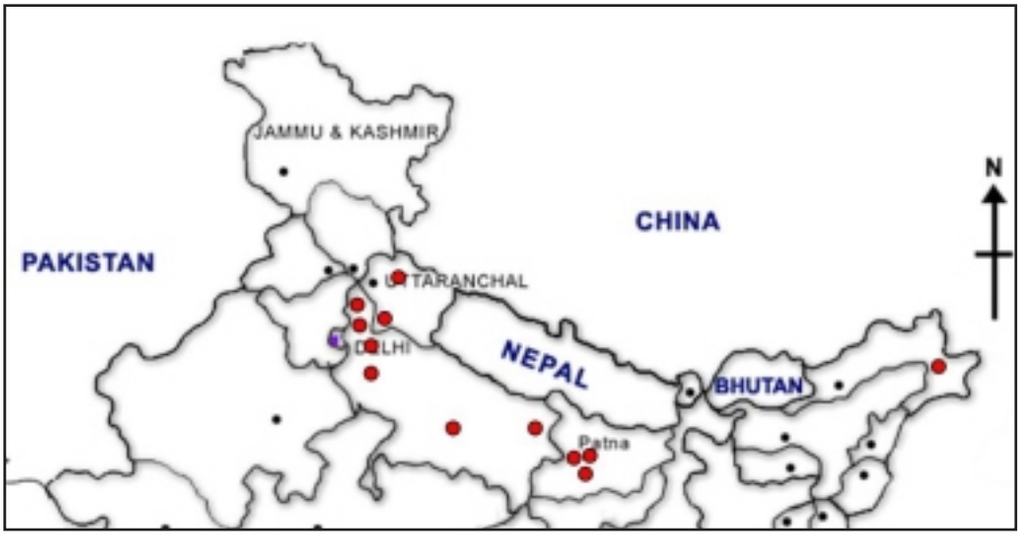


Fig 1. Map depicting area of survey conducted for soil sampling in Indo-Gangetic plains.

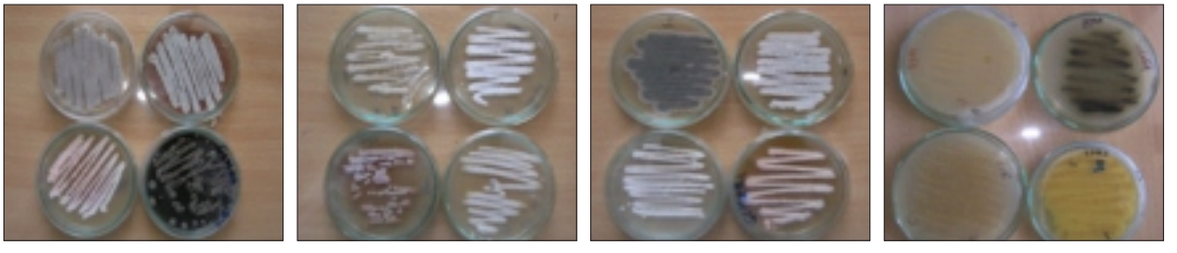


Fig 2. Actinomycetes isolates obtained from Indo-gangetic plains.

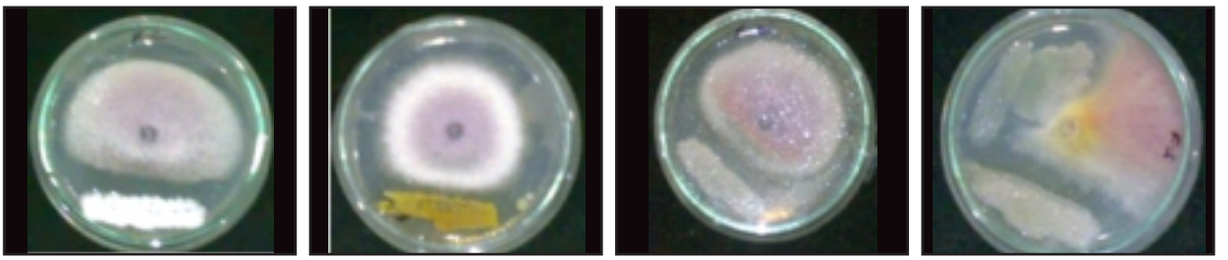


Fig 3. Antagonism towards *F. ciceri* and *F. solani.*

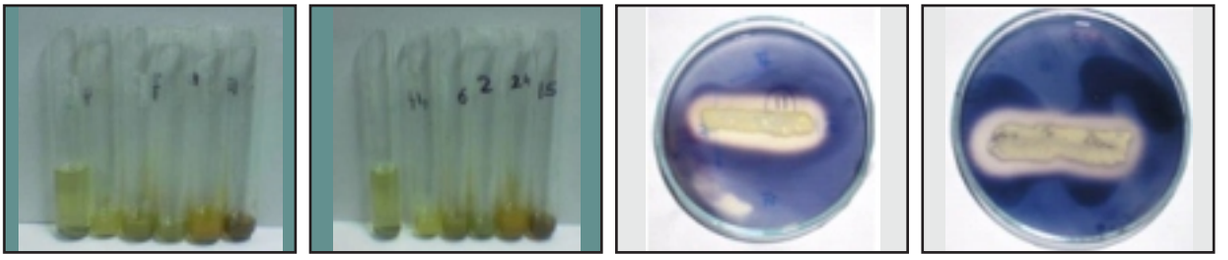


Fig 4. Ammonia production.

Protease activity.

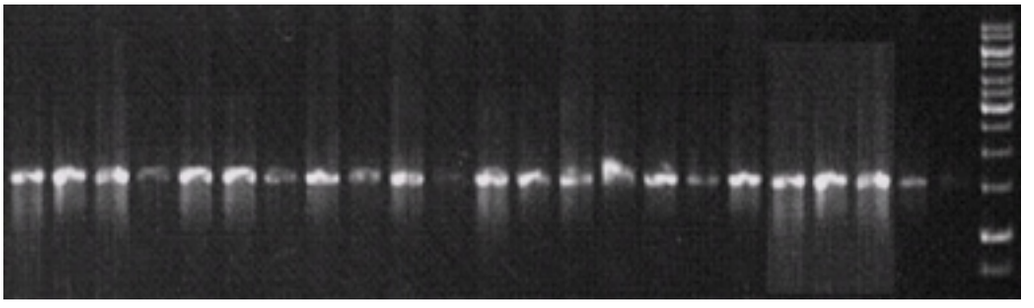


Fig 5. Amplification of 16S rDNA using universal primers from the actinomycetes.

Conclusion :

Actinomycetes isolates obtained from Indo-Gangetic plains were found to be morphologically and biochemically diverse. Diversity was more in Dehradun region as compared to hilly region of Nainital, indicating that soils from forest region harbor more diverse actinomycetes as compared to cultivated lands.

In addition to the structural diversity of actinomycetes, functional diversity was also recorded with respect to biocontrol attributes as well as enzymes involved in biodegradation. This huge database of actinomycetes from IGP region could be exploited for identification of potent biocontrol agents as well as agents for biodegradation.

Diversity of Agriculturally Important Microorganisms in the Western Ghats of Kerala

PI: D. Girija
CoPIs: Sally K. Mathew, S. Surendragopal
Center for Plant Biotechnology & Molecular Biology,
Kerala Agricultural University, Thrissur, Kerala

Objectives:

- Isolation, enumeration & characterization of AIMs (N_2 fixers, P-solubilizers, AMF, fluorescent pseudomonads, *Trichoderma*, chitin, cellulose & lignin degraders) along the Western Ghats in Kerala.
- Assess geographical distribution and develop thematic maps for AIMs.
- Assess functional potentials of each group of organisms for use in agriculture.
- Set up culture bank of potential isolates & deposit with the NBAIM.
- Set up database on the population and diversity of AIMs in the Western Ghats.

Significant Achievements:

- Soil, herbaceous plants and decaying wood were collected from the Western Ghats under different districts viz. Ernakulam, Trivandrum, Wynad and Kannur and Thrissur districts of Kerala, as per the grids developed by Kerala Forest Research Institute, Peechi, Thrissur (fig 1). These were used for isolation of total bacteria, actinomycetes and fungi; and agriculturally important microorganisms (N-fixers, P-solubilizers, fluorescent pseudomonas, *Trichoderma,* cellulose & lignin degraders). A collection of 76 N-fixers, 81 P-solubilizers, 25 pseudomonads, 22 *Trichoderma,* 16 lignin degraders and 3 cellulose degraders was made during the period.

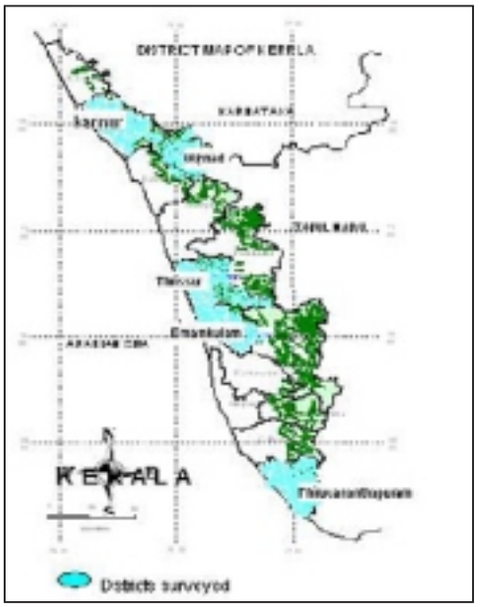


Fig 1. Districts of Kerala surved

according to pigmentation (yellow, white and pigmented) and further, based on size of colonies into large, medium, small and pinhead sized colonies. A total of 52 pigmented bacteria were observed: deep purple, yellow, pink and orange (fig 2).

- Bacteria were subjected to biochemical tests including Gram staining, utilization of carbohydrates, urease, oxidase, catalase, indole production & methyl red reduction, esculin, starch & gelatin hydrolysis;

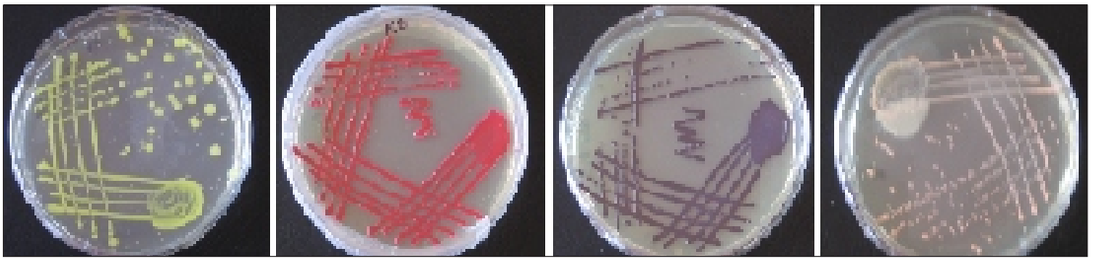


Fig 2. Pigmented bacteria

- A total of 87 samples were analysed to assess the microbial diversity and 1286 bacteria were obtained on nutrient agar.
- Bacteria were classified into three morphotypes intrinsic antibiotic resistance pattern and PGPR characteristics like production of HCN, IAA, siderophore , protease and ammonification.
- The purple coloured bacterium was identified by

morphological, cultural and biochemical characters and 16SrRNA sequence as *Chromobacterium violaceum* and it lost the colour after 3-4 subcultures. The pink pigmented bacteria was identified as *Serratia marsecens* based on 16S rRNA sequence.

- Different groups of agriculturally important microbes isolated on specific media included N_2-fixers, P-solubilising bacteria and fungi, fluorescent pseudomonads, Trichoderma, cellulose degraders and lignin decomposers. The population of AIMs varied in each soil sample. Among these, P-solubilizing bacteria were found to predominate ($15x10^4$ cfu/g soil). Nitrogen fixing bacteria were of the tune of $15x10^3$. Soils collected from Wynad region recorded highest population as well as diversity of AIMs. A total of 76 N-fixers, 81 P solubilizers, 25 *Pseudomonas fluorescens*, 16 lignin degraders, 22 *Trichoderma* and three cellulose degraders were isolated and characterized. Seventy two P-solubilizing bacteria obtained from Waynad and Kannur districts were screened by the qualitative method in Pikovskaya's agar by spot inoculation. Eighteen most efficient bacterial isolates and four fungal isolates were subjected to quantitative assay in liquid medium by spectrophotometric method. The fungal isolates PSF2 and PSF 6 recorded the highest solubilization of 57.6 g/ml.
- Six isolates of lignin degrading fungi were isolated and its degradation efficiency was quantitatively estimated by using pharmaceutical waste and coir pith as substrate. Compared to control the lignin degradation was high in fungal inoculated substrate.
- Fluorescent pseudomonads were grouped based on the size of colony on King's B agar. Six isolates of *Trichoderma* were tested for their efficiency to control *Pythium* and *Phytophthora* sp. Some were very efficient in biological control and completed suppressed growth of these plant pathogenic fungi. They were also studied for the presence on antifungal metabolites, zone of lysis and hyperparasitism. These two isolates are being functionally characterized. More soil samples are being analysed for the diversity of microorganisms.
- Four isolates of P-solubilizing fungi were characterized and identified as *Penicillium glabrum.* Five isolates of *Trichoderma* were identified as *T. viridae* and one as *T. harzianum* at the National Centre of Fungal Taxonomy, New Delhi.

Conclusion

The diversity of agriculturally important microorganisms isolated from the Western Ghats coming under five different districts of Kerala was assessed at morphological, biochemical and functional levels.

Isolation, purification, identification and molecular assessment of microbial diversity from selected districts of Indo-Gangetic Plans.

PI: L. C. Rai
Co-PI: Shashi Pandey
Centre for Advance Study in Botany, Banaras Hindu University, Varanasi-221005

Objectives:

- Survey and Collection of soil and cyanobacterial samples from selected districts of Indo-Gangetic plain like Chandauli, Mirzapur, Varanasi, Allahabad, Ghazipur, Balia, Arrah and Patna over different time frames and study of selected physico-chemical properties of these soil samples.
- Isolation, purification and molecular characterization of nitrogen fixing cyanobacteria and P-solubilizing bacteria from the collected samples.
- Assessment of biodiversity of N_2-fixing cyanobacteria and P-solubilizing bacteria from different soil types using molecular techniques.
- Deposition of characterized/identified organisms to NBAIM.

Cylindrospermum muscicola, Hapalosiphon intricatus, Nostoc muscorum, Tolypothrix tenuis, Anabaena fertilissima, Nostoc species, *Nostoc rivulare, Anabaena torulosa, Nostoc paludosum, Nostoc piscinale* and *Nostoc* species (fig 1).

- Molecular characterization of 14 nitrogen fixing cyanobacteria was carried out and their phylogenetic affiliations were attested using the16S rDNA sequences available in the database (fig 2).
- Six phosphate solubilizing bacteria were identified as *Enterobacter* sp. LCR1, *Enterobacter* sp. LCR2, *Enterobacter* sp. LCR3, *Exiguobacterium* sp. LCR4, *Exiguobacterium* sp. LCR5 and *Exiguobacterium* sp. LCR6 using morphological, biochemical characteristics and partial 16S rDNA sequencing. The

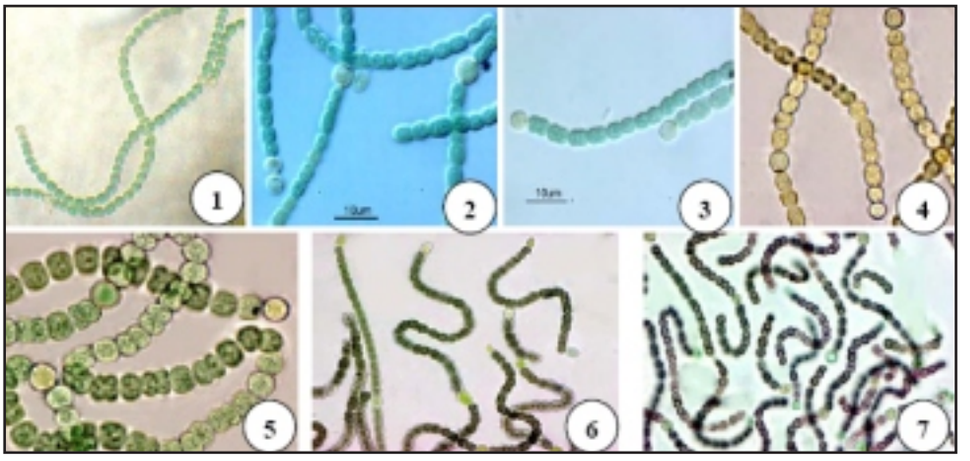


Fig 1. Photomicrographs and accession no. of some of the cyanobacterial isolates of paddy fields; i.e. (1) *Anabaena fertilissima* EU446018, (2) *Nostoc* species EU446009, (3) *Nostoc rivulare* EU446021, (4) *Nostoc paludosum* EU446016, (5) *Anabaena torulosa* EU446013, (6) *Nostoc piscinale* EU446011, (7) *Nostoc* species EU446017.

Significant Achievements:

- Collection of soil sample and cyanobacterial mats from rice fields of different districts of Indo-Gangetic plane covering UP and Bihar such as Varanasi, Mirzapur, Chandauli, Allahabad, Balia, Ghazipur, Arrah and Patna and analysis of their selected physio- chemical properties.
- Isolation, purification and morphological characterization of forteen nitrogen fixing cyanobacteria viz. *Anabaena anomala, Anabaeana doliolum, Anabaeana oryzae, Aulosira fertilissima,*

16S rDNA sequences have been deposited in the GenBank database with accession numbers EU304794, EU304795, EU304796, EU304797, EU304798 and EU304799 respectively.

- 16S rDNA based phylogenetic analysis of the above phosphate solubilizing bacteria were carried out using Neighbour-Joining algorithm (Thompson et al, 1997).
- The above isolates were also characterized for phosphate solubilization under different abiotic stresses such as high pH, high temperature and salt concentration (sodium chloride). *Enterobacter* sp.

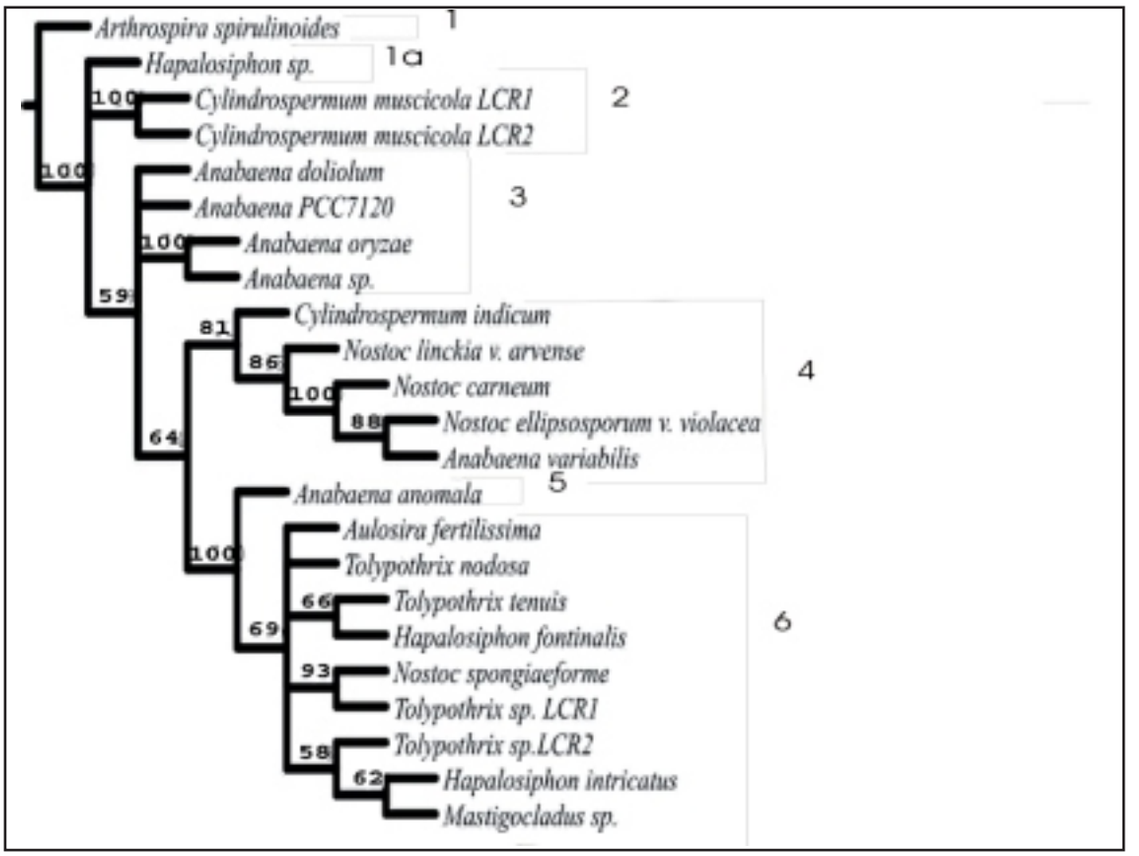


Fig 2. Neighbour-Joining phylogenetic tree of 16S rDNA sequences showing the positions of seven cyanobacterial isolates (The node points values correspond to the respective bootstrap support values)

LCR1, *Enterobacter* sp. LCR2 and *Enterobacter* sp. LCR3 appeared most powerful phosphate solubilizers under the stresses tested. *Exiguobacterium* sp. LCR4 and *Exiguobacterium* sp. LCR5 demonstrated increased phosphate solubilization at high pH (up to 10.0).

- In addition to the above, eight phosphate solubilizing bacteria have been isolated and characterized by partial 16S rDNA sequencing. BLAST homology search has demonstrated them to be strains of *Arthrobacter* sp. and *Acinetobacter* sp.

Agriculturally important microorganisms from soils of rice-based cropping system from agro-ecological zones of east coast of India

PI: T. K. Adhya
Co-PI: T. K. Dangar
Central Rice Research Institute, Cuttack – 753006, Orissa

Objectives:

- Isolation, characterization, identification and inventorization of agriculturally important microorganisms in pure culture from soils of different agro-ecological zones of east coast of India.
- Explore and quantify the microbial diversity in soils and crop rhizosphere in rice-based cropping system by cultivation-based, proteogram and total DNA finger-printing analyses.
- Phylotyping of the isolated microbial species and investigation on the molecular diversity through soil (metagenomic) DNA analysis.
- Study the structural dynamicity of the microbial community in the rice ecosystem by analysis of diversity indices.
- Characterization of functional diversity with reference to growth promoting hormone and toxin production.

Significant achievements:

- Isolation of different physiological groups of bacteria from coastal saline soils planted to rice from Orissa and West Bengal.
- Among 492 bacterial cultures isolated from the rhizosphere and non-rhizosphere soil of rice plants from coastal saline soils, 229 (46%) isolates were showed IAA production in pure cultures (fig 1 -3).
- Among the 18 cultures tested, a bacterial isolate from Ersama non-rhizosphere soil isolated in TSY/1000 medium (ENR-TSY/1000) showed the highest amount of IAA (124.63 μM.ml^{-1} medium) production (fig 1-3).
- Out of a total of twenty isolates of fluorescent *Pseudomonas* sp. tested, three cultures showed growth inhibition against *Rhizoctonia solani*, causal agent of sheath blight and *Xanthomonas oryzae*, the causal agent for bacterial blight of rice.
- Among the other isolates of fluorescent *Pseudomonas* sp., four isolates showed P-solubilization activity, four isolates had hydrocyanic acid production potential and eight isolates demonstrated acetylene reduction activity.
- A total of 326 isolates of *Bacillus thuringiensis* were isolated in pure culture from rice soils of coastal Orissa and West Bengal.
- A novel *Bacillus* sp. with insecticidal property was identified using partial sequencing of 16s rDNA. The bacteria belonged to *B. fumarioli* cluster with swollen sporangia.
- In a study on the interaction of 42 isolates of *B. thuringiensis* and 32 entomopathogenic fungi, one isolate of *B. thuringiensis* was inhibitory against *Metarhizium anisopliae* and another was inhibitory to *Verticillum* sp (fig 4a-d).
- Twelve isolates of *B. thuringiensis* were tested for their virulence against leaf folder under field conditions and compared with the botanical pesticide nimjam and chemical pesticide monocrotophos. One isolate was more effective than the commercial formulations of *B. thuringiensis*, bactospeine and dipel.

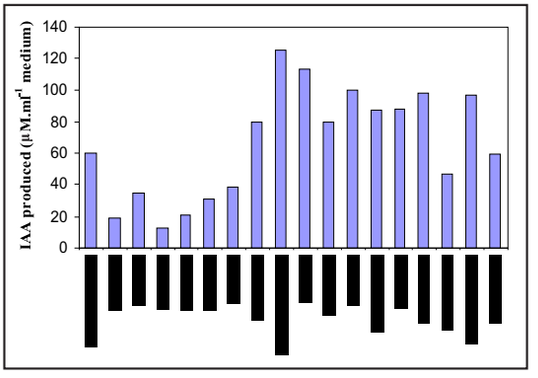


Fig 1. IAA production potential of different microbial isolates

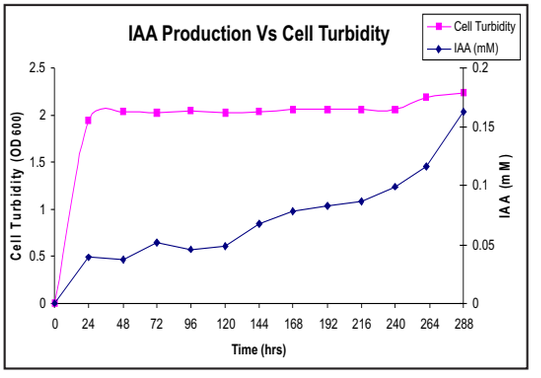


Fig 2. IAA production vs. cell turbidity in bacterial isolate no. ENR-TSY/1000

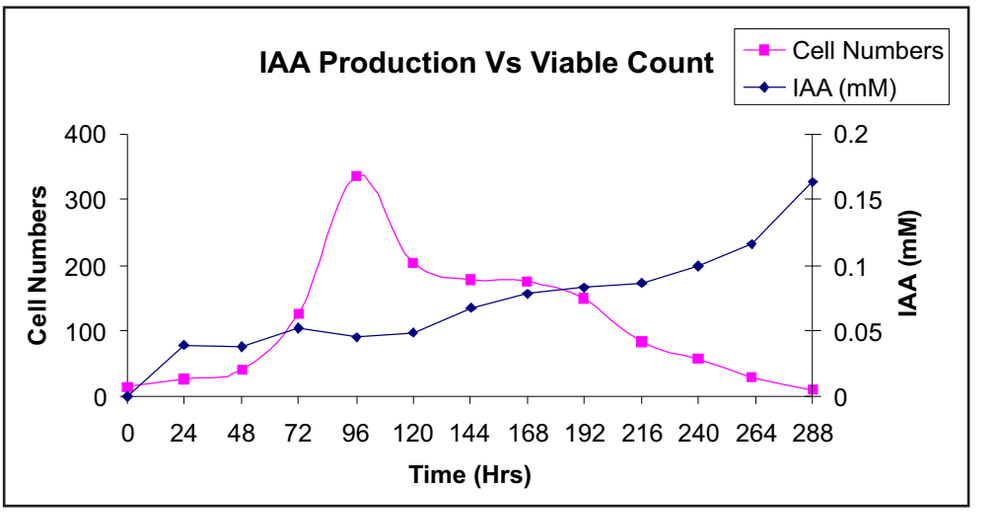


Fig 3. IAA production vs. viable cell count (cfu) in bacterial isolate no. ENR-TSY/1000

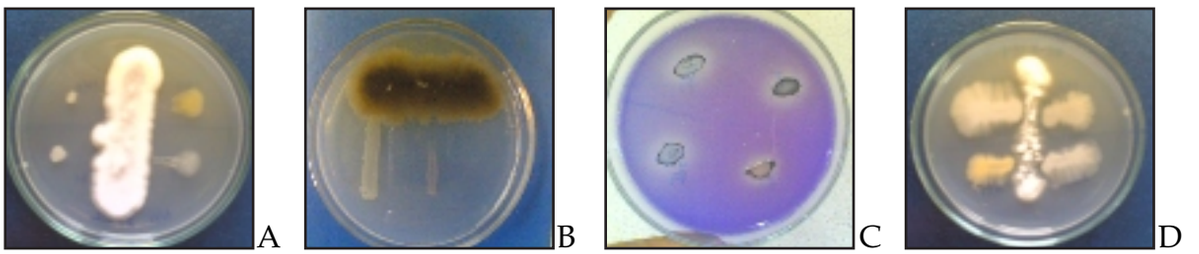


Fig 4a. Inhibition of *Xanthomonas oryzae* against *Pseudomonas sp*
Fig 4b. Inhibition of *Rhizoctonia solani* against *Pseudomonas* sp.
Fig 4c. P-solubilization potential of *Pseudomonas* sp.
Fig 4d. D: Inhibition of *B. thuringiensis* against *M. anisopliae* and *Verticillium* sp.

Conclusion:

In the present project, attempts have been made for isolation, characterization, identification and inventorization of agriculturally important microorganisms in pure culture from soils of different agro-ecological zones of east coast of India covering W. Bengal and Orissa. A total of 524 bacteria were isolated in pure culture by using standard microbiological techniques involving seven different isolation media of which 141 isolates showed positive reactions for IAA production. Bacterial cultures isolated on tryptone soy agar (1/1000 dilution) medium exhibited better results (100% positive for IAA production) than cultures isolated in other media. However, highest IAA production (136.4µM ml^{-1} medium) was recorded in a non-rhizosphere bacterium, isolated from Sukinda soil in LB medium. IAA production in the microbial cultures reached their peak during the maximum stationary phase beyond 6-7 days. Six hundred twenty eight fluorescent *Pseudomonas* sp. were isolated in pure cultures from alluvial soil of CRRI farm and coastal saline soils of Kendrapara and Jagatsingpur districts of Orissa. Twenty most promising isolates of fluorescent *Pseudomonas* spp. were characterized further and the fluorescent pigment produced by the isolates was characterized by UV-Vis spectrophotometry. Three of the isolated *Pseudomonas* sp. showed growth inhibition against plant pathogenic fungi *Rhizoctonia solani*, causal agent of bacterial sheath blight and *Xanthomonas oryzae*, causal agent of bacterial leaf blight of rice. A total of 326 isolates of *Bacillus thuringiensis* from rice soils of coastal Orissa and West Bengal were characterized. Partial sequencing of the 16s rDNA of a new isolate of *B. thuringensis* isolated from coastal saline soil of Gadkujang of Orissa, suggested it to be a novel species. Unlike all other *B. thuringiensis* strains, the present strain does not group with *Bacillus cereus* group and rather with *B. fumarioli* and *B. niacini*. The bacterium has the usual profile of control of lepidopteran insects but has swollen sporangia unlike that of *B. thuringiensis*.

Microbial Diversity Analysis from different Brakiswater System of East Coast of India

PI: T. C. Santiago
Co-PI: Kalaimani N, Shankar V. Alavandi
Central Institute of Brackish Water Aquaculture (ICAR), Chennai

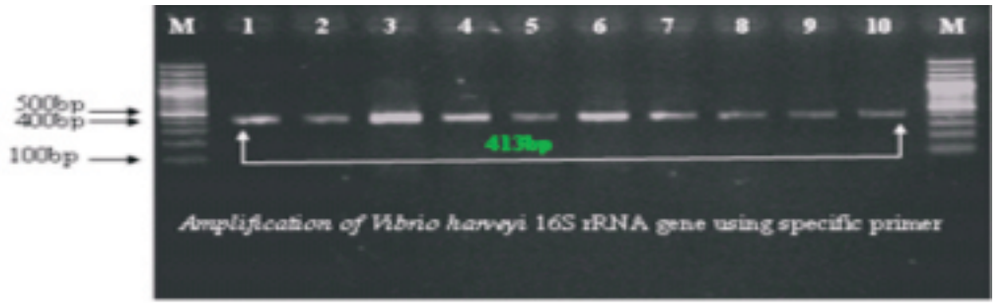
Objectives:

- To isolate bacteria, actinomycetes and fungi from different brackish water system of east coast of India.
- Identification of the microbes isolated from different brackish water ecosystem.
- To screen the economically important microbes, bioactive microbes with special reference to antagonistic activity/ useful metabolites, from the microbial stock
- To evaluate and optimize the production parameters of the economically important microbes.
- To isolate economically important genes from the isolated microbial stock.
- To standardize the expression of the economically important genes using different vector systems.
- To establish a repository of these microbes with bioactive potential for *ex situ* conservation of microbial biodiversity and future biotechnological applications.

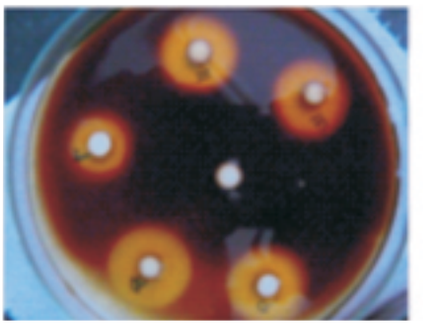
Sampling sites:
Spot-1- Tuticorin
Spot-2- Pichavaram (Chidambaram)
Spot-3- Mahabalipuram
Spot-4- Chennai
Spot-5- Pulicat
Spot-6- Nellore
Spot-7- Vishakapattanam
Spot-8 Sunderban (West bengal)

Significant Achievements:

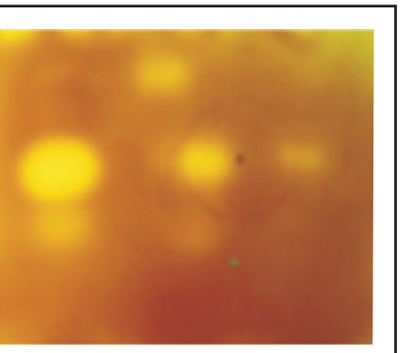
- A total of 620 isolates of microbes were isolated from different geographic environments of brackish water eco-system, which comprises 329 bacteria, 66 actinomycetes, 55 fungi, 21 yeast isolates and 7Archae.
- A total 11 agarolytic isolates, 3 nuclease producing bacteria, 13 sulfur metabolizing bacteria, 4 denitrifying bacteria, 10 protease producing bacteria, 15 lipase producing, 8 chitinase producing, 9 ligninase producing, 7 cellulase producing, 6 chitosanase producing, 20 high salt resistant bacteria, 10 isolates of bioluminescent bacteria, 16 isolates of pigment producing bacteria has been isolated.
- Detailed studies like species level molecular identification, biochemical characterization, and enzyme activity assay has been carried out on 11 agarolytic enzyme producing marine bacteria
- One isolate of algae and 5 isolates of archaebacteria which produce high quantity of β-carotene were isolated and preserved for further characterization.
- One isolate of *Dunaliella salina* has been isolated from salt pan.
- Pure culture of this algae has been cultivated and the molecular identification of the isolate was done based on 18S rRNA *Dunaliella sp* specific primers.



- A total of 21 *Vibrio harveyi* has been isolated from different brackish water system of east coast of India and been identified using species specific 16S rRNA primer, Box, ERIC and RAPD primers has been used for the study of genotypic variation among the species. Detailed antibiogram pattern has also been studied.
- A total of 40 bacterial 16S rRNA gene has been amplified using universal 16S rRNA primer and sequenced. All the sequence has been used for the identification of the bacterial species using data base information.
- A total of 20 16SrRNA gene sequence has been so far submitted in NCBI nucleotide Database.
- A total of 7 archaebacteria has been isolated form salt pan and identified using 16S rRNA primer which is specific for archaebacterium.
- 48 identified bacterial and 2 archaebacterial isolates have been deposited in the NBAIM culture collection bank.



Bacterial filterates showing Agarase activity



Zymogram assay demonstrating Agarase activity

Conclusion :

In two years of the study various surveys were carried out in the different geographic environments of brackish water eco-system and several isolates of bacteria, actinomycetes, fungi yeast and archaebacteria were isolated. These microbes possessed various beneficial traits such as agarolytic activity, nucleases, sulfur oxidation, denitrification, protease, lipase, chitinase, ligninase and cellulase activity, salt resistance, pigment production etc. Detailed studies like species level molecular identification, biochemical characterization, and enzyme activity assay were carried out. The results of the present study revealed high prevalence and diversity of agarolytic bacteria from the aquaculture settings, estuary and coastal regions of south east coast of India. This study focused the presence of *Vibrio* species such as *Vibrio hepatarius*, *Vibrio fortis* and other bacterial species like *Photobacterium rosenbergii, Alteromonas macleodii* and *A.hispanica* which has not been associated with the agarolytic properties so far. One isolate of algae and 5 isolates of archaebacteria were isolated producing high quantity of β-carotene. *Dunaliella salina* was isolated from salt pan**.** Detailed antibiogram pattern of *Vibrio harveyi* was studied. The results of the present study revealed high prevalence and diversity of *Vibrio harveyi* from the aquaculture settings, estuary and coastal regions of south east coast of India..

Isolation of microorganisms from fermented dairy foods and sequencing of 16S rDNA for strain identification

PI: Dinesh Kumar
Co PI: B. K. Joshi
National Bureau of Animal Genetic Resource, Karnal
Co-PIs: Rameshwar Singh; S. K. Tomar
National Dairy research Institute, Karnal

Objectives:

- To isolate microorganisms from fermented dairy foods.
- To characterize microorganisms on technological and taxonomic parameters.
- To sequence 16SrDNA for species identification.

Significant Achievements:

- A total of 40 non starter *Lactobacillus* isolates were isolated from four Churpi samples obtained from four different cheese plants of Arunanchal Pradesh.
- The *Lactobacilli* were found to proliferate in Churpi cheese prepared from yak milk.
- The biochemical characterization, especially sugar fermentation pattern was used as a phenotypic method for species determination of the *Lactobacillus* isolates.
- On the basis of sugar fermentation pattern *Lactobacillus* was identified.
- *Lactobacillus paracasei* was most prevalent in first, third and fourth cheese sample but in sample two *Lactobacillus plantarum* was the most prevalent species which was clearly identified on the basis of sugar fermentation pattern and molecular biology.
- The species of isolates as assigned on the basis of biochemical characterization were confirmed by species specific PCR using primers reported in the literature i.e. *L. paracasei, L casei, L. plantarum, L. brevis, L. helvaticus isolates* (fig 1 A).
- For closely related species *L. paracasei* & *L casei, L. plantarum* & *L. pentosus* which cannot be resolved on basis of partial sequencing of 16s rRNA gene sequence were resolved by species specific primer designed on the intergenic spacer region (fig 1 B).
- Sequencing of all the obtained *Lactobacillus* isolates for 16S rRNA gene sequence was performed. BLAST analysis of obtained sequence was done to find similarity of our sequence with the sequence deposited in GenBank.
- The obtained sequences of each typical species of *Lactobacillus* from Indian Yak cheese (Churpi) (fig 2) are deposited in NCBI (GenBank).

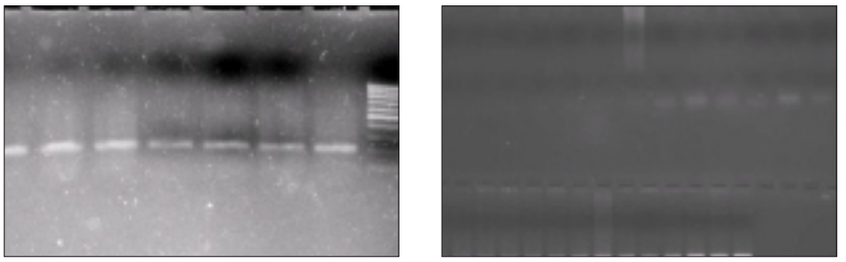


Fig 1. (A) Genus specific PCR of *Lactobacillus* (B) Species-specific PCR to differentiate *Lactobacillus casei* and *Lactobacillus paracasei,* First row- PCR with *Lactobacillus casei* specific primer, Second row- PCR with *Lactobacillus paracasei* specific primer, using same genomic DNA

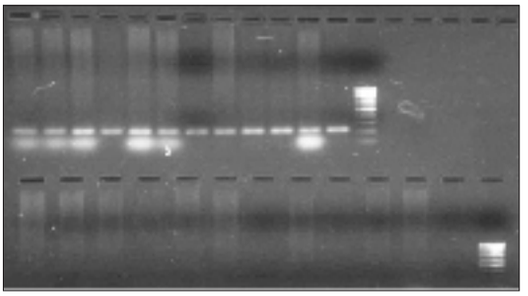


Fig 2. Species-specific PCR to differentiate *Lactobacillus plantarum* and *Lactobacillus pentosus.* Row one PCR with *Lactobacillus plantarum* specific primer, Row two PCR with *Lactobacillus pentosus* specific primer, using same genomic DNA

Conclusion:

Study was undertaken to evaluate the non-starter *Lactobacillus* species diversity, of Churpi cheese of Indian Yak covering areas of Arunchal Pradesh. A total of 40 non starter *Lactobacillus* isolates were isolated. The *Lactobacilli* were found to proliferate in Churpi cheese prepared from yak milk. The biochemical characterization, especially sugar fermentation pattern was used as a phenotypic method for species determination of the *Lactobacillus* isolates. On the basis of sugar fermentation pattern *Lactobacillus* was identified. The five species of isolates as assigned on the basis of biochemical characterization were confirmed by species specific PCR using primers reported in the literature i.e. *L. paracasei, L casei, L. plantarum, L. brevis, L. helvaticus isolates.* For closely related species *L. paracasei* & *L casei, L. plantarum* & *L. pentosus* which cannot be resolved on basis of partial sequencing of 16s rRNA gene sequence were resolved by species specific primer designed on the intergenic spacer region. Sequencing of all the obtained *Lactobacillus* isolates for 16S rRNA gene sequence was performed. BLAST analysis of obtained sequence was done to find similarity of our sequence with the sequence deposited in GenBank. The obtained sequences of each typical species of *Lactobacillus* from Indian Yak cheese (Churpi) are deposited in NCBI (GenBank). Thirty three (30) accession numbers were obtained from NCBI, USA (GenBank accession EU637371-EU637403).

Strengthening, authentication and exploitation of mushroom biodiversity at the National Mushroom Repository for Human Welfare

PI: R. C. Upadhyay
National Research Center for Mushroom, Solan, H.P.

Objectives:

- Collection of wild edible, non edible and wood rotting mushroom species from different ecological regions of India namely western Ghats of Karnataka, Northwest Himalaya, Jharkhand, Aravali hills (Mount Abu, Udaipur, Khairwada etc.) and Thar desert of Rjasthan, Gir and Satpura forest of Gujrat and Panchmarhi hills of Madhya Pradesh.
- To obtain mycelial cultures from the collected specimens and culture conservation in the Gene Bank of NRCM, Solan and at NBAIM, Mau.
- Identification of collected mushroom species following traditional taxonomic anatomical methods and molecular techniques using Random Amplified Polymorphic DNA (RAPD), RFLP and Internally Transcribed Spacer (ITS) regions of the ribosomal DNA.
- Artificial domestication of unexploited indigenous wild edible mushroom species like *Hericium, Tremella, Macrolepiota, Agaricus, Pleurotus* spp., *Lepista, Tricholoma* spp and *Auricularia* spp.
- Conservation of dried mushroom specimens in the Herbarium of NRCM, Solan.

Significant Achievements:

- A total number of 261 wild mushrooms have been collected from the forest of Himachal Pradesh, Uttarakhand (Dehradun only) and Aravali hills of Rajasthan. All the specimens have been preserved in the Herbarium of NRCM, Solan.

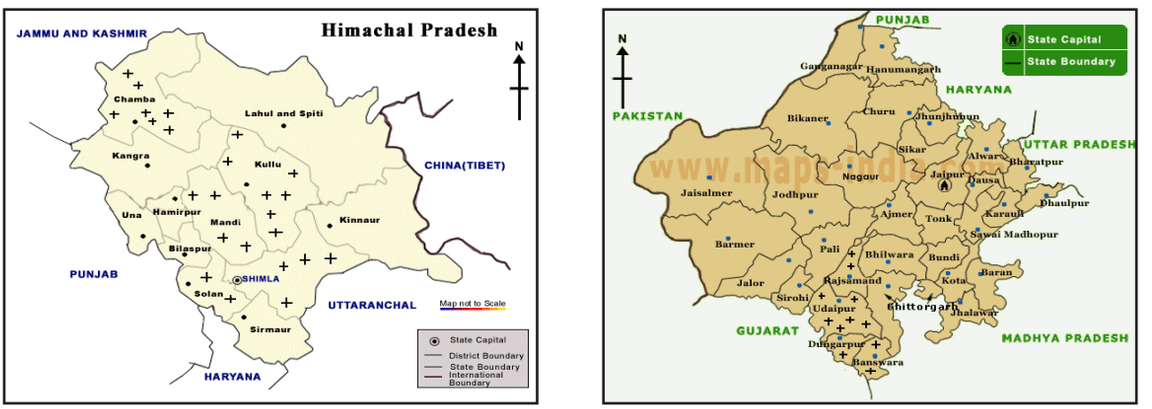


Fig 1. regions covered under survey (shown as +)

- A total number of 160 tissue cultures from wild specimens have been preserved in the Gene Bank of NRCM in liquid paraffin and kept at 4C.
- A wild strain of *Calocybe* spp (X-1003) has been collected which shows similarity with *Calocybe indica* but the mycelial growth is strandy and not cottony as in *Calocybe indica*. The tissue culture has been raised and will be identified using molecular tools.
- Two interesting spp. *Macrocybe giganteum* (X-469) and *Tricholomella constricta* (X-415) have been identified on the basis of molecular studies; however these both species were earlier tentatively identified as *Calocybe* spp. on the basis of morphological studies.
- A new variety of *Flammulina velutipes* (X-249) was identified which was successfully cultivated on wheat straw while European culture could only fructify on saw dust.
- Two wild specimens of *Volvariella* spp. (X-492 and X-881) and three Coremia forming *Pleurotus* spp. (P-62, X-600 and X-652) were collected which have been identified as new species recorded from the world on the basis of anatomical studies and molecular studies including sequencing.
- *Cantharellus applachenesis* (Col No. 281/07) and *C. cibarius* var. *multiramis* (Col No. 113/07) has been reported for the first time from India and the paper is communicated to Mycologia and we also found a new species of *Cantharellus* (Col. No. 159/07) on the basis of classical taxonomy, RFLP and ITS length data and submitted for the sequencing.
- Several specimens of the following genera were recorded for the 1st time, which includes *Phaeocollybia, Hypsizygus, Rickenella, Asterophora, Leucopaxillus* and *Cystoderma.*
- Four wild indigenous *Hericium* spp. were collected from Himachal Pradesh and preparing spawn was prepared on wheat grain for their cultivation on wheat straw and saw dust based substrates and the molecular studies of different *Hericium* spp. is going on.
- We have identified and purified a new oxidase enzyme accelerated by hydrogen peroxide from a *Agrocybe* sp. which could tolerate upto 14mM of H_2O_2 and maximum activity at 0.4mM. The molecular weight of this enzyme is around 47KD. The studies on iso electric focusing point are under progress.

Conclusion:

During the last two years a total number of 573 wild mushroom specimens were collected from Himachal Pradesh, Uttarakhand and Rajasthan. We have identified 434 collections upto the genus level of wild mushrooms. Tissue cultures from 191 specimens were raised and conserved in the Gene Bank of NRCM, Solan. Earlier, we were doing only traditional taxonomic methods for identification of a species by phenotypic and microscopic details. These morphological and anatomical characters are some times slightly variable in two different species and gives wrong results. Now we have standardized the technique for DNA isolation from mushroom tissue cultures and fruit bodies, 5.8S rDNA sequencing and RFLP for molecular identification. We have identified two new lignicolous *Volvariella* spp., a new species of *Flammulina*, which have not been sofar reported from world using 5.8S rDNA sequencing. Two wild *Hericium* spp. (edible and medicinal mushrooms) namely as *H. coralloides, H. racemosum, Cantharellus applachenesis, Cantharellus cibarius ver. multiramis*, three species of coremia forming *Pleurotus* spp., *Macrocybe giganteum* (edible fungi), *Tricholomella constricta* and a new strain of *Calocybe indica* were collected and identified which have not been earlier reported from India. The specimens of 15 *Agrocybe* spp. and a new gasteromycete *Lysurus* sp. are under identification by traditional taxonomy and molecular methods. Artificial domestication for *Flammulina* spp. and *Macrocybe giganteum* on wheat straw were successful which could help in diversification of new mushroom species from India. Interesting chlorinated aromatic metabolites have been found in the culture and mushroom fruit bodies of *Descolea* spp., which is under investigation using High Performance Liquid Chromatography

Exploring the microbial diversity in Western plains and Kachchh eco-region of Gujarat for agricultural and industrial applications

PI: K. K. Pal
Co-PI: Rinku Dey
National Research Centre for Groundnut (ICAR), Junagadh, Gujarat

Objectives:

- Isolation, characterisation and identification of important microorganisms (salt tolerant strains of nitrogen fixers, P-solubilizers, biocontrol strains, etc.) from salt affected areas and salterns of Kutch region of Gujarat.
- To study the diversity of representative groups of microbes obtained from Kutch eco-region.

Significant Achievements:

- A total of 44 soil samples were collected from different locations of Kutch eco-region of Gujarat (fig 1) during kharif, 2007 and analysed for pH and EC. The average pH of soil was 8.6. However, salinity varied significantly from location to location. The EC of cultivated soil ranged from 0.9 to 8.9 dSm^{-1} and that of salt affected soil ranged from 33-139 dSm^{-1}

Fig 1. Places surveyed in Kacchchh region

- A total of 54 endospore forming bacteria, 171 other bacteria including fluorescent pseudomonads, 12 rhizobia, five extreme halophilic bacteria, and six salt tolerant (2.0 M) proteolytic bacilli were isolated, purified, and preserved.
- Eleven fluorescent pseudomonads having multiple traits were identified by biochemical tests as *Pseudomonas putida* biovar A and *Pseudomonas fluorescens* biovar III and *Pseudomonas fluorescens* biovar V.
- Eight fluorescent pseudomonad isolates exhibited multiple plant growth promoting traits including antifungal activities against *A. niger*, *A. flavus* and *S. rolfsii*.
- Twelve rhizobial isolates showing tolerance to more than 5% NaCl were tested and found to have both *nifH* and *nodC* functions.
- Salt tolerant strains (upto 10% NaCl concentration) were obtained which exhibited multiple plant growth promoting characters.
- Five extreme halophilic bacteria were isolated from Salterns of Kutch which can grow at saturated salt concentration.
- Restriction analysis of 16S rDNA of *Pseudomonas fluorescens* biovar A isolates 330 and 357 indicated that these two isolates could be differentiated using the same combinations of restriction enzymes viz. *Rsa*I-*Hha*I and *Msp*I-*Rsa*I.

Conclusion:

The kutch eco-region of Gujarat was surveyed and explored for the isolation of salt tolerant bacteria from the different salt affected ecological niches and crop rhizospheres. A number of salt tolerant bacteria (54 endospore forming and 171 others), including rhizobia (12 having both *nod* and *nif* functions) and plant growth-promoting fluorescent pseudomonads (eleven identified), having multiple traits of plant growth-promotion and nutrient mobilization were obtained. Three cellulolytic and thirteen chitinolytic isolates were also obtained. Besides, five extreme halophiles comprising two archaea and three eubacterial isolates were cultured at saturated salt concentrations. None of the extremophiles used betaine production/accumulation as a mechanism of salt tolerance. Three fluorescent pseudomonad isolates viz. *Pseudomonas fluorescens* biovar V isolate 57, *Pseudomonas fluorescens* biovar III isolate 232 and *Pseudomonas fluorescens* biovar A isolate 357 exhibited multiple plant growth-promoting traits including antifungal activities against *A. niger*, *A. flavus* and *S. rolfsii*. A water soluble bioactive compound has been obtained from *Pseudomonas fluorescens* biovar V isolate 57 active against all three soil borne fungal pathogens of groundnut. Besides, five fluorescent pseudomonas were characterized at molecular level to confirm the presence of different plant growth promoting traits. A number of proteolytic bacilli were isolated which could grow at 2.5 M NaCl and would be the source of salt tolerant proteases.

Mapping of microbial diversity in the marine ecosystem in and around Mumbai

Dr. C. S. Purushothaman
Co-PIs: P. K. Pandey, A. Vennila
Aquatic Environmental Management Division, Central Institute of Fisheries Education, Mumbai

Objective:

- To analyze the bacterial diversity between closely-related bacterial species and strains with reference to the salt-tolerant gene using various molecular techniques

Significant Achievements:

- Cultures isolated from the intertidal zones were tested for their tolerance to increasing concentrations of NaCl (2.5% to 30%). Protein profiling of Gram-positive and Gram-negative isolates tolerant to concentrations of greater than or equal to 10% salt was carried out (fig 1).

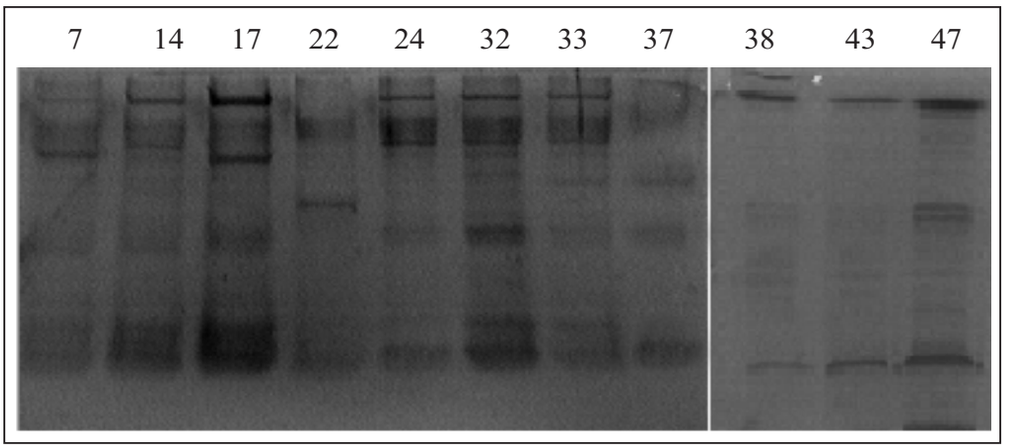


Fig. 1. SDS-PAGE for 1D-protein profiling of 11 of the most salt-tolerant Gram-positive isolates

1D-proteomics of the isolate N2 in the presence and absence of external assistance of compatible solute, trehalose, under salt stress was also done.

- In the salt tolerance experiment performed, only seven isolates tolerated up to 30% NaCl concentration, three tolerated up to 25% and not beyond, ten isolates tolerated up to 15% and not beyond, 42 tolerated up to 10% and not beyond, and 11 tolerated up to 5% and not beyond.
- The known standards include Culture 38 in the first cluster, identified as *Bacillus pasteurii* by biochemical characterization. Culture 17 was identified as *B. macerans* (third cluster). Culture 33 was identified as *B. larvae*. From the two main branches, the major branch bore two main clusters; 33 grouped out of both branches. The minor branch bore three organisms. In conclusion, most of the diversity of the Gram-positive salt-tolerant isolates seems to be limited to the genus *Bacillus*. Although, other Gram-positive isolates are present, including many micrococcal isolates, the *Bacillus* isolates seem to be more effective in withstanding salt stress.
- The tree representing the Gram-negative isolates branches into two clusters. The major cluster holds two smaller clusters. The minor cluster holds four organisms. N6 groups alone in the major cluster.
- The organism N2 was grown in 1%, 2% and 3% NaCl concentrations in Nutrient Broth with no initial concentration of NaCl. The organism was grown with 0.25-mM trehalose (lanes bearing +T sign) and absolutely no trehalose (lanes bearing –T sign). In the first two lanes from the left, a band around 43 kD has become more prominent in the second lane.

Conclusion:

Ninety-three isolates were screened for salt tolerance. Fifty-nine isolates were found to tolerate 10% or more NaCl; 12 of the distinct Gram-positive isolates and 12 of the Gram-negative isolates were subjected to protein profiling and their diversity mapped and visualized using the Neighbour-Joining Algorithm in NTSYSpc 4.0. Most of the tolerant isolates in the Gram-positive list clustered with identified *Bacillus* species: 1D-proteomics was done for N2, grown under salt stress in the presence and absence of externally supplied trehalose.

Isolation and characterization of *Flavobacterium* species from fish and Aquatic Environment

PI: Gaurav Rathore
National Bureau of Fish Genetic Resources, Lucknow, U.P.

Objectives:

- To isolate, identify and characterize *Flavobacterium* from fish and water samples.
- To develop sensitive and rapid method for detection of *Flavobacterium* by PCR.

Significant Achievements:

- A total of 41 presumptive isolates of *Flavobacterium* were used for amplification of 16s rDNA. All these isolates were also characterized by biochemical tests. Approximately 500bp of the downstream region of 16SrDNA gene fragment was sequenced from isolates. The 16S rDNA sequences were compared with sequences deposited in the Genbank database using the BLAST programme and Ribosomal Database Project II (RDP II) for molecular identification of bacteria. On basis of 16s rRNA sequencing, a total of 7 isolates were confirmed as *Flavobacterium* sp, 22 *Chyrseobacterium* sp, 6 *Myroides* sp, 1 *Sejonjia* sp, 1 *Weekesella* sp and 4 *Flectobacillus* sp.
- Phylogenetic analysis showed eight major clades, out of which five clades belonged to *Chyrseobacterium* species. *Flavobacterium* sp and *Myroides* sp were present in the same cluster but different clades, indicating high degree of homology in 16s rDNA sequences.
- Several combinations of forward and reverse primers were used for the selection of *Flavobacterium* sp specific primers for amplification of 16s-23s rDNA ISR. Out of these combinations, only 11 sets of ISR primers were specific to *Flavobacterium* species. Among these 11 sets, AMSEF & AMSER combination resulted in amplification of all *Flavobacterium* sp isolates. PCR detection sensitivity of ISR primers was calculated to be 20pg of genomic DNA of *Flavobacterium* sp. No cross reactivity was observed with many related genus within the *Flavobacteriaceae* family except *Myroides* species due to high homology in the ribosomal operon.
- Three different aquatic environments were screened for the presence of *Flavobacterium* sp by using the ISR specific primers (AMSEF/AMSER & AMSDF/AMSCR). Out of 260 isolates screened by PCR, only 14 isolates were positive for ISR amplification using *Flavobacterium* sp specific primers. Surprisingly, no isolate from marine/ brackish water environment and alkaline lake water were positive for *Flavobacterium* species. The identity of these 14 isolates was re-confirmed by amplification and sequencing of 16s rDNA. All the isolates showed 100 % homology to *Flavobacterium* sp available at RDP-II. These results prove that the new primers designed for amplification of ISR are useful for highly specific and sensitive detection of *Flavobacterium* species.
- A common band of approx 400 bp of box element was observed in the most of the *Flavobacterium* sp as seen by BOX-PCR for genetic diversity analysis.

Conclusion:

Aquaculture system is facing the disease problems globally. *Flavobacterium* species is a primary bacterial pathogen of fish, associated with the gills disease in fish. In the present study, the occurrence of culturable *Flavobacterium* species in different aquatic environments including fish was found to be approximately 3% of the screened yellow pigmented isolates (493 no.). Surprisingly, no isolate from marine/ brackish water environment and alkaline lake water were positive for *Flavobacterium* species. This may be due to low copy number (6 no.) of ribosomal operon in the genome of *Flavobacterium* species. On basis of 16s rDNA sequencing, significant bacterial diversity was observed among yellow pigmented gram-negative isolates. Two sets of new ISR based primers have been developed for rapid and sensitive screening of *Flavobacterium* species from aquatic environment. PCR detection sensitivity of ISR primers was calculated to be 20pg of genomic DNA of *Flavobacterium* sp. No cross reactivity was observed with many related genus within the *Flavobacteriaceae* family except *Myroides* species due to high homology in the ribosomal operon.

Bacterial diversity analysis from different district/ ecological zones of Madhya Pradesh

PI: Kiran Singh
Department of Microbiology, Barkatullah University, Bhopal – 462026

Objectives:

- Sample collection from different eco-zones of Madhya Pradesh
- Isolation of microorganisms and PCR based characterization.
- Characterization of PCR based technique, namely, ERIC, REP and BOX.
- Optimize the use of DNA probe for direct detection of efficient microbial strains from plant parts and soil.

Significant Achievements:

- 350 bacterial strains from agricultural field have been isolated, among these 5 isolates of *Pseudomonas sp.* were obtained and fluorescent pigment produced by the isolates was characterized by UV Vis spectrophotometry; 10 isolates of *Bacillus sp.* has been identified whereas, 80 cultures showed free living as well as symbiotic nitrogen fixing property under laboratory conditions. Out of those 56 cultures, which are biochemically characterized, confirmed the properties of free-living bacterial cells of *Azotobacter.*
- Identification upto species level was done by adding selective compound to liquid Jensen medium. 1). For *A. vinelandii* species L-rhamnose, ethylene glycol, erythritol of D-arabitol as C-source. Alternatively 1.0% Na-benzoate or 0.1% phenol are used to inhibit the growth of other species of *Azotobacter*. 2). For *A.beijerinckii* species – L-tartrate, o-hydroxybenzoate, D-glucuronate or D-galactouronate and pH of 6 favours the growth of bacteria. 3). For *A .armeniacus* species – caprylate is used as carbon source 4). *Azotobacter chroococcum* is one of the most common species which needs no special enrichment.
- 13 strains of *A. vinelandii* were isolated from Bhopal Raisen Neemuch districts of M.P. 11 strains of *A.beijerinckii* were isolated from Betul, Indore, Tikamgarh and Sehore. 8 strains of *A. armeniacus* Bhopal and Nimad.17 strains of *Azotobacter chroococcum* were isolated from Hoshangabad, Indore, Betul and Neemuch.
- 15 strains of *R.leguminsorum* were isolated from Tikamgarh, Nimad, Neemuch, Raisen, Bhopal, Ujjain and Indore : 12 strains *Bradyrhizobium japonicum* from Ujjain, Sehore, Betul, Indore and Hosangabad.
- Bacterial strains isolated from root nodule are being confirmed as *Rhizobium* species by various biochemical test like Hofer's alkaline medium pH (11) test, Glucose peptone agar medium test, Ketolactose test, Staining G-ve, Liquefaction of Gelatin test, Action on Milk test.
- Nodulation ability of these strains was confimed by nodulation test via Jensen's seedling agar method in the laboratory conditions.
- Nodulation frequency of nitrogen fixing isolates will be confirmed using Gas Chromatography for N_2-ase activity in further course of study.

Exploration and Screening of Rainfed Ecosystem Microbial Diversity

PI: P. K. Sharma
Co-PI: R.Gera
Department of Microbiology, CCS HAU, Hisar, Haryana

Objectives:

- To study the soil microbial diversity of nitrogen fixing bacteria under extreme environments (high temperature, low moisture, low nutrients and high salt concentration).
- To characterize the isolates for agriculturally useful traits like nitrogen fixation, growth hormone production and as fungal antagonist.
- To characterize the symbiotic nitrogen fixer for nodulation and nitrogen fixation properties.
- To identify the specific populations associated with abiotic variables and functions.

- *Rhizobium* isolated from rabi legumes were positive for infection on their respective hosts and showed the presence of *nod*C gene.

Conclusion:

RFLP analysis of 16S rDNA indicated wide variability in microbial community structure with respect to host and location.. Two hundreds new isolation of bacteria including free living, symbiotic and actinobacteria were made. Genomic DNA of these isolates was amplified using 16S rRNA gene primers. The amplified 16S rDNA of 30 isolates was given for sequencing and analysis of 8 sequences showed homology to four unidentified Alphaproteobacteria and Gammaproteobacteria while

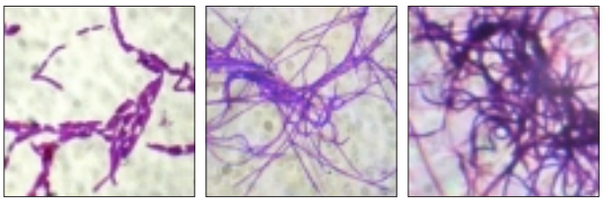


Fig.: Gram staining of selected isolates. The photograph shows Gram +ve rods and Gram +ve filamentous bacteria.

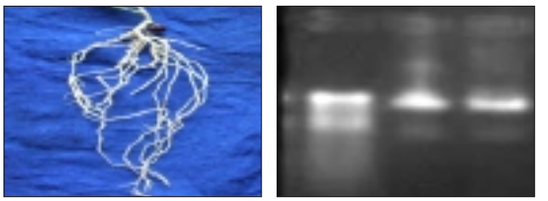


Fig.: Characterization of chickpea rhizobia (a) Nodulation test (b) amplification of *nod*C gene.

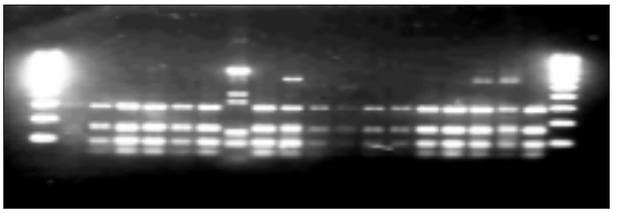


Fig.: Restriction patterns obtained with enzyme *Msp*I.

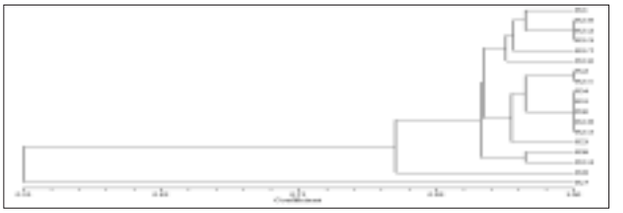


Fig.: Dendrogram depicting grouping of the Actinobacterial isolates.

Significant Achievements:

- RFLP analysis of 16S rDNA indicated wide variability in microbial community structure with respect to host and location.
- Two hundred new isolation of bacteria including free living, symbiotic and actinobacteria were made.
- A bacterial isolate was identified as *Pseudomonas chlororaphis* which is a known biocontrol agents
- Two major cluster of actinobacteria were identified on the basis of RFLP from rhizosphere soils of Haryana

three isolates were identified as *Bacillus* species. A bacterial isolate was identified as *Pseudomonas chlororaphis* which is a known biocontrol agent Seventy isolates of nitrogen fixing bacteria from arid zone soils and salt affected soils showed nitrogenase activity and will be tested for the presence of nif genes. *Rhizobium* isolated from rabi legumes (Chickpea and berseem) were positive for infection on their respective hosts and showed the presence of nodC gene. RFLP analysis of the amplified nodC gene is in progress

Exploration and Screening of Microbial Diversity of Bihar and their Potential Application

PI: V. K. Shahi
Co-PI: Daya Ram; R. K. Pandey; Subodh Kumar Sinha
R.A.U., Pusa, Samatipur-848125, Bihar

Objectives:

- Collection, identification and conservation of agriculturally important microbes from different agro climatic zone of Bihar.
- Characterization of germplasm to ensure broad-spectrum genetic variability.
- Evaluation of potential strains for their use and development of technology for mass multiplication.

Significant Achievements:

- Eight explorations were made with 650 samples which covered 59 villages of 32 block of 12 districts.

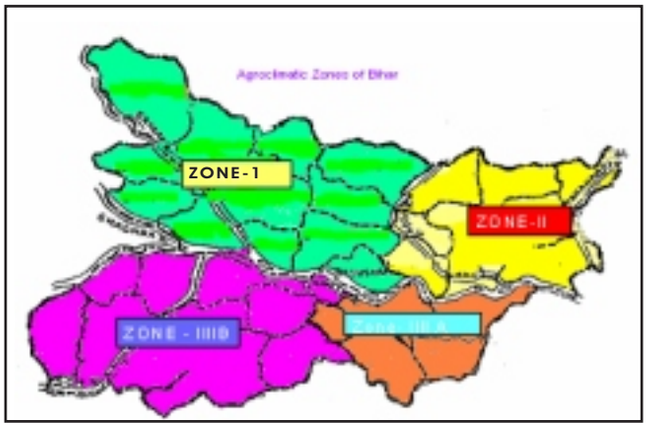


Fig 1. Survey sites in Bihar

- 28 isolates including 8 isolates of fungi and 20 isolate of bacteria was submitted to the NBAIM, Mau, U. P.
- Organic manure rich soils samples contain higher number of organic matter decomposing microorganism.
- Low land soil contains more populations of *Azotobacters*, PSB and N_2 fixing bacteria.
- Light textured soil of different rivers basins shows the presence of microorganisms in low numbers with slow growth and minimum slime production.
- Chaur lands of Hanuman Nagar and Hayaghats blocks of Darbhanga district as well as Mokama Tal of Patna districts, Paru block of Muzaffarpur and few pockets of Kalyanpur block of Samastipur districts has maximum population of *Azotobacter, Rhizobium* and PSB which are fast growing, much shinny and dominating to other found in various blocks of different districts or the same district i.e. Samastipur and Vaishali..
- Vegetable growing areas showed more number of fungal populations than nutrient solubilizing bacteria.
- Plantation crops like litchi grown in light textured soil showed better response than heavy textured soil with maximum population (CFU) of fungi as well as other bacterial species.
- Rabi maize growing soils of Khagaria, Begusarai, Samastipur, Bhagalpur and Muzaffarpur showed higher population of PSB and Azotobacter than fallow or flooded fields of Samastipur, Muzaffarpur, Darbhanga, Lakhisarai, Begusaria, Kaimur, Bojpur and Rohtas districts.

Conclusion:

Eight explorations covering 59 villages of 32 blocks of 12 districts were made and a total of 650 samples were collected. Out of 650 samples, 242 samples were analyzed for microbial diversity. A total of 480 agriculturally important microbes were isolated from different crops. Out of 480 organisms, 243 consisting of 112 Azotobacter, 52 *Rhizobium* isolates, 44 PSB isolates, 27 Azosprillium isolates and 8 *Glucanocatobacter* isolates from Rice, Wheat, Maize, Sugarcane and Pulses crops were considered for further study. Commercial formulation of specific organism will be done after nutritional validity and genetic variability.

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

PI: Ratul Saikia
Co-PI: T. C. Bora
Biotechnology Division, North East Institute of Science & Technology (CSIR), Jorhat, Assam

Objectives:

- Collection of environmental samples (soil, water, plant, decomposed matter etc) from different habitant of North-East States of India.
- Isolation, purification and preservation of bacterial isolates
- Bacterial data base development for future uses
- Screening of bacterial isolates for biocontrol etc.
- Characterization of potential isolates and diversity analysis.

Significant Achievements:

- We have explored and collected environmental samples from various ecological niches of North Eastern States. More than 500 bacterial isolates and 70 *Streptomyces* strains were isolated from 6-NE States; more than 50 nos. of fluorescent pseudomonads were also isolated from different tea gardens of Assam. These fluorescent pseudomonads showed strong antimicrobial properties against microbial pathogens (fungi & bacteria); diversity of these fluorescent pseudomonads were analyzed based on morphological, biochemical and molecular characters.
- We have screened the bacterial and streptomyces isolates for antagonistic activity against different plant pathogens. One of the biocontrol potential bacteria from Garam Pani (a natural hot spring) of Golaghat District, Assam has been identified as *Brevibacillus laterosporus* (BPM3) and the sequence has been submitted to the NCBI GeneBank (Acc. No. EU159585). Process parameters for maximum antagonistic activity of the bacterium was studied and recorded at incubation temperature 30^0C and pH 8.5; in case of carbon and nitrogen source, maximum activity was recorded with dextrose and $(NH_4)_2SO_4$ respectively. Further experiments need to be carried out for field level application.
- Development of a microbial database of North East gene pool is in progress.
- 35 fluorescent pseudononads and 20 Streptomyces isolates have been submitted to NBAIM's Culture Bank.

Collection, identification and characterization of microbial diversity of North Bengal

PI: B. N. Chakraborty
Co-PI: **U. Chakraborty, A. Saha**
Immuno-Phytopathology Laboratory, Department of Botany, University of North Bengal, North Bengal

Objectives:

- Isolation of microorganisms (fungi, bacteria and actinomycetes) from soil (forest, agricultural and riverine) and plant roots of six districts of North Bengal (Darjeeling, Jalpaiguri, Cooch Behar, Uttar Dinajpur, Dakshin Dinajpur and Malda).
- Screening of the isolates for their utilization as bioprotector and biofertilizer.
- Extraction of Genomic DNA, molecular diversity analysis, PCR-RFLP analysis of SSU rDNA for identification.

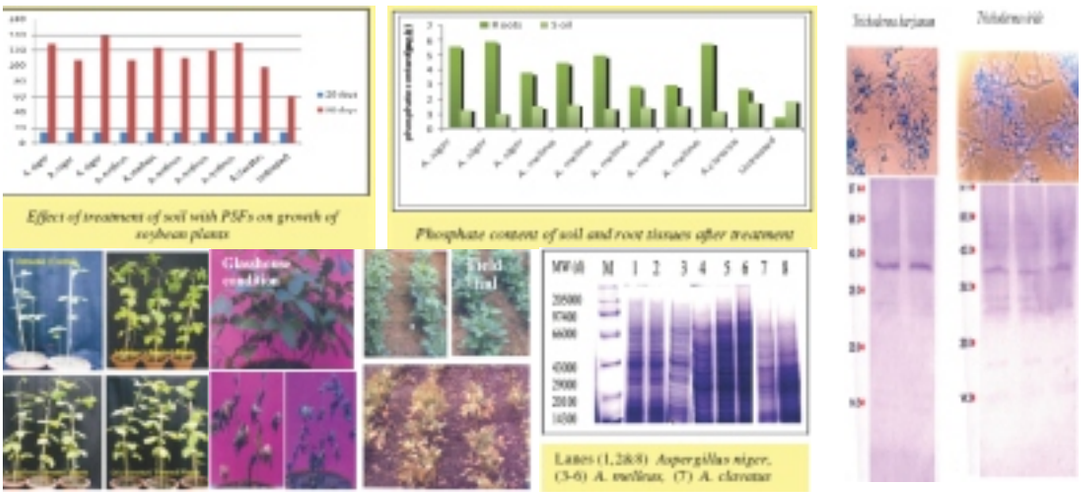
Significant Achievements:

- A total of 442 fungal isolates were obtained from soil samples collected from Darjeeling, Jalpaiguri and Cooch Behar districts of North Bengal. Apart from the fungal isolates, 10 isolates of actinomycetes and 14 bacterial isolates have been obtained from sampling sites of Darjeeling, Malda and Uttar Dinajpur.
- Some of the frequently occurring identified isolates include *Aspergillus niger, A.melleus, A.clavatus, Penicillium sp, Trichoderma viride, Trichoderma harzianum, Acremonium fusidioides, Curvularia lunata, Fusarium chlamydosporum, Rhizopus oryzae, Aspergillus fumigatus, Fusarium oxysporum, Aspergillus candidus,, Aspergillus terreus, Alternaria alternata, Fusarium equiseti, Aspergillus carneus, Aspergillus ustus, Aspergillus flavus, Aspergillus fumigatus, Cladosporium cladosporioides, Drechslera sp., Bipolaris sp., Mortierella*

polycephala, Gliocladium viride, Fusarium solani, Colletotrichum gloeosporioides, Cochliobolus lunatus, Sclerotium rolfsii, Sphaerostilbe repens, Rhizopus oryzae, Fusarium graminearum, Macrophomina phaseolina, Rhizoctonia solani, Sclerotinia sclerotiorum and *Fomes lamaoensis.*

- Nine different types of glomalean spores were recovered from the soil samples, including five from the genus *Glomus*, three from *Acaulospora*, one from *Gigaspora* and few undefined species of *Sclerocystis* and their identification was done using scanning electron microscopy.
- A total of 71 fungal isolates as well as 4 actinomycetes, and 5 bacterial isolates showed phosphate solubilizing activities. *In vivo* tests in glass house condition showed growth promoting activity in soybean plants by the PSFs.

obtained from forest, riverine and agriculture dependent soil samples collected from six districts (Darjeeling, Jalpaiguri, Cooch Behar, Uttar Dinajpur, Dakshin Dinajpur and Malda) of North Bengal. Apart from the fungal isolates, 10 isolates of actinomycetes and 14 bacterial isolates have also been obtained from sampling sites of Darjeeling, Malda and Uttar Dinajpur. Some of the most frequently occurring fungi include species of *Aspergillus,Penicillium, Trichoderma, Acremonium, Curvularia, Fusarium, Rhizopus, Alternaria, Cladosporium, Drechslera, Bipolaris,, Mortierella, Gliocladium, Colletotrichum, Cochliobolus, Sclerotium, Sphaerostilbe, Macrophomina phaseolina, Rhizoctonia , Sclerotinia* and *Fomes.* Nine different types of glomalean spores (5 species of *Glomus*, 3 of *Acaulospora*, 1 of *Gigaspora*) were identified using scanning electron microscopy. Among the isolated



- *Trichoderma harzianum*, *T.viride* and other species of *Trichoderma* showed antagonistic activities *in vitro* against tested phytopathogens (*Fusarium graminearum, Fomes lamaoensis*, *Sphaerostilbe repens*, *Sclerotium rolfsii, Colletotrichum gloeosporioides* and *Macrophomina phaseolina.* Both *T.harzianum* and *T.viride* suppressed root rot of soybean caused by *Fusarium graminearum*.
- Immunological formats were developed for screening biocontrol agents directly from soil.
- PCR amplified products of ITS region of genomic DNA from selected PSFs, BCAs and phytopathogens have been obtained and their RAPD analyses are being conducted.

Conclusion:

Four hundred and forty two fungal isolates were microorganisms, 71 fungal, 4 actinomycetes, and 5 bacterial isolates showed phosphate solubilizing activities as tested *in vitro* and *in vivo*. Of the phosphate solubilizers, 9 showed maximum growth promotion. Based on cellulase activity and antagonistic activities the isolated organisms were categorized. *Trichoderma harzianum* , *T.viride* and other species of *Trichoderma* showed antagonistic activities *in vitro* against tested phytopathogens (*Fusarium graminearum, Fomes lamaoensis* , *Sphaerostilbe repens*, *Sclerotium rolfsii, Colletotrichum gloeosporioides* and *Macrophomina phaseolina*) and suppressed root rot of soybean caused by *Fusarium graminearum*. Immunological formats were developed for screening biocontrol agents directly from soil. PCR amplified products of ITS region of genomic DNA from selected PSFs, BCAs and phytopathogens have been obtained and their RAPD analyses are being conducted.

Biodiversity, characterization & conservation of cyanobacteria of Indo-Burma Biodiversity hotspots (NE Zone of India) for harnessing of value added products

PI: O. N. Tiwari
Co-PI: Sunil Thorat
Institute of Bioresources & Sustainable Development, Imphal, Manipur

Objectives:

- Survey and isolation of cyanobacteria from different ecological habitats of NE region of India (Manipur, Meghalaya, Tripura, Nagaland, Mizoram, Arunachal Pradesh, Assam).
- Cataloguing and Identification of potential strains for natural pigments and biofertilizer.
- Development of optimized protocol for enhanced production of natural pigments.

Significant Achievements:

- More than 150 soil samples were collected from the Meghalaya, Manipur and Tripura for isolation of cyanbacterial diversity (fig 1).

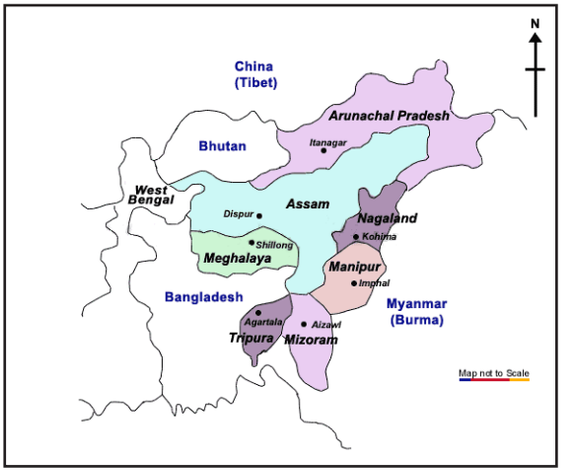


Fig 1. Survey sites in North-Eastern part of India

- Total 110 strains belonging to 16 genera of cyanobacteria have been encountered from different ecological habitats of three states, namely, Manipur, Meghalaya (Garo and Jayantia Hills) and Tripura and added to the pool of existing 200 cyanobacterial strains available in the germplasm of IBSD, Imphal. The strains are *Phormidium* (18), *Limnothrix* (05), *Oscillatoria* (05), *Lyngbya* (13), *Microcoleus* (01), *Plectonema* (23), *Nostoc* (09), *Anabaena* (19), *Scytonema* (01), *Aulosira* (02), *Tolypothrix* (01), *Calothrix* (02), *Microcheate* (06), *Dichlothrix* (02), *Haphalosiphon* (01) and *Westiellopsis* (02).
- Fifteen non - heterocystous filamentous cyanobacterial strains of Manipur state have been morphologically characterized and identified up to species level and their cultural behaviour also documented.
- Fifty eight cyanobacterial strains isolated from Manipur have been studied for their chl-a, carotenoids and phycobiliproteins contents and four stains from each have been selected for further studies.
- Twenty two cyanobacterial strains isolated from Meghalaya have been studied for their chl-a, carotenoids and phycobiliproteins contents and three stains from each have been selected for further studies.
- **Two value added cyanobacterial products namely, Spiro papad and Spirogel (fig 2) have been prepared and launched in markets and highly demandable in local community**.
- Spirogel is a product of cyanobacteria *Spirulina platensis* (fig 3) IBSD 1 & 2 (s). It is non-toxic protein rich product with various pharmaceutical properties. It possess phycobiliproteins, caroteniods, amino acids, fatty acids, exocellular polysaccarides, -lactamase, restriction enzymes, bioactive compounds and γ-linolenic acid (GLA)

Fig 2. Preparation of Spiro papad and spiro gel in plastic cans

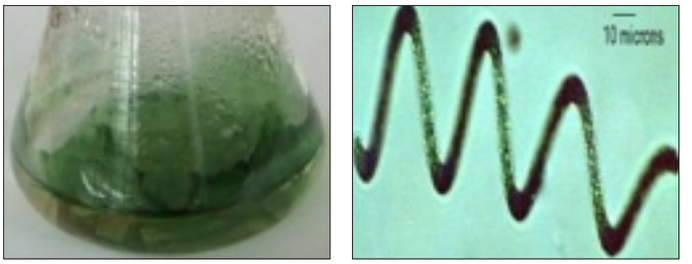


Fig 3. Culture of *Spirulina* and its photomicrograph

Conclusion:

Samples collected from three states of North Eastern India showed tremendous variation in community structure cyanobacterial diversity. Out of total 110 strains characterized biochemically different strains showed their ability to produce Phycocynins, Allo-phycocyanin, chlorophyll-a, and carotenoids. Two value added cyanobacterial products namely, Spiro papad and Spirogel were prepared and launched in markets and is in high demand by local community.

Assessment of molecular diversity and exploitation of plant growth promoting rhizobacteria for sustainable agriculture

PI: Ashok Kumar, School of Biotechnology
Co-PI: M. B. Tyagi, R. P. Sinha, CAS in Botany
Banaras Hindu University, Varanasi

Objectives:

- Screening and isolation of diazotrophic bacteria from Northern Plain especially from rhizospheric soils.
- Assessment of metabolic diversity among various rhizospheric diazotrophic bacteria for plant growth promoting ability such as N_2 fixation, ammonia excretion, IAA and siderophore production and P solubilization.
- Molecular diversity analysis employing various molecular biological techniques.
- 16S rDNA amplification and sequencing for identification of selected important isolates.
- Selection of most efficient strain having combination of all the beneficial characters for use as PGPR.

Significant achievements:

- Out of 12 districts selected sampling of rhizospheric soil from 7 districts of UP and 3 districts of Bihar has been completed (fig 1).

Fig 1. Sampling locations in UP and Bihar

- Based on similarity index altogether 154 diazotrophic isolates have been tentatively identified as distinct isolates and they seem to belong to different species/strains. Among 154 isolates, 124 isolates were found to be endowed with one or other plant growth promoting characters.
- Quantification of IAA production in all the isolates has been made and all the 109 isolates showed differential level of IAA production. IAA production was significantly affected with the supplementation of N sources especially amino acids in the medium. Certain isolates were capable to produce IAA even in the presence of as high as 5% NaCl in the medium. Isolate J2S5 showed the highest level of IAA production (354 µg IAA/mg protein).
- Among all the isolates tested for plant growth promoting characters, the percentage of P solubilizing isolates was lowest (21%). The percentage of isolates showing IAA production was more than 70%.
- Scoring of distinct isolates (154) based on morphological and physiological features became evident when ARDRA of 13 isolates belonging to three districts was done. Based on construction of phylogenetic tree, it was evident that all these isolates indeed belong to 12 ARDRA groups suggesting distinctness of individual isolate. Almost similar results were obtained in isolates belonging to other districts.
- Based on partial sequencing of 16S rDNA, tentative identification of 22 efficient isolates (PGP) has been made. The accession number has also been obtained.
- Ammonia excretion is not common phenomenon in free-living bacteria.
- Inoculation of isolate VA8S1 (isolated from the rice field of Varanasi district) to a rice variety Saryu-52 showed significant increase in total length, fresh weight and chlorophyll a content both in synthetic medium and pot experiment after 4 and 7 weeks respectively.

Conclusion:

In total 485 bacterial isolates were isolated from rice field soils of 10 districts including 7 from UP and 3 from Bihar. Based on similarity index and plant growth promoting properties/activities between 485 isolates, 154 have been characterized as distinct isolates. Results of metabolic diversity revealed that out of 154 isolates, 109 (70.70%) were capable to produce IAA and only 33 (21%) were P solubilizers. Siderophore production ability was recorded in 53 (34%). IAA production was significantly affected with the addition of various amino acids in the medium. Our results suggest that metabolic diversity does occur among various bacterial isolates. Genomic DNA from all the 154 isolates has been isolated. With a view to study diversity and identification of various isolates, 16S rDNA was amplified from all these isolates. ARDRA was performed with selected isolates which suggested that these isolates indeed belong to different groups. Amplification of *nif H* and *pqq* genes from various isolates showed that all the isolates do carry *nif H* but occurrence of *pqq* gene is not a common feature. Obviously cofactor other than PQQ may be involved in P solubilization in certain group of bacteria. Sequencing of 16S rDNA of 22 efficient isolates has been done and tentative identification was made. It appears that occurrence of *Pseudomonas.,Bacillus, Agrobacterium, Serratia,* and *Klebsiella* sp is very common whereas species such as *Microbacterium, Pantoea* and *Cronobacter* are of rare occurrence.

Exploration of Plant Pathogenic and Antagonistic Microbial Resources Associated with Vegetable and Spice crops of Andaman and Nicobar Islands

PI: V. Jayakumar
Co-PI: Kishan Kumar
Central Agricultural Research Institute, Port Blair, Andamans

Objectives:

- To isolate and characterize phyllosphere and soil borne plant pathogens associated with vegetable crops and spices from coastal agro-ecosystems of Andaman & Nicobar Islands.
- To isolate and characterize antagonists (*Pseudomonas* spp, *Bacillus* spp and *Trichoderma* spp) associated with rhizosphere of spices and vegetable crops.
- To evaluate i*n vitro* the isolated antagonistic microorganisms for their biocontrol and plant growth promoting properties.
- Molecular characterization and diversity analysis of isolated plant pathogens and antagonistic microorganisms.

Significant achievements:

- Andaman & Nicobar consists of 3 districts and in that the cultivated areas of 61 villages in South Andaman and 20 villages in Middle Andaman were surveyed in the reported period and collected infected plant samples and rhizosphere soil samples from vegetable and spice crops (Fig 1and Table 1). From the initiation of the project a total of 86 villages were surveyed for collection of samples.

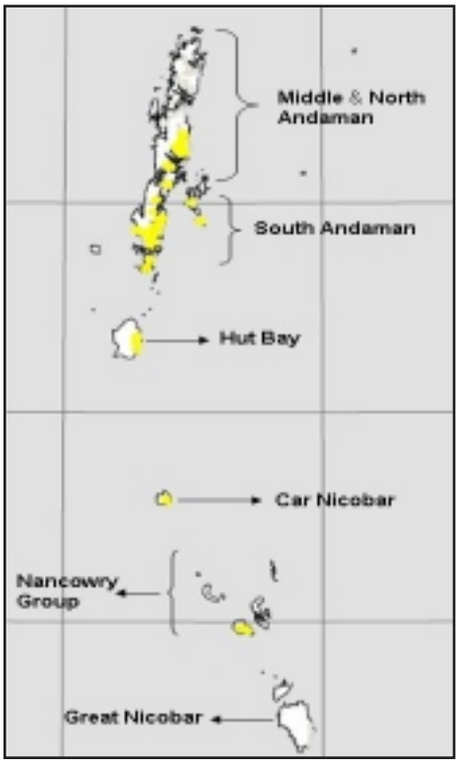


Fig 1. Andaman and Nicobar surveyed area (Yellow marking)

Table 1. Geographical locations surveyed for collection of samples

Name of the district	Total no. of villages surveyed		
	2006-07	2007-08	Total
South Andaman	35	26	**61**
Middle & North Andaman	-	20	**20**
Nicobar	5	-	**5**
Total	**40**	**46**	**86**

- A total of 285 organisms were isolated from the samples collected during 2007-08, i.e., from the initiation of the project so far 543 microorganisms were isolated and maintained in slants at Central Agricultural Research Institute, Port Blair. The detailed list of microorganism isolated and maintained at CARI, Port Blair is listed in Table 2.

Table 2. Microorganisms isolated from vegetable and spice crops

Isolate	Year		Total
	2006-07	2007-08	
Fungal pathogens	20	13	**33**
Bacterial pathogens	23	8	**31**
Bacterial antagonist (isolated on KB)	104	100	**204**
Bacterial antagonist (Isolated on NA)	90	105	**195**
Mycoparasitic fungi (Isolated on TSM)	22	58	**80**
Total	**259**	**284**	**543**

- Of the 543 microorganisms isolated, 33, 31, 339 and 80 were fungal pathogens, bacterial pathogens, bacterial antagonistic microorganisms and mycoparasitic fungi respectively.
- Among the 33 fungal pathogens 10 were identified as *Colletotrichum* spp, 1 is *Sclerotium rolfsii* and 1 is *Fusarium* sp. three *Colletotrichum* spp were identified as *C. gloeosporioides*.
- 23 of the 31 bacterial pathogens were identified as *R. solanacearum*
- Among the 399 bacterial antagonistic microorganisms 54 were identified as *Pseudomonas* spp and 47 as *Bacillus* spp by morphological and biochemical characterization
- The thorough study on morphological, cultural and microscopic studies revealed that 63 of the mycoparasitic fungi belong to *Trichoderma* spp and in that 10 were identified at species level, i.e., *T. hamatum*-6, *T. pseudokoningii*-2, *T. koningii*-1 and *T. harzianum*-1
- Pathogenecity test for the 23 *R. solanacearum* isolates and 10 *Colletotrichum* spp were carried out in the respective host crop under glass house condition and the Koch's postulates were proved.
- The antagonistic property of 47 *Bacillus* spp isolates are being tested *in vitro* against i.e., *Sclerotium rolfsii*, *C. capsici* and *R. solaneacearum* by dual culture assay.
- The total DNA of 10 *Colletotrichum* spp was isolated and their ITS region was amplified in PCR with ITS1 and ITS4 primers, purified the amplicons and submitted for gene sequencing. RFLP analysis of PCR-ITS amplicons with *Hinf* I restriction enzyme revealed presence of 3 gropus of *Colletotrichum* spp.
- Among the 23 isolates of *R. solanacearum* 14 were amplified for their 16Sr DNA region with eubacterial primers and ARDRA analysis was performed with three different restriction enzymes *viz. Hinf* I, *Hpa* II and *Taq* I. The results revealed the occurrence of 7 groups of *R. solanacearum*.
- After confirming the species, a total of thirteen microorganisms (10 *Trichoderma* spp and 3 *Colletotrichum* spp) were submitted to NBAIM, Mau.

Microbial Diversity and Identification of Western Ghats: Collection, Mapping, assessment of the geographical distribution and in vitro conservation of agriculturally important microorganisms of the Western Ghats

PI: D. Radhakrishna
Co PI: B. C. Mallesha
Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore

Objectives:

- Isolation, enumeration, characterization and inventorization of AIMs (N2 fixers, P-solubilizers, VAM, PGPRs, fluorescent pseudomonads, chitin decomposers, cellulose and lignin degraders) along the central regions of Western Ghats in Karnataka).
- Assess the geographical distribution and developing thematic maps for the above groups of organisms.
- Assess the functional potentials of each group of organisms for use in agriculture
- Set up the culture bank of the potential isolates under each group and deposit with the NBAIM
- Set up the Western Ghats region specific database on the population and diversity of AIMs.

Significant Achievements:

- Soil samples collected from Western Ghats of Hassan forest region recorded a population density of microorganisms in the range of 10^4 cfu /g to 10^5 cfu/g as influenced by organic carbon.
- Phosphate solubilizing bacteria of bacillus type was predominant followed by nitrogen fixing bacterial population
- Fungi isolated from decaying wood belonged to Basidiomycetes .A few were isolated and maintained in the laboratory. They failed to produce fruiting bodies in *invitro.* Cell free extracts of these fungi showed plant growth promoting activity.
- Lignin degrading fungi (fig 1) were tested for effective decomposition and maintained for further activity.
- Bacterial and fungal Isolates showed very effective antibacterial and anti fungal activities (fig 2). Many of the Pseudomonad's were having plant growth promoting activity.

Conclusion:

The research work on microbial diversity and identification of Western Ghats has been carried out in the forest area. Western Ghats of the central range covering the districts of Hassan, Kodagu, was covered for the sampling. Each soil sample is analyzed for the population density of bacteria, fungi, cellulose degraders, chitin degraders, phosphate solubiliser, nitrogen fixers and *Pseudomonas fluorescence*. Microbial sample collected were categorized into three types: forest, floor and Soil samples. Using specific and enriched media different fungal isolates were obtained having lignin degrading ability. Different bacterial isolates having PGP attributes such as P solubilization and N fixation were also obtained. The mechanisms that are regulating the diversity of species and traits of microorganisms are solid surface, strongly fluctuating moist condition, variable and often limiting levels of organic and inorganic substances and interaction with the soil microorganisms including the influence of plant roots. Identification and functional characterization of the isolates will be done by BIOLOG or conventional biochemical methods.

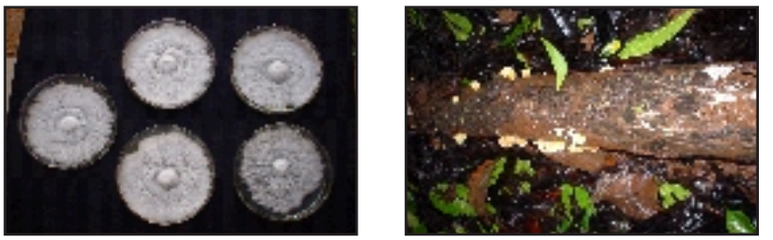


Fig 1. Lignocellulolytic Fungal isolates from decaying wood

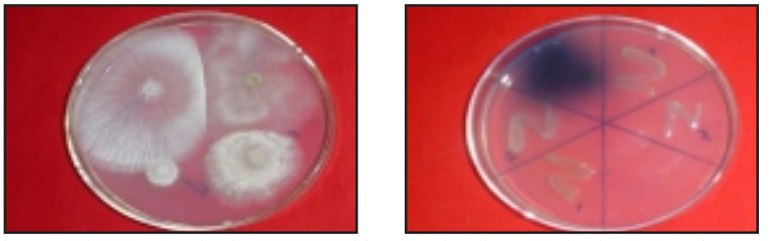


Fig 2. Fungal isolates with antimicrobial activity and bacterial isolates with plant growth promoting attributes

Isolation and Characterization of Microorganism from Freshwater Ecosystem

PI: N. K. Maiti
Co-PI: Sriprakash Mohanty
Central Institute of Freshwater Aquaculture (ICAR), Bhubaneshwar, Orissa

Objectives:

- To isolate different bacterial species from soil water and fish.
- To identify the isolated species by phenotypic characterization.
- To study the intra and inter species genomic diversity by molecular tools.
- To find out the correlation between phenotypic and genomic characteristics.
- To evaluate the comparative efficacy of molecular tests to study the diversity of bacteria.

Significant Achievements:

- For isolation of cellulolytic bacteria from freshwater ecosystems 200 samples were collected from the ponds sediments and water in and around CIFA, Kausalyaganga, Bhubaneswar and different village ponds in Khurda district.
- Screening was carried out, by spreading the pre-enriched samples performing a serial dilution procedure in CMC agar plates. Different morphologically distinct colonies were pure cultured in CMC agar plates (cellulose enrichment broth + 2% agar). Around 41 positive isolates were obtained and preserved in 40% glycerol at –20^{0}C for further use.
- Production of indole, citrate utilization, H_2S production, methyl red reaction, acetyl methyl carbinol production, phenylalanine deamination, arginine dehydrolase activity, motility and different agriculturally and industrially important enzymes *viz*. amylase, gelatinase, lipase, lecithinase, urease, oxidase, catalase were detected .
- The organisms could utilize carbohydrates and related compounds (monosaccharides, disaccharides, trisaccharies, polysaccharides, polyhydric alcohols, glycosides and organic acids) such as arabinose, glucose, sucrose, raffinose, fructose, inulin, rhamnose, lactose, mannitol, sorbose, sorbitol, salicin.
- Effect of physiological parameters on growth including temperature and salinity was studied and results are shown in table 1 and 2.
- Identification was carried out by the conventional biochemical methods, which includes enzymatic activity, salt tolerance, and temperature resistance of the different isolates following Bergey's manual of systematic bacteriology. All the isolates belongs to genus *Bacillus*
- Genomic DNA was extracted. The DNA quality was checked and quantified spectrophotomectrically.
- 16s rDNA was amplified using the universal 16s primer and the amplified products were analysed to be around 1500bp.
- PCR product purification was carried out from 0.8% gel and each aliquot were dissolved in 20µl TE buffer and stored at –20^{0}C.
- The purified PCR product was sequenced for nBLAST

Table 1. Salt tolerance assay of the different isolates.

SAMPLE	2%	5%	7%	10%
C1G	+	+	+	+
C1H	+	+	-	-
C2B	+	+	+	-
C2F	+	+	+	-
C3E	+	+	+	+
C3F	+	+	+	+
C4B	+	+	+	+
C5A	+	-	-	-
C5F	+	-	-	-
C5K	+	+	+	-
C5M	+	+	+	-
C5R	+	+	+	-
C6C	+	+	+	+
C7A	+	+	+	-
C7E	+	+	+	+
C8K1	+	+	+	-
C8K3	+	+	+	-
C8M	+	+	+	-
C8N	+	+	+	-
C8P	+	+	+	-
C9E	+	+	-	-
C9EP	+	+	-	-
C10F	+	+	+	-
C11A	+	+	+	-
C11B1	+	+	+	-
C11B2	+	+	+	-
C11C	+	+	-	-
C11D	+	+	+	-
C11E	+	+	-	-
C12C	+	+	-	-
C12I	+	+	-	-
C12IP	+	+	-	-
C12L	+	+	-	-
C13A	+	+	+	-
C14A	+	+	+	-
C14D	+	+	-	-
C14E	+	+	+	+
C14J	+	+	+	+
C14K	+	-	-	-
C14L	+	+	+	-
C14N	+	+	+	-

Table 2. Effect of temperature on the growth of the isolates.

SAMPLE	30 ^{0}C	37 ^{0}C	40 ^{0}C	45 ^{0}C	50 ^{0}C	55 ^{0}C	60 ^{0}C	65 ^{0}C
C1G	+	+	+	+	+	+	+	-
C1H	+	+	+	+	+	+	+	-
C2B	+	+	+	+	+	+	+	-
C2F	+	+	+	+	+	+	+	-
C3E	+	+	+	+	+	+	+	-
C3F	+	+	+	+	+	+	+	-
C4B	+	+	+	+	+	+	+	-
C5A	+	+	+	+	+	+	+	-
C5F	+	+	+	+	+	+	+	-
C5K	+	+	+	+	+	-	-	-
C5M	+	+	+	+	+	+	+	-
C5R	+	+	+	+	+	+	+	-
C6C	+	+	+	+	+	+	+	-
C7A	+	+	+	+	+	+	+	-
C7E	+	+	+	+	+	+	+	-
C8K1	+	+	+	+	+	+	+	-
C8K3	+	+	+	+	+	+	+	-
C8M	+	+	+	+	+	+	+	-
C8N	+	+	+	+	+	+	+	-
C8P	+	+	+	+	+	+	+	-
C9E	+	+	+	+	-	-	-	-
C9EP	+	+	+	+	-	-	-	-
C10F	+	+	+	+	-	-	-	-
C11A	+	+	+	+	+	+	+	-
C11B1	+	+	+	+	+	-	-	-
C11B2	+	+	+	+	+	-	-	-
C11C	+	+	+	+	+	+	+	-
C11D	+	+	+	+	+	+	+	-
C11E	+	+	+	+	-	-	-	-
C12C	+	+	+	+	-	-	-	-
C12I	+	+	+	+	-	-	-	-
C12IP	+	+	+	+	+	+	+	-
C12L	+	+	+	+	-	-	-	-
C13A	+	+	+	+	+	+	+	-
C14A	+	+	+	+	+	+	+	-
C14D	+	+	+	+	+	+	+	-
C14E	+	+	+	+	+	+	+	-
C14J	+	+	+	+	+	+	+	-
C14K	+	+	+	+	+	-	-	-
C14L	+	+	+	+	+	+	+	-
C14N	+	+	+	+	+	+	+	-

Conclusion:

From freshwater ecosystems of Bhubaneshwar and Khurda, water and sediment samples were collected. Using enrichment culture technique 41 bacterial isolates were obtained showing CMCase activity. Using conventional morphological tools and sequencing of 16S rDNA region the isolates were identified as *Bacillus*. Effect of salinity and temperature was also studied.

Collection, identification and characterization of microbial diversity of Punjab

PI: S. K. Gosal, Dept. of Microbiology
Co-PIs: **M. Gangwar,** Dept. of Microbiology; **G. S. Saroa,** Dept. of Soils
Punjab Agricultural University, Ludhiana

Objectives:

- To study the taxonomical and functional diversity for diazotrophs in different agroclimatic regions of Punjab for sustainable agriculture.
- Conservation of microbial diversity for their commercial/genetical exploitation in future.

Significant Achievements:

- Soil samples representing six different agro climatic regions of Punjab were collected (fig 1) by recording GPS position and analysed for physio chemical properties. The soil texture varied from loamy sand to loam. The ammonical and nitrate nitrogen of Punjab soils ranged between 40-112 mg/kg and 10-192 mg/kg, respectively, pH from 6.20-9.28 and electrical conductivity from 0.095-1.70 dS/m. The per cent organic carbon range for soil varied in various regions i.e. Central plain 0.22-1.22%, Western plain 0.22-0.64 %, Undulating plain 0.32-1.35%, Sub mountain undulating plain 0.23-0.88%, Western region 0.42-0.50% and Flood plain region 0.44-0.89% respectively.

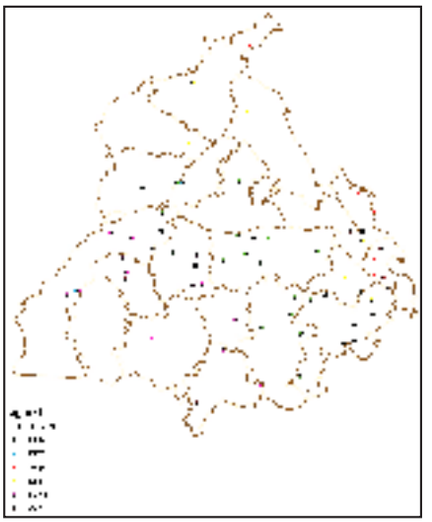


Fig 1. Soil sampling sites in the different agro-ecological zones of Punjab

- A total of 122 diazotrophic isolates were obtained from soils of different agroclimatic regions of Punjab on nitrogen free media.
- Culturally majority of isolates exhibited appearance of white transparent colonies however few had creamy and transparent yellow colonies and few were transparent pinhead. Most of colonies were protruding, mucoid, smooth margined, while few were highly protruded with wavy margins.
- Morphologically isolates were gram-negative motile bacilli, while some typical spiral shape and a few non-motile cocci. All the isolates were non-endospore forming, positive for metachromatic staining and showed absence of capsules.
- Biochemically some of the isolates of Central Plain region were capable of utilizing all five sugars (glucose, adonitol, lactose, arabinose and sorbitol) as carbon source however isolates of Western plain, Undulating plain and Flood plain region exhibited positive reaction for glucose and arabinose utilization.

Conclusion:

Soil samples representing six different agro climatic regions of Punjab were collected by recording GPS position and analysed for physio chemical properties. The soil texture varied from loamy sand to loam. The ammonical and nitrate nitrogen of Punjab soils ranged between 40 - 112 mg/kg and 10 - 192 mg/kg, respectively, pH from 6.20 - 9.28 and electrical conductivity from 0.095 - 1.70 dS/m. The per cent organic carbon varied in different regions and it ranged in Central plain from 0.22 - 1.22%, in Western plain from 0.22 - 0.64 %, in Undulating plain from 0.32 - 1.35%, in Sub mountain undulating plain from 0.23 - 0.88%, in Western region from 0.42 - 0.50% and in Flood plain region from 0.44 - 0.89%. A total of 122 diazotrophic isolates were obtained from different agroclimatic regions on nitrogen free media. Culturally majority of isolates exhibited appearance of white transparent colonies however few had creamy and transparent yellow colonies and few were transparent pinhead. Most of colonies were protruding, mucoid smooth margined, while few were highly protruded with wavy margins. Morphologically isolates were gram-negative motile bacilli, while some typical spiral shape and a few non-motile cocci. All the isolates were non-endospore forming, positive for metachromatic staining and showed absence of capsules. Biochemically some of the isolates of Central Plain region were capable of utilizing all five sugars (glucose, adonitol, lactose, arabinose, sorbitol) as carbon source however isolates of Western plain, Undulating plain and Flood plain region exhibited positive reaction for glucose and arabinose utilization

Identification and Diversity of Lactobacillus spp. from Fermented Dairy Foods

PI: S. De
Co-PI: Rameshwar Singh
Animal Biotechnology Center, NDRI, Karnal, Haryana

Objectives:

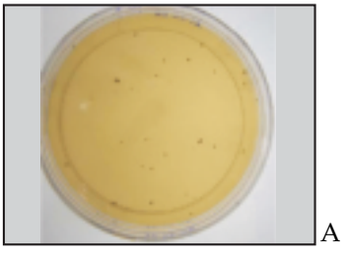
- Microbial diversity of Indian fermented dairy foods
- Molecular typing of new isolates and available NCDC (National Collection of Dairy Cultures) cultures.
- Screening of microorganisms for novel probiotic and functional properties and their application.

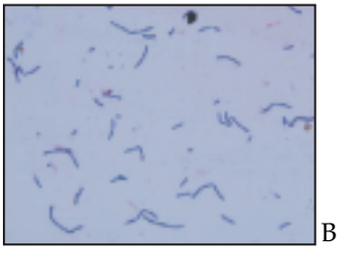
Significant Achievements:

- In various samples obtained from four markets curd sample, lactic acid bacteria (LAB) were the predominant microorganisms, ranging from log 8.0 to log 9.0 cfu g^{-1}. *Lactobacillus* strains were found to be predominant and no other microorganisms were present to such a level. A total of 85 strains of *Lactobacillus* were selected for further study at the genetic level. The identity of the culture was based on characteristics of the strains of *Lactobacillus* spp. as presented in Bergey's Manual of Determinative Bacteriology, carrying out colony morphology in MRS media, microscopy (morphology), Gram straining, growth at 45^{0}C, and fermentation of different carbon sources. Genomic DNA was isolated from all these new 85 isolates. A genus specific PCR was also performed to identify these isolates for genus *Lactobacillus*. The genus specific PCR was successfully performed in 300 isolates. On the basis of all of the identification tests these isolates were found to belong to genus *Lactobacillus* (fig 1 A-D)

Conclusion:

A total of 10 samples were collected (7 samples from Haryana [Karnal, Yamunanagar, Chandigarh, two from Delhi and one sample from Uttar Pradesh [Mathura]) and used for isolation of different Lactobacillus isolates. A total of ~325 isolates of Lactobacillus isolates were obtained. Primary screening for *Lactobacillus* was done using selective growth in MRS media, Gram's reaction and catalase reaction. Biochemical characterization (Sugar fermentation), predicted species of *Lactobacillus* (*L. acidophilus, L. rhamnosus, L. casei, L. plantarum, L. fermuntum, L. bulgaricus, L. brevis, L. lactis, L. viridescens,*). Genomic DNA was isolated from all isolates. A genus specific PCR was also performed to identify these isolates for genus *Lactobacillus*. The genus specific PCR was found positive for more than 95 % of the isolates. *Lactobacillus* strains were found to be predominant and no other microorganisms were present to such a level. On the basis of all of the identification tests these isolates were found belongs to genus *Lactobacillus*.


A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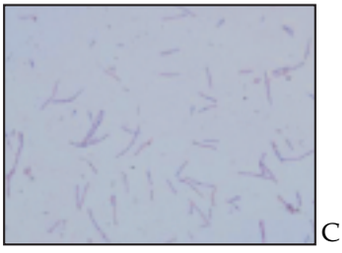

C

D

Fig1 A: Characteristic colonies of *Lactobacillus* isolates in MRS agar plate in 10 -8 dilution.
Fig 1B-C: Microphotograph of Gram positive, rod *Lactobacillus* isolates.
Fig 1D: PCR based genus specific screening of Lactobacillus spp. Each lane (lane1 to 18) indicates a positive band (~550 base pair) for genus *Lactobacillus*.Lane M, 100 bp DNA ladder.

Exploration and Screening of Fish Microbial Diversity

PI: Imelda Joseph
Central Marine Fisheries Research Institute (CMFRI), Ernakulam, Kochi, Kerala

Objectives:

- To screen bacterial groups from selected marine fishes (from skin, gills and gut).
- To identify the selected groups by physiological and biochemical tests.
- To identify microorganisms using ribosomal RNA gene sequences.
- To develop 16S group probes for assessing the diversity of bacterial groups.

Significant Achievements:

- Completed identification of 8 pure bacterial isolates from milk fish, *Chanos chanos* up to genus level. The morphological, physiological and biochemical details of the 8 strains isolated from *Chanos chanos*are. From the skin isolates of milkfish *Flavobacterium, Streptococcus, Enterobacteriaceae, Planococcus* and *Alkaligenes* were identified. From the gill isolates, *Moraxella, Acinetobacter*; and *Flavobacterium* were identified.
- Completed identification of 17 pure bacterial isolates from catfish *Arius* sp up to genus level. The morphological, physiological and biochemical details of the 17 strains isolated from *Arius* sp. from the skin isolates of Arius, one *Staphylococcus* and two *Vibrio*, From visceral isolates three *Alcaligenes*, one *Moraxella*, , one *Aeromonas*, two *Bacillus*, one *Photobacterium*, one *Vibrio*, one *Pseudomonas*, two *Alcaligenes* and one Enterobacteriaceae were identified.
- Completed identification of 32 pure bacterial isolates from Dhoma *Johnius* sp up to genus level. The morphological, physiological and biochemical details of the 32 strains isolated from Dhoma *Johnius* sp. From the skin isolates of Dhoma, three Bacilli, seven *Pseudomonas*, three *Acinetobacter*, two *Moraxella*, seven *Alcaligenes*, three Enterobacteriaceae, and one *Arthrobacter* strains were isolated. From the visceral isolates, three *Alcaligenes*, two *Bacilli* and one *Lactobacillus* strain were identified.

Conclusion:

The fish microbial diversity is a virgin area which has so much of potential in various applications including, pollution or productivity of a particular locality, feeding habits or indication of climate change. It requires further requires elaborate studies to come to any concrete conclusion. However, since the bacterial strains isolated from fish include many different groups, the isolated and partially identified (up to genus level) ones could be screened for their application for production of metabolites or for its own characteristic traits (salinity, pH and temperature tolerances). Study on fish microbes have not been done so far in India and the study will help in mapping the strains which are inherent to different marine species of different locations and later for mapping of pathogens or benevolent strains.

Conservation of beneficial fungi of Himalayan Ecosystem of Jammu Province

PI: P. K. Raina
Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir

Objectives:

- Isolation of microorganisms (fungi- mushroom) from western Himalayan ecosystem of Jammu Province.
- Identification of micro-organisms using conventional/biochemical methods.
- Screening of the strains for disease suppression.

Fig 1. Map of sampling site in Jammu region.

Significant Achievements:

- The soil and plant samples collected during the survey were the representative samples of diverse agroclimatic regions in Jammu region (Fig 10).
- The surveyed areas were located in sub-tropical plain zone, intermediate low hill zone and Temperate zone. The fungal diversity of 36 locations surveyed revealed the presence of three *Trichoderma* species viz. *T. viride*, *T. harzianum*, and *T. virens* and there frequency distribution is 3.66 : 2 : 1 respectively. Among these, *Trichoderma harzianum* was the fastest growing species followed by *T. viride* and *T. virens* with growth rate of 1.30, 1.06 and 0.60 mm/h at 25° C on PDA. Besides three species of *Trichoderma* , 9 fungal pathogens were purified and identified. These include two isolates of *Bipolaris sorokiniana* isolated from wheat leaves and wheat grain, two isolates of *Fusarium oxysporum* isolated from strawberry and mango seedlings , 3 isolates of *Colletotrichum gleosporioides* isolates from mango, guava and walnut leaves, one isolate of *Chaetomium globosum* isolated from mushroom compost and one isolate of *Ampelomyes quisqualis* isolated from Ber leaves infected with powdery mildew fungus.
- While studying the toxicity of three fast growing *Trichoderma* species one each of *Trichoderma viride*, *T. harzianum* and *T. virens* against *Fusarium oxysporum* , *Colletotrichum gleosporioides* and *Phomopsis vexans* isolated from strawberry, guava and mango leaves reveals that the non volatile metabolites restricted the growth of *Fusarium oxysporum* by 55.38-72.38% , *Colletotrichium gleosporioides* by 44.5-49.2% and *Phomopsis vexans* by 39.0-69.4% . However, the volatile metabolites were toxic only upto 6th day, thereafter the test pathogen stabilized there growth and was equal to that of control by 15th day.

Theme 2: Nutrient Management, PGPR, and Biocontrol

Improving yields and nutrient uptake of selected crops through microbial inoculants in Vertisols of Central India

PI: D. L. N. Rao
Co-PI: S. S. Manna
Indian Institute of Soil Science, Nabi Bagh, Bhopal-462 038, M.P.

Objectives:

- To study the culturable microbial diversity of vertisols for selection of promising rhizobial and plant growth promoting rhizobacterial strains for soybean, chickpea and wheat.
- To develop the best consortia of rhizosphere competent and compatible strains of N fixers, P solubilizers, PGPR and PGP-B for inoculation of above crops.
- To study the microbial interactions in the crop rhizosphere in relation to CNP transformations and bio-availability of nutrients.
- To test the best performing combination of inoculants for soybean, chickpea and wheat in farmers fields.
- To make multiple-repositories of the elite strains of microorganisms.

Significant Achievements:

- Out of 93 rhizobial strains screened *in vivo* in green house in vertisols, 45 effective strains were short-listed (36 of soybean and 9 of chickpea).
- Of the 247 PGPR strains screened *in vivo* in green house in vertisols, 47 were found to be effective (17 for soybean, 16 for chickpea and 14 for wheat).
- Rhizobia and non-rhizobial contaminants showed differential resistance and sensitivity patterns to different antibiotics, of which ampicillin, carbencillin, neomycin, rifampicin and ciprofloxacin proved to be most discriminatory.
- Base on germination enhancement *in vitro* as well as *in vivo* screenings in green house, it was found that plant growth was promoted by a greater number of isolates from soybean rhizosphere as compared to chickpea and lowest no. of isolates from wheat rhizosphere. Greater number of isolates made from standard media stimulated the growth compared to those from minimal media.
- Out of 306 bacterial isolates, 19 showed anti-fungal activity (PGP-B). Importantly, it was revealed that all of them showed PGPR activity and enhanced the plant growth.
- Inoculation of PGPR alongwith *Rhizobium* in soybean and chickpea produced dramatic increases in nodulation.

Conclusion:

Culturable microbial diversity in vertisols of central India was assessed by isolations from soils at a dozen sites in 8 different media including standard and minimal media. PGPR isolates (247 no.) from soybean, chickpea and wheat growing in vertisols of central India, and from vermicompost and vermicast were screened in vivo in green-house and 47 isolates effective in promoting shoot yield were identified. Only 3% of the isolates showed `obligate oligotrophy' or `obligate copiotrophy'. Nearly 90% of the isolates were facultative in their mode of nutrition. A higher proportion of the isolates made from standard media were effective and stimulated the growth compared to those from minimal media. Plant growth was promoted by a greater number of isolates from soybean rhizosphere as compared to that from chickpea rhizosphere and the lowest number of effective isolates was from wheat rhizosphere. Out of 306 bacterial isolates screened, 19 isolates showed anti-fungal activity (PGP-B) against *Fusarium oxysporum* f. sp. *ciceri*. Most of these antagonists also showed PGPR activity and enhanced the plant growth. Rhizobial isolates (93 no.) of soybean and chickpea were screened and 45 effective strains were identified. Best nodulation of chickpea was observed from lands which lie submerged during monsoon (`havelis') in

the chickpea belt of Dt. Narsinghpur. M.P. Rhizobia and non-rhizobial contaminants showed differential resistance and sensitivity patterns to different antibiotics, of which Ampicillin, carbencillin, neomycin, rifampicin and ciprofloxacin proved to be the most discriminatory. Inoculation of PGPR in soybean produced significant increase in nodulation by native rhizobia.

Exploration, Collection and Characterization of some Agriculturally Important Biocontrol Agents Suitable for Disease Managemnet

PI: D. K. Arora
Co-PI: Alok Srivastava; Sudheer Kumar
National Bureau of Agriculturally Important Microorganisms, Mau, UP.

Objectives:

- To isolate and characterize antagonistic organisms from diverse agro climatic/cropping system for disease management.
- To screen potential isolates by using different antibiosis assays against major soil borne plant pathogens like *Macrophomina* and *Fusarium oxysporum* f. sp. ciceri.
- To develop formulations by using mass multiplication technique suitable for various delivery systems and their evolution against target diseases.

Significant achievements:

- Soil and plant samples were collected from Assam (Kaziranga national biodiversity park and Jorhat district), Uttaranchal, West Bengal (Sunderban mangrove forest areas) adn Uttar Pradesh (western Uttar Pradesh).
- Total 260 fungal isolates, 134 bacterial isolates (54 *Pseudomonas* and 84 *Bacillus*) and 80 actinomycetes were isolated from these samples using different selective and semi selective media. *In vitro* screening of the fungus and bacterial isolates were carried out for antagonistic activities by using dual culture plate and cellophane paper method.
- After primary screening through dual culture 14 *Pseudomonas fluorescens* and 14 *Bacillus* were selected for evaluation of their secondary metabolite against *Fusarium oxysporum* f. sp. *ciceri* and *M. phaseolina*.
- The selected bacterial isolates were characterized for the volatile and non volatile compounds involved in antagonism like siderophore, HCN, ammonia, hydrolytic enzymes (Table 1). Among these many were found positive in qualitative assay for the production of such compounds.
- These selected bacterial isolates were further evaluated through culture filtrate technique against test pathogens, out of them 2 bacterial isolates (one of Pseudomonas fluorescence and 1 of Bacillus) gave very high growth inhibition and selected for characterization for active principle of antagonism and rhizospheric competence (Table 2).

Table 1: Number of isolates found positive for qualitative test for metabolite and hydrolytic enzyme production

Bacterial isolates	Number of bacterial isolates positive for production of						
	Siderophore	HCN	Ammonia	Chitinase	Pectinase	Cellobiose	Endoglucanase
***Pseudomonas* isolates (14)**	8	11	10	2	11	5	5
***Bacillus* isolates (14)**	5	9	12	3	4	-	1

Table 2:- Screening of bacterial secondary metabolites against *M. phaseolina* and *Fusarium oxysporum* f. sp. ciceri.

Bacterial isolates	Number of antagonists against plant pathogens	
	Macrophomina phaseolina	*Fusariumoxsporum* f.sp. *ciceri*
***Bacillus* spp (14)**	1	1
***Pseudomonas fluorescens* (14)**	1	1

- After preliminary screening by dual culture and cellophane paper technique 9 fungal isolates have been selected for further secondary metabolite screening, among then 6 were found potent against the test pathogens (*M. phaseolina* and *Fusarium oxysporum* f. sp. *ciceri*). The fractionation and characterization of the active molecule responsible for growth inhibition is under progress.
- The selected fungal isolates were subjected for the qualitative and quantitative assay of hydrolytic enzymes like chitinase, protease (fig 1), cellobiase and cellulase. Out of these three produced high quantity of chitinase, the main enzyme that hydrolyse the chitin and is involved in mycoparasitism (fig 2).

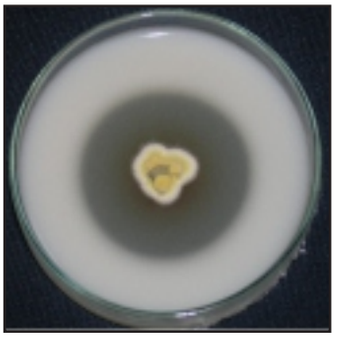


Fig1 Protease activity by fungal isolate

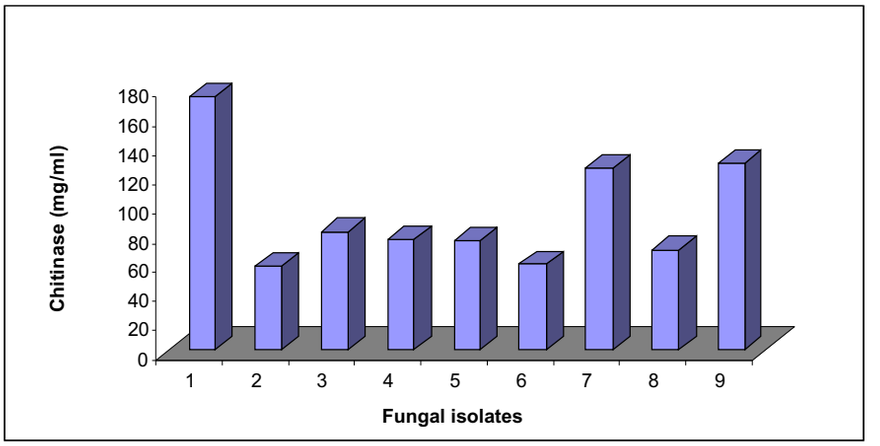


Fig 2. Quantitative analysis of chitinase in selected fungal antagonists

Conclusion:

The findings suggest that one strain of each Pseudomonos and Bacillus and few fungal isolates have several biocontrol traits. Which, may be further examined for disease suppression in soil.

Application of AIMS for nutrient management and plant growth promotion in rainfed agro ecosystem

PI: B. Venkateswarlu
Co-PI: S. Desai
Division of Crop Sciences, Central Research Institute for Dryland Agriculture, Hyderabad

Objectives:

- To study the culturable microbial diversity of soils from different agro-ecological sub regions, production systems and land use practices, including stressed ecosystems.
- To characterize the isolated microorganisms for their nutrient mobilization (N, P and micronutrients).
- To evaluate the establishment of isolates, particularly in mixed cropping systems and select isolates for multiple crops and geographical locations.
- To standardize methods for mass multiplication and identify appropriate delivery systems and improve the formulations, quality, shelf life of the above bio-agents with superior delivery systems.
- To carry out multi-location testing for evaluation of the promising formulations.
- To make multiple-repositories of isolated microorganisms.

Significant Achievements:

- During the year, 33 *Pseudomonas,* 25 *Bacillus,* 10 *Bradyrhizobium* and 10 *Azospirillum* isolates were isolated from rhizosphere of rainfed crops from arid and semi arid zones.
- Nine and 18 isolates of *Pseudomonas* promoted more than 50% growth over control of sorghum and pigeonpea seedlings, respectively. Similarly, four and five isolates of *Bacillus* promoted more than 50% growth over control of sorghum and pigeonpea seedlings, respectively.
- One isolate of *Pseudomonas viz.,* P22 (isolated from rhizosphere of sorghum production system of Andhra Pradesh) and GASRB4 and HASRB25 *Bacillus* isolated from rhizosphere of sorghum production system of A.P were found to be promising plant growth promoters of sorghum in pot culture by 60% over control.
- Out of 74 isolates of *Pseudomonas,* P17, P74, P75 possessed more than 2 PGP traits, off which P17 could promote growth of both sorghum and pigeonpea.
- Six isolates of *Pseudomonas* showed more than 50% P solubilization and also enhanced biomass of sorghum and pigeonpea seedlings in the range of 25-80%. Similarly, one isolate of *Bacillus* (GASRB13) showed 23% P solubilization and also enhanced biomass of sorghum and pigeonpea seedlings in the range of 25-50%.

Conclusions:

From semi arid locations of the country promising strains of *Peudomonas* were identified that promoted growth of sorghum (14 strains) and pigeonpea seedlings (19 strains). Similarly, 5 strains of *Bacillus* promoted growth of both sorghum and pigeonpea seedlings respectively. An isolate of *Pseudomonas* (P22) and GASRB4 and HASRB25 *Bacillus* (all isolated from rhizosphere of sorghum production system of A.P) promoted plant growth of sorghum in pot culture by 60%. Four isolates viz. P12, P14, P37, P67 possessed tolerance to abiotic stress as well as promoted growth of sorghum and pigeonpea in terms of drymass by 10-65%. Eight isolates of *Pseudomonas* showed more than 50% P solubilization and also enhanced biomass of sorghum and pigeonpea seedlings in the range of 25-80%. Similarly, one isolate of *Bacillus* (GASRB13) showed 23% P solubilization and also enhanced biomass of sorghum and pigeonpea seedlings in the range of 25-50%.

Development of a Cold Tolerant Phosphate Solubilizing Bacterial (PSB) Inoculant

PI: G. Selvakumar
Co-PI: Pankaj Mishra
Vivekananda Parvatiya Krishi Anusandhan Sansthan (ICAR), Almora, Uttarakhand

Objectives:

- To isolate PSB cultures from the rhizosphere of various hill crops and screen them under *invitro* cold conditions (4 and 15 °C) for their P solubilizing ability.
- To develop comprehensive biomarkers for the quality control and field detection of the elite PSB strains.
- To evaluate various locally available low cost organic raw material for use as a carrier material for the PSB inoculant, and identify a suitable combination of efficient PSB culture(s) and carrier material.
- To evaluate the performance of the PSB inoculant with graded levels of P fertilization in select winter crops.
- To conduct large scale field demonstrations with the developed bio- inoculant and commercialize the developed technology.

Significant Achievements:

- The P solubilizing ability of *Pseudomonas fragi* and *Pseudomonas lurida* under cold incubation temperatures has been reported for the first time.
- The rock phosphate solubilizing ability of all the fourteen isolates was determined at 4 ^{O}C, over a fourteen day incubation period.
- Multiple antibiotic resistance markers have been determined for individual elite isolates, this will facilitate the quality control of the bio-inoculants produced using these strains and also help in the field detection of these isolates when released in to the soil.
- Other PGP traits of these elite cultures have been elucidated under cold temperature conditions, it has been observed that most stains posses more than one functional PGP trait at 4 ^{O}C.
- Nine elite isolates were identified based on their 16S rRNA sequences *viz., Pseudomonas fragi* strain CS11RH1, *Pseudomonas poae* strain NS12RH(1), *Pseudomonas poae* strain RT5RP2 , *Pseudomonas lurida* strain M2RH3, *Pseudomonas* sp strain PCRP7 (2), *Pseudomonas* sp strain RT6RP, *Pseudomonas* sp strain CS11RP1, *Pseudomonas sp* strain PB2RP2 and *Pseudomonas* sp strain PB2RP1.
- Six isolates were deposited at NBAIM, Mau and GenBank accession numbers were obtained for all the deposited isolates.

Conclusions:

Based on TCP solubilizing ability at 4 ^{O}C fourteen isolates were selected and designated as elite isolates. The P solubilizing ability of *Pseudomonas fragi* and *Pseudomonas lurida* under cold incubation temperatures have been reported for the first time. The isolate *Pseudomonas poae* strain RT5RP2 registered the highest level of soluble P (26.64 ppm) from Tri Calcium Phosphate (TCP). Multiple antibiotic resistance markers were identified for each of the individual isolates to facilitate their quality control during inoculant production and environmental detection when applied in soil. Apart from solubilizing P most isolates were found to harbor more than one plant growth promoting trait at 4 ^{O}C. Nine elite isolates were identified based on their 16S rRNA sequences *viz., Pseudomonas fragi* strain CS11RH1, *Pseudomonas poae* strain NS12RH (1), *Pseudomonas poae* strain RT5RP2, *Pseudomonas lurida* strain M2RH3, *Pseudomonas* sp strain PCRP7 (2), *Pseudomonas* sp strain RT6RP, *Pseudomonas* sp strain CS11RP1, *Pseudomonas sp* strain PB2RP2 and *Pseudomonas* sp strain PB2RP1. Six isolates were deposited at NBAIM, Mau and GenBank accession numbers were obtained for all the deposited isolates. It is further proposed to determine the P supplying potential of these isolates in conjunction with insoluble sources of phosphorus, and standardize the inoculum production technology of these isolates.

Isolation, identification, evaluation and exploitation of PGPR for Spices

PI: M. Anandraj
Co-PI: R. Dinesh, N.K. Leela, A. Kumar
Indian Institute of Spice research, Calicut, Kerala

Objectives:

- Isolation, characterization, evaluation of microbes for nutrition, mobilization, growth promotion and biological control.
- Screening isolates for the desirable characters.
- Studies on compatibility and ecological fitness and development of consortium.
- Studies on rhizobacteria mediated induced systemic resistence.
- Studies on mechanism of rhizobacteria mediated growth promotion in crop plants.

Significant Achievements:

- One hundred and nineteen rhizobacterial isolates were isolated from healthy black pepper (*Piper nigrum* L.) and ginger (*Zingiber officinale* Roscoe) from different geographical regions in Kerala and Karnataka. Five cultivars of black pepper and six cultivars of ginger were used for isolation of rhizobacteria. The samples were serially diluted upto 10^{-10} and isolations were made using Tryptic Soy Agar (TSA), Nutrient Agar (NA), Basal medium amended (Glucose, Mannitol, Sorbitol, Inositol and Sucrose). Maximum diversity and abundance of rhizobacteria were obtained in TSA followed by NA. The individual bacterial colonies were selected and subcultured on NA and cryopreserved at –80°C in 20% glycerol. Isolates were tentatively grouped based on phenotypic characteristics such as colour, form, elevation, margin, diameter, surface, opacity and texture. Motility, cell morphology, size, Gram reaction and spore formation were also recorded using standard procedures. Rhizobacterial isolates of black pepper (50) and ginger (69) were screened using dual plate culture technique against *Phytophthora, Pythium* and *Fusarium*. Rhizobacteria of black pepper isolates showed more than 50% inhibition were short-listed. Five isolates from black pepper and 6 isolates from ginger inhibited all 3 pathogens. These isolates were also tested for their ability to promote plant growth, for production of Indole Acetic Acid, HCN, nitrogen fixation, and phosphate solubilization. Out of 119 rhizobacterial isolates tested, 53 produced IAA, 38 solubilized phosphate and only 4 isolates produced HCN. Two isolates BRB 28 and BRB 37 were positive for all and 16 isolates were positive for IAA and phosphate solubilization. Out of 119 rhizobacterial isolates tested, 22 produced α-amylase, 12 produced cellulase, 27 produced pectinase and 38 isolates produced protease.

Conclusions:

One hundred and nineteen rhizobacteria have been isolated from black pepper (50) and ginger (69) from different geographical regions such as Peruvannamuzhi and Wyanad in Kerala and Kodagu District in Karnataka. Five isolates from black pepper and 6 isolates from ginger inhibited 3 pathogens namely *Phytophthora, Pythium*, and *Fusarium*. These isolates were also tested for their ability to promote plant growth, for production of Indole Acetic Acid, HCN, Nitrogen Fixation, and Phosphate solubilization. Two isolates BRB 28 and BRB 37 were positive for all.Among 119 isolates, 22 produced amylase, 12 produced cellulase, 27 produced pectinase and 38 isolates produced protease.

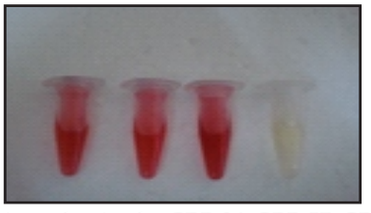


Fig.A: IAA production by GRB-36, GRB-57, GRB-58 and Negative control GRB-25.

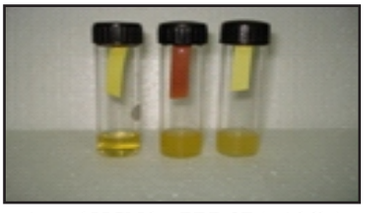


Fig B. Production of HCN by BRB-37 (middle) and negative strain BRB-21 (right) and medium control (left).

Fig.C, D & E: Production of enzyme by rhizobacterial isolates; Ginger isolate GRB-35 producing Pectinase (C), Cellulase (D) and Protease by GRB-2 and GRB-4 (E).

Development and application of PGPR formulations for growth improvement and disease suppression in coconut and cocoa

PI: G. V. Thomas
Co-PI: Murali Gopal, Alka Gupta, M. Gunasekaran
Central Plantation Crops Research Institute, Kudlu P.O., Kasaragod, Kerala – 671 124

Objectives:

- To characterize PGPR (rhizosphere soil dwelling and endophytic) associated with coconut and cocoa grown in different agro-climatic conditions using multi-phase techniques involving microbiological, biochemical and molecular protocols.
- To screen and select efficient rhizobacterial strains possessing beneficial traits like production of IAA, HCN, nitrogen fixation and phosphate solubilization.
- Development of mass multiplication techniques and consortia formulations of compatible microorganisms.
- To evaluate the selected strains for growth promotion of coconut and cocoa in green house studies with seedlings.
- To evaluate the biocontrol potential of the PGPR against stem bleeding disease of coconut caused by *Thielaviopsis paradoxa* and *Phytophthora* diseases of coconut and cocoa in green house environment.
- To develop the PGPR based biofertilizer technology with appropriate quality control measures.

Significant Achievements:

- A total of 1031 PGPR were isolated from rhizosphere and roots of coconut and cocoa from major coconut growing tracts in Kerala, Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra.
- Coconut rhizosphere harboured more PGPR population (upto 2×10^6) than cocoa rhizosphere (upto 98×10^4).
- *Bacillus* spp. formed the major group of PGPR in both the crops, irrespective of the place or agro-climatic conditions, when compared to the fluorescent pseudomonads (66% of the total isolates belonged to *Bacillus* spp. as compared to 31% fluorescent pseudomonad isolates).
- The numbers of PGPR found as root endophytes were more in cocoa (190 isolates) than in coconut (133 isolates).
- The coconut roots had only *Bacillus* spp. as endophytes whereas; cocoa roots possessed both fluorescent pseudomonads and Bacillus spp. as endophytes.
- Mineral phosphate solubilizing and ammonifying bacteria were preponderant PGPR in the rhizosphere of coconut in Kerala.
- N-fixation capability was seen mostly in bacilli. Three of the isolated fluorescent pseudomonads from coconut showed N-fixation potential whereas only one fluorescent pseudomonad from rhizosphere and one from roots of cocoa showed N-fixation potential.
- Among bacilli, 50% of endophytic bacilli showed N-fixation in coconut as compared to 10% of rhizospheric isolates whereas, in cocoa, 50% of rhizospheric isolates showed N-fixation as compared to 25% of endophytic bacilli.
- Higher percentage of fluorescent pseudomonads showed P-solubilization (94%), siderophore (97%) and IAA production (85%) than bacilli (39, 15 and 27%, respectively for the three characters).
- Among the bacilli, 95% showed antagonism against *Thielaviopsis paradoxa* and *Ganoderma* sp., fungal pathogens of coconut, as compared to 8% of fluorescent pseudomonads. The PGPR-mediated antagonism was found to be medium-dependent.
- Three PGPR isolates from coconut rhizospheres from Thopumpady, Kunnumkkai and Pollachi produced high levels of IAA and showed early germination and stimulated root growth in paddy.

Conclusion :

Rhizosphere soil and root samples of Coconut and Cocoa were collected from Coimbatore and Pollachi of Tamil Nadu; Kidu and Vittal of Karnataka, Kasaragod, Kozhikode, Wayanad of Kerala. It was observed that Coconut roots harboured more of bacilli than fluorescent pseudomonads. The Bacilli also showed N-fixation capability as compared to fluorescent pseudomonads. Among bacilli, higher % of endophytic bacilli showed N-fixation than rhizospheric isolates. Higher % of fluorescent pseudomonads showed P-solubilization, siderophore and IAA production than bacilli. Similar results were obtained in different soil samples collected from Cocoa rhizosphere.

Microbial Control of Insect Pests-II

PI: B. Ramanujam
Co-PI: S. Sriram
Project Directorate of Biological Control, Bangalore

Objectives

- Germplasm collection of entomogenous fungi from insect hosts for control of sucking pests of vegetable crops.
- Screening and identification of potential isolates of entomogenous fungi against sucking pests of vegetable crops (laboratory bioassay, glass house studies).
- Molecular characterization of promising isolates of entomogenous fungi.
- Development of efficient mass production and formulation techniques including oil-based formulations of promising isolates of entomogenous fungi.
- Field evaluation/ Commercial glasshouse testing of promising isolates of entomogenous fungi against sucking.

Significant Achievements:

- Nine isolates of *Beauveria*, one isolate of *Beauveria brongniartii*, two isolates of *Verticillium lecanii*, one isolate of *Nomuraea rileyi*, one isolate of *Fusarium pallidoroseum*, two isolates of *Cladosporium oxysporum* and five isolates of *Fusarium* sp. were isolated from different insect hosts and soils from different locations of the country and added to the germplasm collection of entomofungal pathogens.
- Bb4, Bb-7 and Bb-12 isolates of *B. bassiana* and Ma-3, Ma-4, Ma-5, Ma-6, Ma-9, Ma-10, Ma-11 isolates of *M. anisopliae* were identified as promising isolates against *Aphis craccivora*. These isolates will be used for field testing.
- Against *Bemisia tabaci,* Bb-1, Bb-2, Bb-7, Bb-9 and Bb-12 isolates of *B. bassiana*, Ma-11 isolate of *M. anisopliae* and Vl-3e, Vl3g, Vl-6 and Vl-10 isolates of *V. lecanii* were identified as promising isolates.
- Standardized the procedure for production of dry conidia of promising isolates of *B. bassiana* and *M. anisopliae* on solid substrate for preparation of oil-based formulations.

Conclusion:

Eighteen isolates of entomofungal pathogens comprising ten isolates of *Beauveria bassiana,* two isolates of *Metarhizium anisopliae,* three isolates of *Verticillium lecanii,* two isolates of *Nomuraea rileyi* and one isolate of *Fusarium pallidoroseum,* have been collected from different insect hosts and soil from Karnataka, Tamilnadu, Assam and Himachal Pradesh and added to the germplasm collection of entomofungal pathogens. Extensive surveys will be made during next year in the wet and humid areas like North-East states, East and West coast areas to collect a rich germplasm of entomofungal pathogens which can be used for establishing a repository and development of data bases of strains of entomopathogenic fungi. Promising isolates of entomogenous fungi have been identified against sucking pests like, *Aphis craccivora, Scirtothrips dorsalis, Bemisia tabaci* and *Myzus persicae* based on laboratory bioassay studies. These promising isolates will be field tested and potential fungal bioagents will be identified against sucking pests as an alternative to chemical pesticides. Mass production and formulation technologies will be developed for the promising entomopathogenic strains of sucking pests that can be passed on to the commercial agencies for large scale production.

Developing PGPR consortia for enhanced crop and soil productivity of rice-wheat cropping system

PI: Lata
Co-PI: Radha Prasanna
Division of Microbiology, IARI, New Delhi – 110012

Objectives:

- To isolate and screen bacterial/cynobacteria from the rhizosphere soil samples of rice and wheat crop.
- To evaluate the effect of the selected set of strains on crop yield and soil fertility.
- To develop PGPR consortia for rice and wheat crop.

Significant Achievements:

- Screening of cyanobacterial and bacterial isolates under laboratory conditions for their PGP ability, followed by their evaluation with rice and wheat crop in Phytotron (Fig. 1), led to the identification of promising strains for use as inoculants in rice/ wheat pot experiments.
- Synergistic interactions among the PGPR strains (bacteria-cyanobacteria) were observed in terms of significant enhancement in the soil microbiological and plant growth/yield parameters in both rice and wheat pot experiments.
- Biochemical characterization of the selected strains revealed promising PGPR traits such as IAA production, P-solubilization, ammonia production.

Conclusion:

More than hundred cyanobacterial, and bacterial plant growth promoting microorganisms were isolated from wheat and rice rhizosphere. All the PGPR strains were screened for their plant growth promoting ability by seedling germination test followed by their evaluation with rice and wheat crop in phytotron. Synergistic interactions among the PGPR strains (bacteria-cyanobacteria) were observed in terms of significant enhancement in the soil microbiological and plant growth/yield parameters in both rice and wheat pot experiments. Biochemical characterization of the selected strains revealed promising PGPR traits such as IAA production, P-solubilization, ammonia production. Further testing in rice and wheat crop would be undertaken, with the promising combinations of PGPRs. On the basis of field trials and multilocational trial , a consortium of cyanobacterial and rhizobacterial PGP consortium will be identified for rice –wheat cropping system.

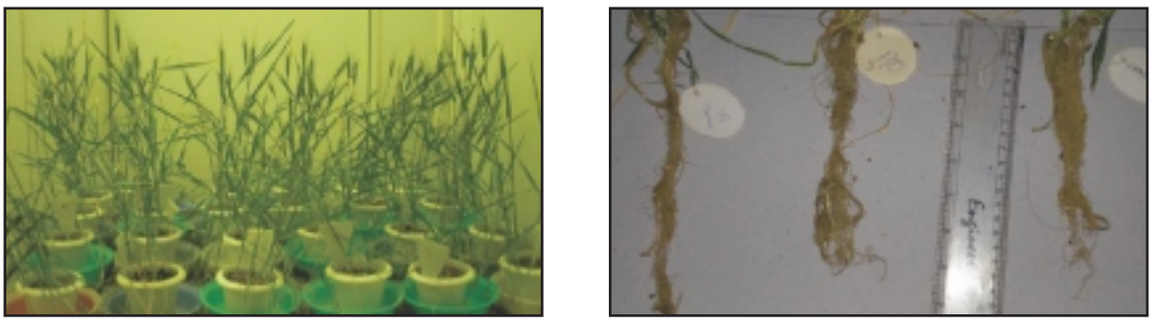


Fig 1. Phytotron experiment showing effect of rhizobacterial inoculants on plant biomass and root length.

Structural and functional dynamics of the microbial isolates in biogeochemical cycling of C, N, P and S in rice ecosystem

PI : T. K. Adhya
Co-PI : V.R. Rao
Central Rice Research Institute, Cuttack – 753006, Orissa

Objectives:

- Isolate agriculturally important microorganisms from rice soils varying widely in physico-chemical properties.
- Explore and quantify the microbial diversity in rice soils by cultivation-based, proteogram and total DNA finger-printing analyses.
- Characterize the functional diversity analysis of the microbial isolates in the biogeochemical cycling of C, N, P and S and integrate them in the nutritional management of rice ecosystem.
- Confirm efficacy of isolated microorganisms for nutrient acquisition and maintenance of sustainability under rice cultivation.
- Standardize methods of mass multiplication, improved formulations and delivery systems particularly for rice cultivation.

Significant achievements:

- Microorganisms were isolated from flooded rice soils of the coastal districts of Orissa with two target groups *viz.* heterotrophic nitrifying bacteria and P-solubilization bacteria. Out of a total of 78 heterotrophic bacteria isolated earlier, five cultures were characterized for their nitrification potential following standard incubation techniques with acetylene. All the candidate microbial cultures exhibited significant nitrification activity in terms of NO_2 production that was found to be related to growth of the organisms. Two bacteria were further identified through 16s rDNA sequencing technique and following analysis of corrected sequences online by nBLAST (www.ncbi.nih.gov.in) to find maximum identity, genus was assigned on the basis of existing 16S rRNA gene sequences in database. The 16S sequences have been submitted to NCBI and assigned accession nos. EU 169572 and EU 169573 respectively (Fig 1).
- Out of a total of 513 P-solubilizing bacteria isolated in pure culture following the formation of clear zones in Pikovskaya's medium, 20 selected P-solubilizing bacteria exhibiting higher P-solubilization potential were further characterized for their abilities under different incubation conditions. Among these cultures, three bacteria were characterized by 16s rDNA sequencing and assigned phylotype (Fig 2). The bacteria included *Bacillus megaterium* isolated from the rhizosphere of rice *cv.* IR 64, *Enterobacter* sp. isolated from isolated from rice field soil at Talchua and *Mycobacterium* sp. isolated from rice field soil at Khola. The 16s rDNA sequences have been submitted to NCBI and accession nos. EU 169569, EU 165970 and EU 165971 were annexed. Subsequently, the bacteria were characterized for their sugar utilization patter.

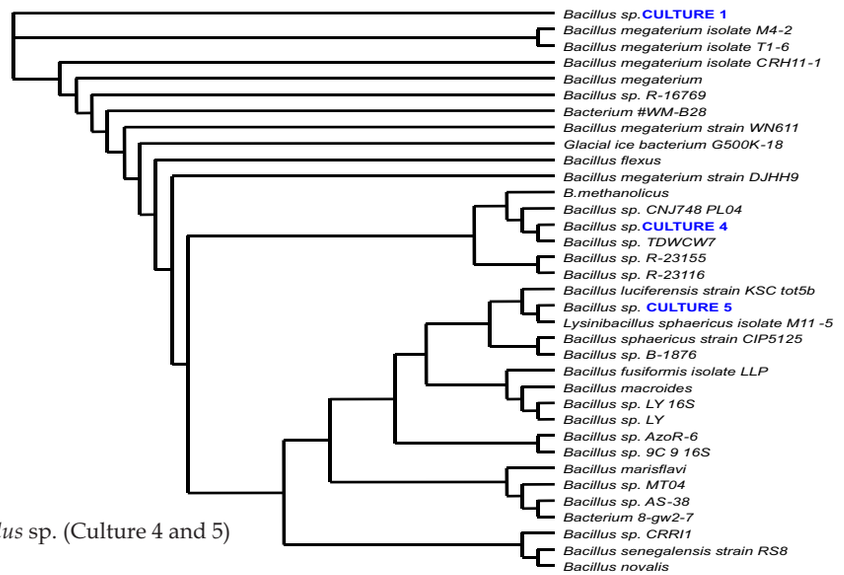


Fig 1. Phylogenetic tree of *Bacillus* sp. (Culture 4 and 5)

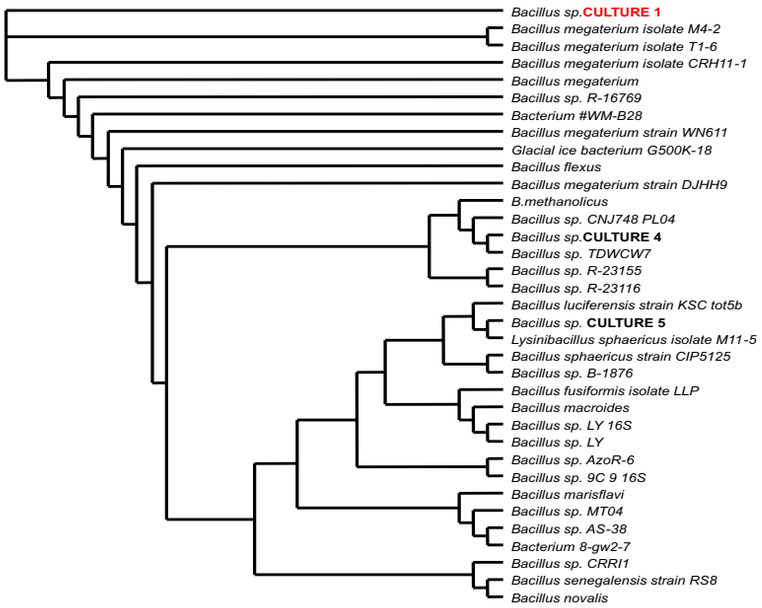


Fig 2. Phylogenetic tree of the P-solubilizing *Bacillus* sp. (Culure no.1)

Conclusion:

A total of 76 heterotrophs, 78 heterotrophic nitrifiers, 8 denitrifiers, 14 oligotrophs and 6 *Azospirrilum* isolates were obtained in pure culture. Standardization of functional analysis through assay of methanogenesis, methane oxidation potential, nitrogenase, denitrification enzyme assay (DEA) and nitrification enzyme activity was done. Methane oxidation potential of 15 soils from rice-based cropping system of Orissa was measured. Methane oxidation potential expressed as $t_{1/2}$ (d) varied from 6.16 to 81.53 indicating high variability. Twelve pure cultures of methanotrophs with particulate methane monooxygenase (pMMO) activity were isolated for further molecular analysis. Two heterotrophic nitrifying bacteria were identified through 16s rDNA sequencing. Results indicate that both the cultures belonged to *Bacillus* sp. while one culture formed a single clad with *Lysinibacillus*sp. The heterotrophic nitrifying bacterial cultures isolated from saline soils could tolerate salinity of 10% NaCl and still retain nitrifying activity. A total of 539 P-solubilizing bacteria were isolated in pure culture from coastal saline soils of Orissa and West Bengal. The isolates expressed wide variability in their P-solubilization potential with tricalcium phosphate as P-source. Several bacterial cultures isolated from rice soils exhibited P-solubilizing optima both at acidic (pH 6.0) and alkaline (pH 8.0) ranges. Based on 16s rDNA sequencing three P-solubilizing bacteria were identified as *Bacillus megaterium*, *Enterobacter* sp. and *Mycobacterium* sp. Cultures of *B. megaterium* and *Enterobacter* sp. when grown in mineral salts medium with samples of rock phosphate as source of P, exhibited an wide variability in their P-release potential. Results indicate that *Enterobacter* sp. was more effective in releasing soluble-P from different rock phosphate samples.

Promoting Zinc Nutrition in Maize using Mycorrhizal Symbiosis

PI : K. S. Subramanian, Department of Soil Science & Agriculture Chemistry
Co-PI : M. Thangaraju, Department of Microbiology
Tamil Nadu Agricultural University, Coimbatore

Objectives:

- To examine the nutritional, physiological and biochemical responses of maize, rice and tomato to mycorrhizal inoculation under varying intensities of Zn stress conditions.
- To study whether mycorrhizal symbiosis alters the plant water relations in rice under aerobic, semi-dry and flooded ecosystems.
- To examine the long-term effects of cropping and fertilization on soil microbial population and biochemical changes.
- To study the response of maize to mycorrhizal inoculation under varying levels of fertility gradients in LTFE soils.
- To assess the carbon sequestration pattern in the presence or absence of mycorrhizal symbiosis.

Significant Achievements:

- Mycorrhizal inoculation orchestrates biochemical changes such as increased biomass C, acid phosphatase and glomalin that favorably enhance the availability of Zn.
- Enhanced availability of Zn increased the anti-oxidant enzyme activities in the mycorrhizal plants conferring tolerance to Zn deficient conditions.
- The responses to mycorrhizal colonization was more pronounced under P60 than P30 suggesting that optimal P fertilization is essential to derive fullest potential of the symbiosis.
- Mycorrhizal soil had improved biochemical changes that favor the availability of Zn. Addition of FYM increased all the soil biochemical indicators and the response was more pronounced when FYM was applied optimally.
- Acid phosphatase activity increased by 20-40% due to mycorrhizal colonization that acidifies the rhizosphere besides improving the availability of Zn.
- Concomitant use of organics and inorganics increased C fractions such as organic C, water soluble C and glomalin and the values were the highest among the fertility gradients tested.
- Mycorrhizal colonization appears to translocate the aerial C assimilated into the soil where the substrate serves as the sink for C. These processes enhances the C sequestration pattern and soil organic C.

Conclusion:

Field experiments were conducted in order to assess the interaction effects between Zn vs P and Zn vs organics in the presence or absence of mycorrhizal inoculation. The data have shown that mycorrhizal inoculation orchestrates soil biochemical changes such as increased biomass C, acid phosphatase and glomalin that favorably enhanced the availability of Zn. Acid phosphatase activity increased by 20-40% due to mycorrhizal colonization that acidify the rhizosphere besides improving the availability of Zn. These changes facilitate lowering of pH which resulted in the release of Zn from the fixed pool. Further, glomalin serves as an adsorbitive site for Zn fixation and contributed for the organically bound fraction of Zn. The improvement in availability of soil Zn reflected on the increased anti-oxidant enzyme activities in the mycorrhizal plants conferring tolerance to Zn deficient conditions. The responses to mycorrhizal colonization was more pronounced under P60 than P30 suggesting that optimal P fertilization is essential to derive fullest potential of the symbiosis. Further, application of FYM @ 12.5 t ha^{-1} in conjunction recommended doses of fertilizers improved the responses to mycorrhizal inoculation. Concomitant use of organics and inorganics increased C fractions such as organic C, water soluble C and glomalin contents that further increased by mycorrhizal colonization. The colonized plants appear to translocate the aerial C assimilated into the soil where the substrate serves as the sink for C. Overall data suggest that mycorrhizal symbiosis alleviates Zn deficiency in crops by improving the biochemical changes in the soil and the host plant and the responses were more pronounced when the host plants fertilized optimally.

Harnessing Agricultural Beneficial Microorganisms for production and protection of Sorghum and Rice

PI : O. P. Rupela
International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh

Objectives:

- To access agriculturally beneficial microorganisms (ABM) from relatively new microbiological and crop niches in relevant crop husbandry system(s) involving sorghum and rice, evaluate and use them in field conditions.
- Laboratory evaluation of traditional knowledge products/protocols involving agriculturally beneficial microorganisms.
- To submit promising accessions of beneficial microorganisms to NBAIM, after due evaluations and characterization (including nomenclature using molecular level methods).

Significant Achievements:

- Eighteen out of total 335 bacterial isolates suppressive to *Macrophomina phaseolina* (causes charcoal rot in sorghum) were identified as most promising due to bigger size of hallow (in dual culture method) than the others. Seven of these were evaluated for ability to promote growth in pot culture conditions in glasshouse. CDB 58 and CDB 59 were identified as most promising due to their positive effect on root and/or shoot growth. We thus have bacteria with ability to promote growth of sorghum to some extent and at the same time suppress a disease of sorghum.
- Twenty nine bacterial isolates from rice field (root rhizosphere soil) and 13 isolates from sorghum had at least two PGPR traits were screened for ability to suppress Mp and solubilization of rock-phosphate. Two out of 29 bacterial isolates from rice and five out of 13 from sorghum were antagonistic to Mp and one isolate from rice and seven from sorghum had ability to solubilise rock-P. The results indicate a scope of identifying bacteria with multiple PGPR traits and that can perform across crops, a major emphasis at ICRISAT.
- Two long term field experiments (multi-disciplinary and multi-institutional) were initiated one each on rice and sorghum. While the focus for this project remains accessing agriculturally beneficial microorganisms from contrasting treatments/environments, the experiment on rice is focused on studying scope of saving water, enhancing use of PGPR and inclusion of legumes in rice-rice cropping systems and the experiment on sorghum is focused on managing charcoal rot (caused by *Macrophomina phaseolina*) by using multiple and varied application of most relevant PGPR and antagonists of this disease.
- Ten out of thirteen spore forming bacteria with PGPR traits available with the Microbial Culture Collection at ICRISAT were further studied and tentatively identified as *Bacillus thuringiensis*.

Conclusion:

Traditional knowledge items used by farmers such as compost and Amrit Paani (a ferment involving cowdung, urine and jaggary) were rich (generally $>10^{-4}$ per g or mL of the materials) in agriculturally beneficial microorganisms - P-solubilizers, siderophore producers (known to promote plant growth), nitrogen fixers and *Pseudomonas fluorescens* (indicator of ability to manage pests). This suggested potential of these items in protecting plants from pests, besides helping in crop nutrition. Natural occurrence of P-solubilizing microorganisms measured using Pikovskaya medium was generally high but those with ability to solubilize Rock-P were very less in the niches that were studied (rice fields, Vertisols). Antagonists of *Macrophomina phaseolina* (a fungus that causes charcoal rot of sorghum) were present even in soils that did not grow sorghum in its known history of about 30 years. For example, fields growing rice at ICRISAT campus had antagonists of this fungus.

Isolation, identification, evaluation and exploitation of microorganisms for management of important pathogen and having PGPR potential for vegetable crops

PI : T. K. Bag
CO PI : A. B. Rai
Indiant Institute of Vegetable research, Varanasi, UP

Objectives:

- Isolation and purification of dominant root colonizing bacteria from rhizosphere and rhizoplane of tomato, brinjal and chilli by soil extract dilution method.
- Selection of potential isolate of rhizobacteria with disease suppressing (antagonistic) properties by *in vitro* screening through dual culture techniques on the basis of hyphal growth inhibition of pathogen and lysis.
- Selection of potential isolate of rhizobacteria having PGPR properties by *in vitro* screening of IAA production and siderophore production.
- Green house evaluation of PGPR response in relation to seed germination, seedling vigour, root growth, shoot growth and biomass production in tomato and cowpea).
- *In vitro* evaluation of consortia for compatibility among the potential microbes.

Significant Achievements:

- Rhizospheric soil was collected from southern region of the country, from the tomato, brinjal and chilli and other vegetable. Dominant bacterial colony was isolated, purified and maintained from the vegetable crops. Isolation was done through serial dilution method on soil extract agar medium. 18 new bacterial isolates have been isolated and maintained and will be used in subsequent studies. The potential isolates will be identified later on.
- The bacterial isolates compatible with each other grew on the medium containing the culture of other bacteria. Those bacteria which were not compatible with other bacterial culture in the medium showed the halo region around the filter disc. Bacterial cultures showing the compatibility were mixed in all the combination and consortium was prepared and tested in the field.
- The PGPRs which were found effective for growth were grown in broth and each of the effective cultures were mixed with each of the other cultures and before mixing they were checked for the compatibility with each other through filter paper disc method and the compatible cultures were mixed and checked on one transplanted vegetable crop (tomato) and one non transplanted vegetable crop (cowpea). The result showed that the consortium number 12b was found to be the most beneficial for tomato followed by 2e, 13g, and 12c. While in cowpea most of the consortium was showing the better result in comparison to the control in which the consortium no. 5c was found to be the best followed by 5h, 2a, 6b and 6c.

Conclusion:

Eighteen new rhizobacteria were isolated from the rhizospheric soil of vegetables. Result of PGPRs consortium showed that consortium number 12b was found most beneficial followed by 2e, 13g, and 12c in tomato. In cowpea, most of the consortium was showing the better result in comparison to the control in which the consortium no. 5c was found best followed by 5h, 2a, 6b and 6c.

Application of PGPRs, Biofertilizers and Biocontrol Agents for Disease Managemnt and Sustainable Production of Tuber Crops

PI : R.S. Misra
Co-PI : Susan John K; M. L. Jeeva
Central Tuber Crops Research Institute, Thiruvananthapuram – 695 017

Objectives:

- To collect and maintain the PGPR's, VAM fungi and bio control agents from the rhizosphere of tropical ecosystem, tuber crops and wild relatives of tropical tuber crops.
- To evaluate the microbes under laboratory and controlled conditions for nutrient use efficiency, bio control ability, growth promotion and metabolites of industrial use.
- Field evaluation of the efficacy of selected microbes individually and in combination to tuber crops for yield, quality of produce, nutrient uptake, soil health, disease management and cost effectiveness.
- To characterize the selected microorganisms and develop suitable microbial consortium for tropical tuber crops.
- To study the compatibility of the microbes in the consortium and competitive survival ability of the introduced microbes in the field.
- To produce selected microorganisms as biofertilizer and biocontrol agents for application to crops on a large scale at institute and on farm level.
- To strengthen the national capacity of agriculturally important microorganisms for research and protection of microbial resources.

Significant Achievements:

- Survey was carried out for assessing the distribution of microbes in rhizosphere of tropical tuber crops, its wild relatives and other ecosystems of the high biodiversity biosphere area was carried out at 140 locations.
- A total of 505 microorganisms were isolated (341 bacteria, 80 fungi, 75 actinomycetes, and 9 EPN bacteria).
- Out of the 505 isolates, 169 isolates showed phosphate solubilizing capacity (fig 1). Among these 70 isolates were bacteria, 55 were fungi, 42 were actinomycetes and 2 were EPN cultures.
- Most potent phosphate solubilizers were identified by vanadomolybdophosphoric yellow colour estimation method. Two of them were formulated in vermicompost / coir pith/charred rice husk + sand based inoculum which is under trial for cassava at Salem and for sweet potato at CTCRI.
- 194 bacterial cultures showed N fixing ability, out of which 96 have been quantified for their Nitrogen fixing efficacy.
- All the isolates were tested for the production of certain enzymes viz., cellulase, protease, pectinase, urease and amylase. Among these, there were 239 cellulase producers, 229 protease producers, 114 pectinase producers, 53 urease producers and 210 amylase producers.
- Eight of the isolates were found to produce siderophore.
- Out of the 80 fungal isolates, 17 were identified as *Trichoderma* sp. These isolates were screened for their antagonistic property against *Phytophthora colocasiae*, *P. palmivora* and *Sclerotium rolfsii* by dual culture technique (Table 1).
- The most effective *Trichoderma* sp. is to be mass multiplied for field trials.

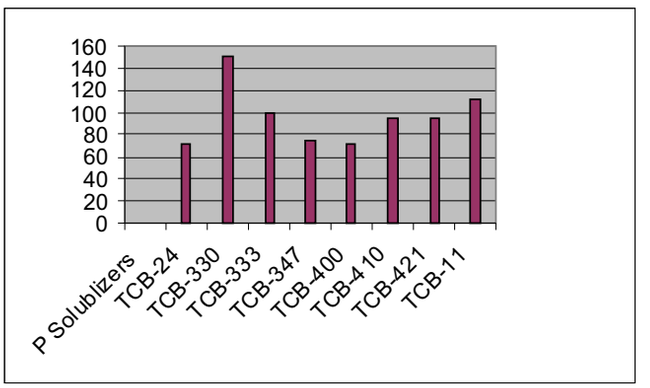


Fig 1. Phosphate solubilizing potential of selected isolates

Table 1. Growth Inhibition of *Sclerotium rolfsii, Phytophthora colocasia* and *P. palmivora* by *Trichoderma* isolates

Isolate No.	Percentage Suppression against		
	Sclerotium rolfsii	*Phytophthora colocasiae*	*P. palmivora*
TCF 2	41.11	67.77	75.55
TCF 6	-	67.22	83.33
TCF 10	-	66.66	76.66
TCF 16	47.22	64.44	65.00
TCF 24	-	-	40.00
TCF 26	55.00	76.11	80.55
TCF 28	-	70.55	76.66
TCF 229	-	-	-
TCF 232	50.55	65.00	77.22
TCF 239	-	-	-
TCF 311	33.88	46.66	68.33
TCF 320	-	-	-
TCF 324	-	-	-
TCF 430	45.00	62.22	65.00
TCF 457(b)	-	41.66	41.66
TCF 459	39.44	67.22	63.33
TCF 460	-	-	-

Conclusion:

Survey, collection and analysis of soil samples from the high biodiversity areas of Kerala, Tamil Nadu and Andhra Pradesh for different microbes led to the isolation of around 505 agriculturally important microorganisms comprising of bacteria, fungi and actinomycetes. Biochemical characterization of these microbes revealed most of them as potent phosphate solubilizers, nitrogen fixers as well as capable of producing certain enzymes like cellulase, urease, amylase, protease and pectinase. The phosphate solubilizing capacity of the isolated phosphate solubilizers were quantified and two potential strains having phosphate solubilization efficacy to the tune of 150 and 112.5 ppm respectively were identified and formulated into biofertilizers which is being tried for cassava at Salem and sweet potato at CTCRI. Different species of *Trichoderma* isolated from the collected soil samples were tested for its disease control efficacy against *Phytophthora palmivora*, *Phytophthora colocasiae* and *Sclerotium rolfsii* by dual culture method under *in vitro* condition. The best performing strains are identified and will be mass multiplied for further field trials.

Plant growth promoting rhizobacteria (pgpr) for chickpea and pigeonpea

PI : K. Swarnalakshmi
Co-PI : Mohan Singh, R.G.Chaudhary
Indian Institute of Pulses Research, Kanpur

Objectives:

- Screening of microorganisms having potential to improve nutrient use efficiency, disease suppression, growth and yield of pigeon pea and chick pea.
- Developing consortium of PGPR for chick pea and pigeon pea.
- Evaluating the field performance of PGPR consortium on chick pea and pigeon pea.

PGPR for chick pea

- 23 PGPR strains (9 *Azotobacter,* 4 *Pseudomonas* and 10 PSB) selected from primary screening (during *Rabi* 2006-07 by plant bioassay) were subjected for secondary screening under field conditions (*Rabi* 2007-08). Five PGPR strains (A1, A11, P3, PSB 9 &PSB 11) which inhibited plant growth during 45 DAS. However strain A1, P3, PSB 9 &PSB 11 enhanced the growth after 75 DAS. The inhibitory effect may be attributed by carbon depletion at initial stage due to their effective root colonization. These strains can be used as a potential component in PGPR consortium.

Table 1. Effect of PGPR on pigeon pea under plant bioassay

Azotobacter strains	Biomass (mg/plant)	% increase over control	PSB strains	Biomass (mg/plant)	% increase over control
A1	165.60	-10.6	PSB2	176.63	-4.8
A6	160.48	-13.3	PSB4	139.27	-30.4
A7	178.95	-3.4	PSB6	172.10	-7.12
A9B	200.09	8.1	PSB9	208.85	12.8
A10A	229.16	23.8	PSB10	199.90	7.9
A13A	235.36	27.1	PSB11	243.45	31.46
A13B	217.58	17.5	PSB21	224.48	21.2
A17A	219.43	18.5	PSB25	235.09	26.9
A17B	202.66	9.4	PSB26	206.40	11.5
A18	195.05	5.3	PSB27	191.05	3.2
Azotobacter chrococcum	233.01	25.8	PSB30	173.54	-6.3
Pseudomonas strains			PSB32	199.45	7.7
P2	243.53	31.5	PSB33	206.01	11.24
P3	251.45	35. 8	PSB34	183.09	-1.1
P4	216.83	17.1	PSB35	189.11	2.1
P6	225.24	21.6	PSB36	150.85	-18.5
P7	247.23	33.5	PSB37	158.45	-14.4
P10	228.34	23.3	PSB38	140.89	-23.9
P14	235.06	26.9	PSB40	170.19	-8.1
P15	207.74	12.18	PSB41	264.19	42.7
P18	205.24	10.83	Uninoculated	183.65	
B3	176.59	-4.6			
Uninoculated	183.65				

Mean values are average of 8 plants

Significant Achievements:

PGPR for pigeon pea

- 40 (which are fast growing, positive for the biochemical attributes like siderophore production, HCN production, ammonia production) out of 86 isolates were screened for their growth promoting effect in pigeon pea using plant bio assay during kharif 2007. Out of 40 strains tested, 9 strains enhanced the plant growth to the tune of 23 to 43% in pigeon pea (Table 1).
- The % increase over control varied from 22% (PSB 21) to 98% (PSB26) at 75 DAS.
- All 23 stains inhibited the growth of *Fusarium oxysporum,pv ciceri* under *in vitro* condition (3 strains showed inhibition and rest showed nutritional competition (Fig 4).

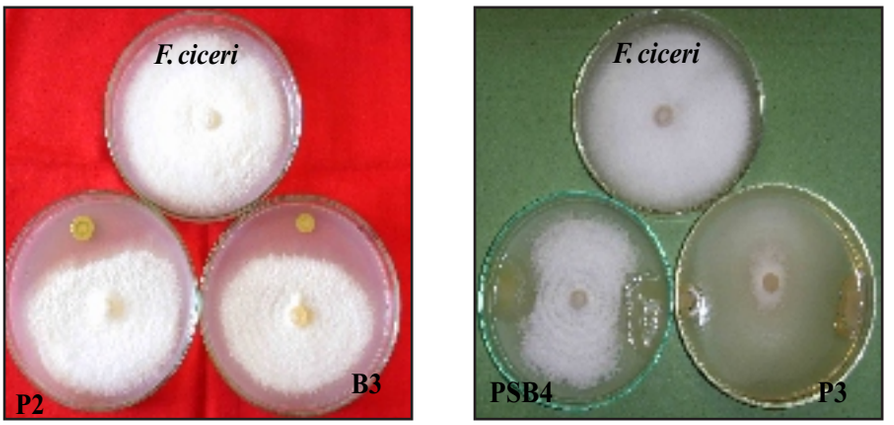


Fig 4:Biocontrol action of PGPR against *Fusarium oxysporum pv. ciceri*

- In addition, 76 new PGPR strains (37 *Azotobacter*, 29 *Pseudomonas* and 10 PSB strains) were isolated from rhizosphere soil samples of different varieties of pigeon pea (collected from different locations viz Kanpur, Jabalpur, Fatehpur) & chick pea using enrichment technique. 37 out of 76 were subjected for their plant growth promoting activity in chick pea using plant bioassay. Out of 37 new isolates, 20 elite strains were selected for their growth promoting activity using plant bioassay in chick pea.

Conclusion:

Plant growth promoting microorganisms were isolated from endorhizosphere of different varieties of pigeon pea. Isolated strains were also subjected to various biochemical characterization *viz* HCN production, siderophore production and ammonia production. Potential strains were evaluated for plant bioassay. Among the tested strains, five *Azotobacter* strains improved chickpea growth with maximum increase of 52% produced with Strain A7 within 4 weeks of plant growth. Out of 15 PSB strains tested, PSB 24 and 27 accumulated significantly greater biomass. Based on plant bioassay & biochemical attributes 23 elite strains were selected for the further studies in chick pea. In chickpea, 23 selected PGPR strains were subjected for secondary screening under field conditions. The % increase over control varied from 22 (PSB 21) to 98% (PSB26) after 75 DAS. All 23 stains showed inhibition against *Fusarium oxysporum,* f. sp *ciceri* under *invitro* condition. In addition, 76 new PGPR strains (37 *Azotobacter*, 29 *Pseudomonas* and 10 PSB strains) were isolated from rhizosphere soil samples of different varieties of pigeon pea & chick pea using enrichment technique.

Development of suitable formulations of potential bioagents for management of important diseases of Sunflower, Safflower and Castor

PI : R. D. Prasad
Co-PI : M. A. Raoof, M. Santha Lakshmi Prasad, P. S. Vimala Devi
Directorate of Oilseeds Research, Rajendranagar, Hyderabad – 500 030

Objectives:

- Screening of fungal and bacterial biological control agents in *vitro* and *in vivo* against *Botrytis ricini*, *Alternaria helianthi, Macrophomina*, *Sclerotium*, *Rhizoctonia* and selection of potential agents with good biocontrol traits.
- Development of formulations of consortia of potential agents for seed and foliar application against selected diseases of castor, sunflower and safflower and Bt plus *Beauveria bassiana* against *H. armigera* and *S. litura.*
- Studying shelf-life, persistence, phylloplane and rhizosphere competence of potential formulations.
- Field-testing of the formulations against Botrytis gray rot in castor, Alternaria blight in sunflower and *H. armigera* and *S. litura* on sunflower crop for determination of the effective field dose, persistence and safety to parasites/predators.
- To generate data and registration of potential agents for field use.

Significant achievements:

- *In vivo* screening of all 85 *Trichoderma* strains and 13 bacterial strains against the selected diseases of sunflower, safflower and castor had been completed. Few bioagents have been found promising and *Trichoderma* strains *viz*., *Trichoderma viride* 5, *Trichoderma* sp. S12 against *R. solani* and *S. rolfsii* and strains *viz*., *Trichoderma viride* 2, *Trichoderma* sp.33 against *S. rolfsii* were found very effective with no disease incidence. Among bacterial strains *B. subtilis* 1 and *B. megaterium* 1 against *R. solani* were found to achieve 100 % reduction of disease incidence over pathogen check and *B. subtilis* 1 was the common bioagent controlling the activity of both *R. solani* and *A. helianthi. Trichoderma* strains *viz*., *Trichoderma* S12 and *Trichoderma* S4 were found effective against both *A. helianthi* and *R. solani.* Likewise, *T. viride* 2 and *T. viride* 5 recorded 100% reduction of *S. rolfsii* and *R. solani*. *T. viride* 32 was found to be common in reducing disease incidence by *A. helianthi*, *B. ricinii* and *S. rolfsii*. Screening of potential bioagents in all possible combinations of consortia and re screening of potential bioagents by single inoculation of powder formulation has been initiated. Standardization of oil formulation is in progress and shelf life studies have been started. Mineral oil based *Bacillus thuringiensis* + *B. bassiana* combination formulation was found very effective on 10 day-old larvae of *Helicoverpa armigera* in terms of mortality of 83.3-86.6 % at the end of 5 days after treatment and were superior to Bt (66.6% mortality) or *B. bassiana* (50.0 % mortality) alone treatments.

Conclusion:

Trichoderma strains *viz*., *Trichoderma viride* 5, *Trichoderma* sp. S12 against *Rhizoctonia solani* and *Sclerotium rolfsii* and strains *viz*., *Trichoderma viride* 2, *Trichoderma* sp.33 against *S. rolfsii* were found very effective. Among bacterial strains *Bacillus subtilis* 1 and *B. megaterium* 1 against *R. solani* were found to achieve 100% reduction of disease incidence and *B. subtilis* 1 was the common bioagent controlling both *R. solani* and *Alternaria helianthi. Trichoderma* strains *viz*., *Trichoderma* S12 and *Trichoderma* S4 were found effective against both *A. helianthi* and *R. solani. T. viride* 2 and *T. viride* 5 recorded 100% reduction of *S. rolfsii* and *R. solani*. *T. viride* 32 was found to be common in reducing disease incidence by *A. helianthi, Botrytis ricinii* and *S. rolfsii*. Mineral oil based *Bacillus thuringiensis* + *B. bassiana* combination formulation was found very effective on 10 day-old larvae of *Helicoverpa armigera* and gave more than 80% mortality of larvae.

Trichoderma treated sunflower showing no root rot (*S. rolfsii*) incidence

Larval bioassays

Dead larvae showing symptoms of Bt infection and fungal infection.

Unravelling the pathogenic mycoflora of grassy weeds in India and development of a mycoherbicide-based biological control strategy for Cyperus rotundus

PI : P. Sreerama Kumar
Project Directorate of Biological Control, Bangalore

Objectives:

- To unravel the pathogenic mycoflora of grassy weeds, including *Cyperus rotundus*, *Echinochloa* spp. and *Phalaris minor*.
- To select a mycoherbicidal candidate(s) for *C. rotundus* commencing from intensive search for its pathogens through established screening procedures for the actual potential of the organism(s).
- To determine the potential of the candidate organism(s) in unformulated and formulated forms against *C. rotundus* in semi-field and field situations.
- To accommodate the method into the existing weed management programme for *C. rotundus* based on the trials.

Significant Achievements:

- Explorative surveys were carried out for fungal pathogens of a range of grassy weeds across India. The main species that exhibited disease symptoms in Karnataka were *Brachiaria eruciformis*, *Buchloe dactyloides*, *Cynodon dactylon*, *Cyperus rotundus*, *Cyperus iria*, *Dactyloctenium aegyptium*, *Digitaria sanguinalis*, *Echinochloa colona*, *Eleusine indica*, *Ergostis* sp., *Fimbristylis miliacea*, *Iseilema laxum* and *Sorghum halepens.* In north India, weed-specific surveys were done in villages falling under Gautam Buddha Nagar, Ghaziabad and Meerut districts of Uttar Pradesh and Faridabad district of Haryana.
- Nine fungal pathogens, isolated earlier from diseased *C. rotundus* plants collected from Punjab and Karnataka were identified. The fungi isolated from the diseased weed parts were confirmed to be pathogens by satisfying Koch's postulates. All the isolated fungi were pathogenic to their respective hosts.
- The test pathogens were grown on various solid (agar-based) media, including dehydrated and freshly prepared, and assessed for different growth parameters, viz. culture/ colony characteristics, radial growth, conidial numbers and germination of conidia. The candidate pathogens were also tested in a range of liquid media, including proprietary and newly developed broths. The fungi were assessed based on the parameters such as culture characteristics, conidial numbers just after completion of incubation period, colony-forming units, dry/wet weight and numbers of conidia/20-mm mycelial mat were studied. These exhaustive studies have been completed for four pathogens, viz. WF(Cr)92, WF(Cr)93, WF(Cr)97 and WF(Cr)101.
- Since the pathogen WF(Cr)101 was isolated from the tuber of the target weed, a new tuber-decoction-based media were developed and named as tuber-decoction agar (TDA). Four variants (TDA-2.5, -5, -10 and -15) of the newly developed tuber decoction-agar (TDA) were also found to support the fungal growth (10.5mm/day) appreciably. With the increase in the concentration of TDA, a gradual increase in the numbers of conidia was observed. The fungus produced maximum conidia (267.64 x 10^4/6-mm mycelial disc) on TDA-15 and minimum (43.56 x 10^4/ 6-mm mycelial disc) on TDA-2.5 after a 15-day growth period.
- The four liquid variants (TDB-2.5, -5, -10 and –15), called tuber-decoction broth (TDB) were studied for conidial production, CFU, wet and dry weights. The maximum conidial production, CFU, wet and dry weights were observed for TDB-15 followed by TDB-10, -5 and -2.5.
- Laboratory studies on the effect of the fungal pathogen [WF(Cr)101] on the tubers of *C. rotundus* showed promising results. There was a considerable reduction in the overall potency of the tuber (shoot bud emergence, number of rhizoids, etc). The tubers, both treated and untreated were also sown in pots for long-term observations.
- In the long-term experiment in the greenhouse, the tuber pathogen [WF(Cr)101] was found to have sustained mycoherbicidal effect on *C. rotundus*.
- A preliminary experiment on soil and coconut coir pith (CCP) incorporation of shake-flask culture of the biomass of the tuber pathogen [WF(Cr)101] obtained from malt extract broth indicated an insignificant reduction in the mean number and maximum number of shoots borne by the mother tuber.
- The results from the second experiment indicated that the tubers, which grew in the pathogen-incorporated soil and CCP showed reduction in the number and shoot (longest) length of the weed. The inoculated pathogen grown in TDB-15 was able to decrease the longest shoot length by 90.4% and 49.2% soil and CCP, respectively, in comparison with control.
- In the third experiment, when the mean number of

shoots emerged were counted and compared with control, all the treatments bore less number of shoots. However, there was no specific trend in the maximum number of shoots emerging from each mother tuber. A 37.8% and 80.7 % reduction in the longest shoot by the TDB-15 grown pathogen was observed in coir and soil substrates respectively, in comparison with control.

- In the wide host-range studies that have been initiated for *C. rotundus* pathogens, collateral hosts were not observed for any of the test pathogens that were handled. The greenhouse evaluations included important cereal crops and vegetables, apart from some of the most commonly occurring weeds belonging to Cyperaceae. The *C. rotundus* rust has been found to infect at least one more host, namely, *C. esculentus.*
- The plant growth-suppressing rhizobacteria (PGSR) isolated and found to be effective against *C. rotundus* were identified based on 16s rDNA sequence data.

Conclusion:

Different explorative surveys were carried out for fungal pathogens of a range of grassy weeds across India. The main species that exhibited disease symptoms are *Brachiaria eruciformis*, *Buchloe dactyloides*, *Cynodon dactylon*, *Cyperus rotundus*, *Cyperus iria*, *Dactyloctenium aegyptium*, *Digitaria sanguinalis*, *Echinochloa colona*, *Eleusine indica*, *Ergostis* sp., *Fimbristylis miliacea*, *Iseilema laxum* and *Sorghum halepens.* The fungi isolated from the diseased weed parts were confirmed to be pathogens by satisfying Koch's postulates.The exhaustive studies have been completed for four pathogens, viz. WF(Cr)92, WF(Cr)93, WF(Cr)97 and WF(Cr)101. Four variants (TDA-2.5, -5, -10 and -15) of the newly developed tuber decoction-agar (TDA) were also found to support the fungal growth (10.5mm/day) appreciably.The four liquid variants (TDB-2.5, -5, -10 and –15), called tuber-decoction broth (TDB) were studied for conidial production, CFU, wet and dry weights. In laboratory studies considerable reduction in the overall potency of the tuber (shoot bud emergence, number of rhizoids, etc) was observed. In the long-term experiment in the greenhouse, the tuber pathogen [WF(Cr)101] was found to have sustained mycoherbicidal effect on *C. rotundus*. The plant growth-suppressing rhizobacteria (PGSR) isolated and found to be effective against *C. rotundus* were identified based on 16s rDNA sequence data.

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

Assessing spatial and temporal shift in soil microbial communities of paper mill effluent contaminated soils and effective utilization of microflora of these sites for crop growth promotion and reclamation effluent contaminated soils

PI : Rajeev Kaushik
Co-PI : A. K. Saxena
National Bureau of Agriculturally Important Microorganisms, Mau

Objectives:

- Isolation of microorganisms from agricultural soils irrigated/contaminated with wastewater of medium and large scale paper mills.
- To assess the functional shift in soil microbial population as a result of long term irrigation or dumping of paper mill effluent on the agricultural lands.
- Screening of the isolates from selected sites showing major shifts in soil functional and structural microbial communities for their ability to produce plant growth promoting attributes
- Characterization of the isolates obtained from selected contaminated sites for their ability to produce enzymes that can be used in paper mills for effluent treatment at different physiological and nutrient conditions.
- Optimization of process parameters for the large scale production of these enzymes in economic way.

Significant Achievements:

- Survey of the farmer's fields, which are being irrigated with paper mill effluent for over 20 years in succession, was carried out. Survey was carried out in the farmers field which are being irrigated with pulp and paper mill effluent of Century paper mill, Lal Kuan, Udham Singh Nagar, Uttranchal (fig 1) in two cropping season i.e. *Rabi* and *Kharif*.
- For collecting soil samples, the fields were selected in the identified regions, where effluent is being used for irrigation since last 20 years. For the sampling of soil and plants three fields were selected (i) Control field where effluent irrigation was not done at all and is being irrigated only with fresh water, (ii) Diluted

Fig 1. Map of the survey site in Udham Singh Nagar

effluent irrigated field (DEIF) and (iii) Concentrated effluent irrigated field (CEIF). Soil samples were collected from rhizosphere, and non-rhizospheric regions of the crops growing in the region.

- The pH of the paper mill effluent contaminated soil and the control soil from the same region was above 7.0, however, the effluent treated soils showed alkaline pH as compared to control field soil. The presence of Na in the effluent could be the main reason for the increase in the soil pH over control field soil. The soil organic carbon content in CEIF soil was significantly higher that the CF soil (Table 1). The higher OC content of the effluent irrigated field over the control soil might be due to the incorporation of lignocellulosic and hemicellulosic fractions of effluent into the soil on regular basis.
- For distinguishing spatial and temporal changes in microbial communities as a result of effluent application the community level physiological profiling was done by inoculating the soil samples in BIOLOG Eco plates. The BIOLOG Eco Plate contains 31 of the most useful carbon sources for soil community analysis. These 31 carbon sources are repeated 3 times to give the scientist more replicates of the data.
- The plates were inoculated after the soil treatment with ion exchange resin and the plates were incubated for 1 week, the OD at 560 nm of each well was taken at an interval of 8 hrs and the data obtained thus is being analyzed by PCA.
- In order to isolate a potential xylan degrader, a main component of the effluent causing functional shifts, large numbers of isolates were screened for xylanase activity at thermophilic range and alkaline pH. Two potential isolates, one bacteria and one actinomycetes, were isolated with maximum xylanase activity (fig 2).
- Using BIOLOG microbial identification system the bacterial isolate was identified as *Burkholderia glumae* (fig 3) and was submitted to the NBAIM culture Collection.

Table 1. The soil properties as influenced by pulp and paper mill effluent irrigation.

Soil Parameters	Control Field	Concentrated Effluent Irrigated Field	Diluted Effluent Irrigated Field
% OC	0.87	1.24	0.91
pH	7.33	8.16	7.93
EC	0.29	0.73	0.51
Available N (Kg ha-1)	57.3	57.9	64.5
Soil Na (Kg ha-1)	132.11	682.31	460.71

Table 2. The total culturable count of the microorganism as influenced by effluent irrigation

Media Used	Soil Sampling Sites*		
	CEIF	DEIF	Control
Crystal violet agar (CV Agar) Gram -ve	4500	3600	16800
Methyl red agar (MR Agar) Gram +ve	12300	19700	4500
Jensen's N free Agar	2.5	8.8	2600
Actinomycetes isolation agar	320	286	220
Potato dextrose agar	33	29	62
King's B	5	6	4

Table 3. The count of specific group of bacteria as influenced by effluent irrigation

Specific Group of bacteria	Soil Sampling Sites*		
	CEIF	DEIF	Control
Phosphorous solubilizers	30	15	48
Rees mineral medium with cellulose	960	173	43
Rees mineral medium with Xylan	490	355	67
Azospirillum	3400	9400	17000
Mercury Tolerant bacteria	770	97	17

*CEIF: Concentrated effluent irrigated field; DEIF: Diluted effluent irrigated field

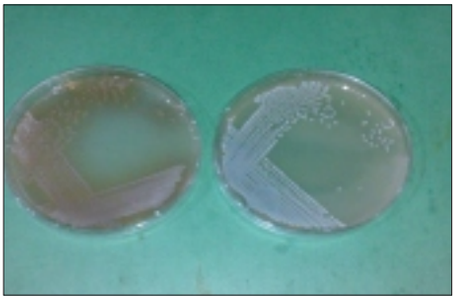
Fig 2. Halo Zone formed by extracellular Xylanase on Congo Red Medium supplemented with Xylan

Fig 3. BIOLOG Window showing the identification of isolate as *Burkholderia glumae.*

- An isolate of actinomycetes was fast growing and could utilize xylan in 12-18 hrs and reach the stationary phase. It is an uncommon property of actinomycetes to grow at this rapid rate (fig 4) and showed different colony pattern and pigmentation on different media (fig 5) and spiral sporulation pattern (fig 6).
- Actinomycetes isolate was characterized at molecular level. PCR amplification of 165 & DNA followed by sequencing was carried out to identify the isolate. The isolate at the initial stage seems to be noval species of *Streptomyces* as reveled by NCBI Blast search of the 16 S rDNA sequence. The further characterization of the isolate and purification of its xylanases is in progress.

the soil samples were collected from the selected control and effluent irrigated sites in Uttranchal state. Initial studies reveled certain changes in the native microbial population aas compared to the control soil. The Gram positive and negative bacterial population showed increase and decrease in their numbers respectively over the period of 20 years of continuous irrigation with paper mill effluent. As effluent is rich in hemicellulosic, cellulosic fraction along with mercury the irrigated soils showed increase in Xylan and cellulose utilizing microbial population, also the Hg tolerant bacterial population increased over period of 20 years. For effective utilization of the allochthonous population developed in the soil for the plant growth promotion in soil irrigated with effluent several isolates were isolated and in preliminary stage encouraging results are obtained which needs further authentication. Subsequently the soils in other parts of India which are being irrigated with pulp and paper mill effluent will be explored.

Fig 4. The actinomycetes isolate completely utilizing media contents and settling down in < 24 hrs



Reese Mineral Media Nutrient Agar

Fig 5. Colony characteristics and pigmentation on different media

Fig 6. Spiral sporulation pattern

Conclusion:

In order to decipher the functional changes in the soil microbial community as result of long term application of pulp and paper mill effluent onto the agricultural lands,

Bioremediation of Polycyclic aromatic hydrocarbons (PAH) through microbial consortia

PI : Lata, Division of Microbiology
Co-PI : Anju Arora, CCUBGA; **Shashi Bala Singh,**
Division of Agril Chemicals, Indian Agricultural Research Institute, New Delhi – 110012

Objectives:

- Isolation and characterization of PAH degrading Microorganisms from soil.
- Screening of microorganisms for their PAH degrading potentialities.
- Evaluation of selected microorganisms for *in situ* decomposition of PAH.
- Development of practical method for application of consortia to degrade PAH in soil.

Significant Achievements:

- Amongst 66 bacterial cultures isolated from contaminated soil samples of four different geoclimatic locations in India, fifteen bacterial isolates were found capable of utilizing all the five polycyclic aromatic hydrocarbons tested (i.e. Naphthalene, Phenanthrene, Anthracene, Fluorene and Pyrene) at concentration as high as 100 ppm.
- Tremendous biochemical and functional diversity was observed among the 66 bacterial isolates.
- Five novel partial gene sequences of 16 S rRNA gene of PAH utilizing bacterial cultures have been submitted the GenBank of NCBI (PubMed) under Accession Nos. EF591777, EF591778, EF584108, EF584109 and EF590317
- PAH degrading Isolate L-11 identified as a strain of *Serratia marcescens* (fig 1 & 2) is being reported as a PAH-degrading organism for the first time, as per the literature available.
- All the five isolates identified to date on the basis of 16S rDNA-based molecular characterization, namely *Bacillus subtilis* [PAH-21 (Ch-58)], *Brevibacterium* sp. [PAH-14 (Ma-40)], *Serratia marcescens* [isolate L-11], *Acinetobacter haemolyticus* [PAH-9 (Ma-2)] and *Bacillus subtilis* [isolate P-3] show high motility which may help them in sequestering the PAH-metabolites from soil for their metabolism.
- Conditions were standardized for recovery and quantification of PAH degradation by HPLC method. Best elution was found with 60:40 :: Acetonitrile: water mobile phase.

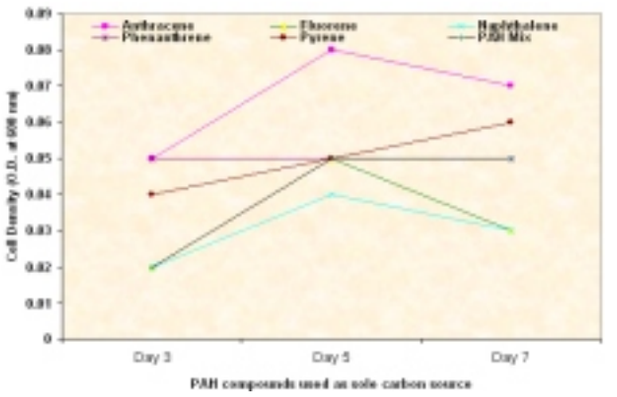


Fig 1. Cell density of PAH L-11 during growth on minimal medium with PAH compounds as sole source of carbon.

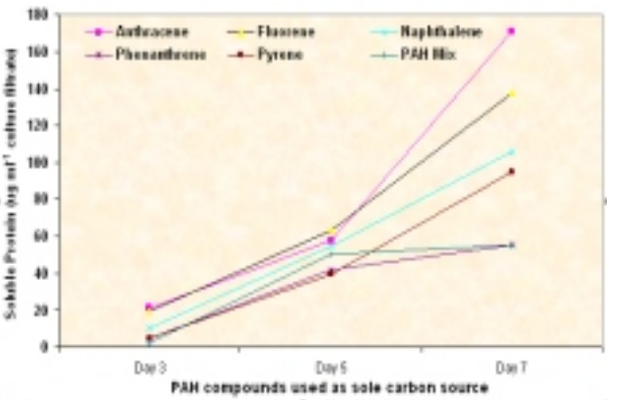


Fig 2. Soluble protein content of PAH L-11 during growth on minimal medium with PAH compounds as sole source of carbon.

Conclusion:

From the soil samples contaminated with PAH 15 bacterial strains were found to have ability to degrade Anthracene, Phenanthrene, Fluorene, Naphthalene and Pyrene at concentration as high as 100 ppm. Five partial gene sequences of 16 S rRNA gene of PAH utilizing bacterial cultures have been submitted to the GenBank of NCBI. PAH degrading Isolate L-11 identified as a strain of *Serratia marcescens* is being reported as a PAH-degrading organism for the first time. Conditions were standardized for recovery and quantification of PAH degradation by HPLC The promising strains will be evaluated for degradation of PAH mixture individually by HPLC and finally a consortium will be developed for bioremediation of PAH contaminated soil.

Genotyping and Isolation of Sphingomonads from HCH Contaminated Agricultural Soils and their Application in Bioremediation

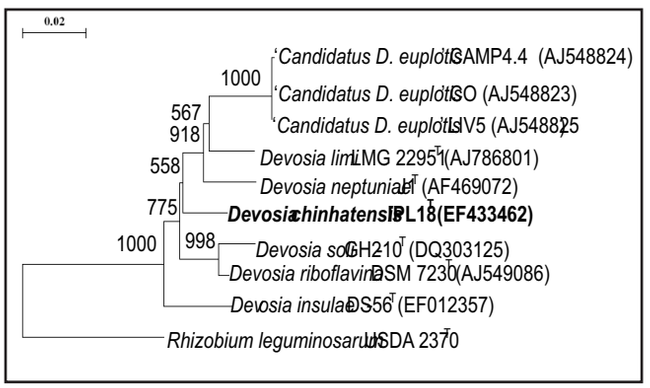
PI : Rup Lal
Department of Zoology, University of Delhi, Delhi

Objectives:

- Identify heavily contaminated agricultural sites with HCH isomers.
- Enrichment of soil samples for the isolation of sphingomonads that degrade HCH or other pollutant.
- T-RFLP genotyping of soils for presence of sphingomonads.
- PCR amplification and cloning of degradative genes.
- Microcosm/mesocosm studies to determine efficacy in the field.

Significant Achievements:

- On analysis of HCH residues from different soil samples, it was found that agricultural soils of adjoining area of HCH manufacturing unit and open dump site are highly contaminated. The HCH levels were varied between 2 to 4200 µg/g of soil.
- Three HCH non-degrading strains IPL-18, Esp-1 and IP-10 are classified on the basis of 16S rRNA gene sequences and constructed phylogenetic tree (Fig 1 and 2).
- A total of 25 sphingomonad/non-sphingomonads strains have been isolated from different enrich, out of 25 strains only 7 strains are HCH-degrader.
- Gas chromatographic analysis of HCH contaminated soil selected for MNA experiment showed 18,250 µg/g of sum of all the HCH isomers. Composite soil sample (after mixing) that was taken for biostimulation experiment was slightly alkaline (pH-7.7) with 45.8% sand, 32% slit and 13.2% clay. Electrical conductivity of soil was 4.5 ds/m (measure of total soluble salt) with 0.58% total available organic carbon while available nitrogen (N), phosphorus (P) and potassium (K) content of the soil was found to be 156.8, 58.93 and 389 kg/ha (kilogram /hectare) respectively. Data of residual analysis and total hetertrophs count are given below (Table 1) while study of community composition and quantification of lin genes copy number by total soil DNA is under standardization.
- Strains UM1, UM3, UM4, F2, IP01 and IP26 have high efficiency to degrade HCH than earlier reported strains (B90A, Sp+ and UT26).
- *lin* genes have been identified in all isolated HCH-degrading strains



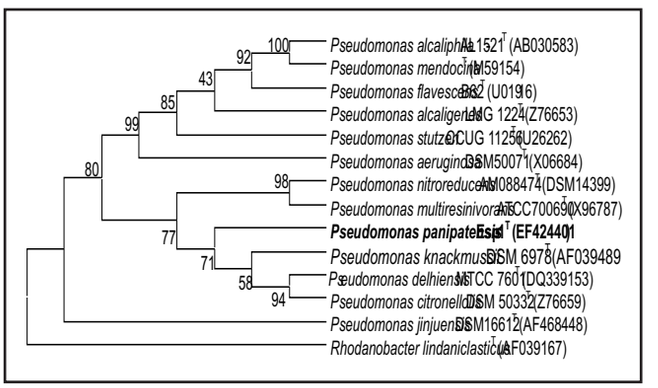


Fig 1. Phylogenetic tree based on complete 16S rRNA gene sequences showing the relationship among the strain IPL18^T, Esp-1^T and other *Devosia* and *Pseudomonas* species respectively

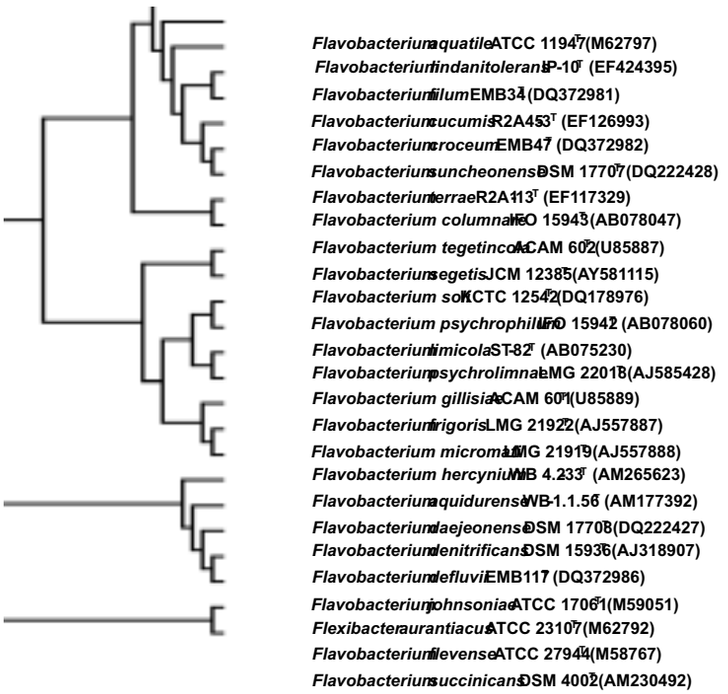


Fig 2. Phylogenetic tree based on complete 16S rRNA gene sequences showing the relationship among the strain IP-10^T and other *Flavobacterium* species

Table 1. Data from MNA Experiment after 30 days

PITS	Σ-HCH (µg/ml)	CFU/ g soil
Control	81.40	3.5x 10^6
Water treated	94.92	1.3x 10^7
$(NH4)_2HPO_4$	86.06	1.8x10^7
Molasses treated	57.15*	2.7x10^7

Conclusion:

Highly contaminated agricultural soil was selected for isolation of HCH-degrading bacteria. A total of 25 strains were isolated by enrichment method. Off which, only 7 strains were found to have HCH-degrading capabilities. Southern blot hybridization has proved that all the seven HCH-degrading strains have *lin* genes. Only three strains (IPL-18, IP-10 & Esp-1) have been classified by polyphasic approach. A total number of 12 bacterial strains have been submitted in NBAIM culture collection. Terminal restriction fragment length polymorphism (TRFLP) has been standardized and diversity of the HCH contaminated sites are being studied.

Refinement in indoor compost technology for white button mushroom using thermophilic organisms

PI : B. Vijay
Co-PI : R. P. Tewari, M. P. Sagar
National Research Center on Mushrooms, Solan, HP

Objectives:

- To conduct surveys at compost units in different corners of India for the isolation of thermophilic organisms for their screening towards their potential in converting agro wastes into white button mushroom compost.
- Molecular characterization, physiological and enzymatic studies on different thermophilic organisms.
- Refinement in environmental friendly indoor compost using above strains by combination of both Anglo Dutch (cold process) and by INRA method (hot process).
- Development of microbial inoculation technology package for bulk production of different composts for commercial purpose.

Significant Achievements:

- A large collection of different thermophilic fungi have been created at the National Centre and among them 8 and 6 strains of *S. thermophilum* and *H. insolens* respectively have been identified.
- Strain 3 and 5 of *S. thermophilum* were found growing at mesophilic temperature ranges an important finding reported for the first time.
- *S. thermophilum* and *C. thermophile* were most important thermophiles with respective to production of extra cellular enzymes. Supplementation of urea in the wheat straw +wheat bran substrates significantly stimulated the enzymes production.
- 45 C was found to be the optimum temperature for the growth of *S. thermophilum* and the stain 8 was the fastest growing fungus
- *S. thermophilum* can tolerate wide fluctuations in the pH, however it does not favour highly acidic pH.
- Wheat straw substrate is the best and cheap source for the growth and multiplication of *S. thermophilum*.
- Strain 6 of *S. thermophilum* can better be utilized for compost production as this is a fast cellulose decomposer.
- Eleven thermophilic bacteria were isolated from different compost samples.

Conclusion:

A large collection of different thermophilic fungi has been created at the National Centre Strains of *S. thermophilum* were found growing at mesophilic temperature *S. thermophilum* and *Chaetomium. thermophile* were most important thermophiles with respective to production of extra cellular enzymes. Supplementation of urea in the wheat straw + wheat bran 45^0 C was found to be the optimum for enzyme production. Strain of *S. thermophilum* can better be utilized for compost production, as this is a fast cellulose decomposer.

Optimization of parameters for utilization of paddy straw, kinnow pulp and pea pods for production of cellulases, ethanol and feed supplements

PI : Harinder Singh Oberoi
Co-PI : D. S. Uppal, V. K. Bhargava
Central Institute of Post Harvest Engineering and Technology, Ludhiana

Objectives:

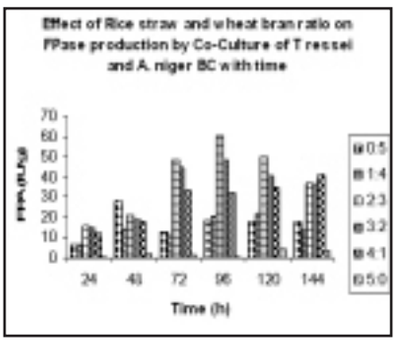
- Development of microbial consortium of organisms for cellulose degradation, hexose and pentose fermentation.
- Optimization of operating parameters such as pelletization process, substrate and inoculum composition, pH, temperature, aeration etc for cellulase and ethanol production
- Optimization of downstream processing parameters for recovery and purification of cellulase and ethanol
- Evaluation of residues after fermentation for suitability as feed supplements.

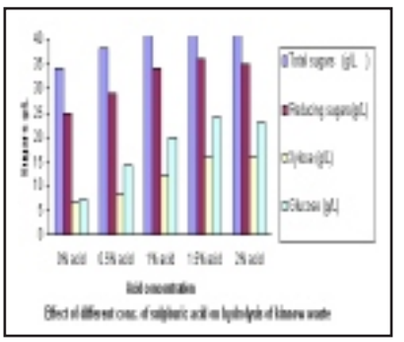
Significant Achievements:

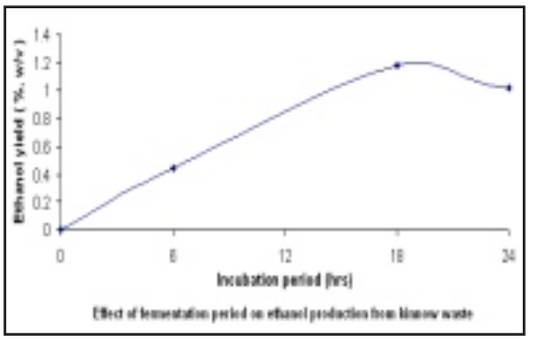
- Primary hydrolysis using oxalic acid treatment resulted in about 25% hydrolysis of rice straw and the secondary hydrolysis resulted in further hydrolysis of about 13%, thus about 38% sugars were produced from the two stage hydrolysis of paddy straw. The furfural, furan and phenolic compound concentration was relatively much less even at 4% oxalic acid (w/v) concentration as compared to the production of furfural, furan and phenolic compound even at lower concentrations of strong acids such as HCl and H_2SO_4. Supplementing kinnow pulp with wheat bran in 3:2 in simple distilled water resulted in FPase and β- glucosidase activity of 13.2 and 12.8 IU/gds and a ratio of nearly 1:1 which is considered to be most appropriate for achieving ideal saccharification efficiency of pretreated lignocellulosic material. Employing co-cultures of *Trichoderma reesei* RC-30 and *Aspergillus niger* BC-1 in the ratio 1:1 on paddy straw and wheat bran combination of 3:2 resulted in FPase, CMCase and β- glucosidase activity of 28 IU/gds, 46IU/gds and 25 IU/ gds respectively. Acid hydrolysate of kinnow waste using mild HCl without any detoxification treatment resulted in an ethanol concentration of 12g/l from total sugar concentration of 44g/l obtained by acid hydrolysis process with a fermentation efficiency of about 58%.

Conclusion:

Out of the 42 strains including the eleven isolates isolated from different environments for cellulase production, pentose and hexose sugars fermentation screened for different activities, 5 isolates (3 for cellulase production and one each for hexose and pentose fermentation) were found to be efficient and were used for further studies. Primary hydrolysis using oxalic acid treatment resulted in about 25% sugars and the secondary hydrolysis resulted in additional sugar yield of 13%, thus about 38% sugars were produced from the two stage hydrolysis of paddy straw. The furfural, furan and phenolic compound concentration was relatively much less even at 4% oxalic acid (w/v) concentration as compared to the production of furfural, furan and phenolic compound even at lower concentrations of strong acids such as HCl and H_2SO_4. Supplementing kinnow pulp with wheat bran in 3:2 using simple distilled water resulted in FPase and β- glucosidase activity of 13.2 and 12.8 IU/gds respectively and a ratio of nearly 1 :1 which is considered to be most appropriate for achieving ideal saccharification efficiency of pretreated lignocellulosic material. Employing co-cultures of *Trichoderma reesei* RC-30 and *Aspergillus niger* BC-1 in the ratio 1:1 on paddy straw and wheat bran combination of 3:2 resulted in FPase, CMCase and β- glucosidase activity of 28 IU/gds, 46IU/gds and 25 IU/ gds respectively.







Bioremediation of commonly used pesticides in tropical rice ecosystem

PI : T. K. Adhya
Central Rice Research Institute, Cuttack, Orissa

Objectives:

- Develop an understanding of the structural and functional diversity analysis of microbial communities and their dynamics in response to anthropogenic stress including xenobiotic application.
- Determine the biochemical mechanisms including enzymatic pathways involved in aerobic and anaerobic degradation of pesticides.
- Expand understanding of microbial genetics as a basis for enhancing the capabilities of microorganisms to degrade polluting xenobiotic.
- Conduct microcosm/mesocosm studies of new bioremediation techniques to determine in a cost-effective manner whether they are likely to work in the field, and establish dedicated sites where long-term field research on bioremediation technologies can be conducted.
- Develop, test and evaluate models for assessing efficacy of bioremediation technologies.

Significant Achievements:

- ARDRA analysis of the genomic DNA of the two novel *Bacillus* sp. with *AluI* and *MspI* showed that the two isolates had almost similar restriction sites indicating species closeness for the two bacterial isolates (fig 1).
- Utilization of different substrates by the *p*-nitrophenol degrading *Bacillus* sp. indicate that both PS-5 and PS-6 utilized glucose (1 and 2%) for their growth and converted *p*-nitrophenol to *p*-aminophenol but did not utilize maltose, arabinose and xylose.
- PCR amplification of the total metagenomic DNA with '*linA*' primer and their size separation on agarose gel showed several bands indicating diversity in microbial population with genomic structure analogous with 'linA'.
- Consistent trends were observed in the DGGE fingerprints of enriched soils (fig 2). The fingerprints reflected selection for successively fewer populations in the sequential enrichment cultures. Interestingly, enrichment with commercial formulation of HCH indicated dense bands as compared to specific isomers.
- A bacterial isolate (CH 13) isolated from flooded planted soil retreated with chlorpyrifos degraded 10 µg ml^{-1} chlorpyrifos in mineral salts medium within 9 days.
- Agarose gel electrophoresis of PCR products of metagenomic DNA from an alluvial soil untreated or retreated with chlorpyrifos indicate that all the DNA fractions extracted from soils of different treatments had positive PCR amplification with a single band each indicating presence of effective microbial population in chlorpyrifos treated or untreated pots.
- A novel *Bacillus* sp. having 92% homology with *Bacillus djibelorensis* degraded vinclozolin in mineral salts medium using it a source of C and energy has been isolated and identified through complete 16s rDNA sequencing and FAME analysis.
- A novel bacteria has been isolated from flooded alluvial soil retreated with α-isomer of hexachlorocyclohexane that degraded β-HCH in a mineral salts medium within 6 days but could not degrade any other isomers of HCH. Morphological and molecular identification of the bacteria is in progress.

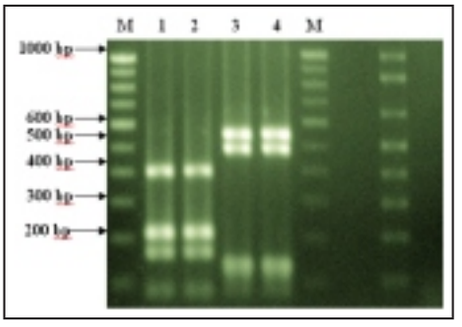


Fig. 1 : ARDRA analysis of 16s rDNA of two *Bacillus* sp. degrading *p*-nitrophenol.

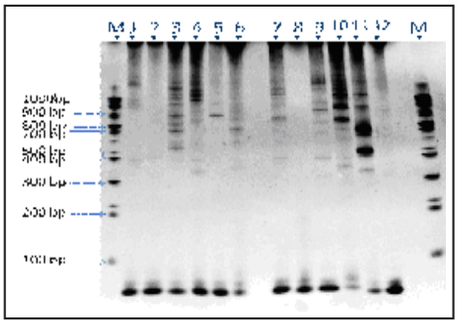


Fig.2 : DGGE of the PCR amplicons from different treatments.

Conclusion:

Two novel *p*-nitrophenol degrading *Bacillus* sp. and one *o*-nitrophenol degrading *Bacillus* sp. were isolated from flooded rice soils retreated with respective isomers of nitrophenol. Both the *p*-nitrophenol degrading bacteria degraded *p*-nitrophenol as the sole source of carbon and energy releasing stoichiometric amount of nitrite. The *o*-nitrohenol degrading bacterium, however, oxidized nitrite to nitrate suggesting the bacteria to be a heterotrophic nitrifier. A novel *Bacillus* sp. having 92% homology with *Bacillus djibelorensis* degraded the organochlorine fungicide vinclozolin in mineral salts medium. PCR amplification of the total metagenomic DNA with '*linA*' primer and their size separation on agarose gel showed several bands indicating diversity in microbial population with genomic structure analogous with '*linA*'. A bacterial isolate from flooded alluvial soil planted to rice and retreated with chlorpyrifos degraded 10 $\mu g.ml^{-1}$ chlorpyrifos in mineral salts medium within 9 days.

Development of Bacterial Consortia for Bio-Processing Agricultural Wastes and Bioremediation of Aquaculture Effluents

PI : C. S. Purushothaman
Co-PI : A. Vennila, P. K. Pandey
Aquatic Environmental Management Division, Central Institute of Fisheries Education, Mumbai

Objectives:

- To develop bacterial consortia capable of decomposing lignocellulosic agro-waste.
- To develop bacterial consortia to bio-remediate toxic materials from aquatic farms.

Significant Achievements:

- The isolates were screened based on their CZ: CS ratio. The Berhampur isolates were found to have a better average CZ: CS ratio as compared to the Bharuch isolates.
- The 10 isolates from Bharuch were characterized by ARDRA (fig 1), with three restriction enzymes, *Alu*I, *Hae*III and *Mbo*I. The dendrogram analysis yielded two clusters. Of one cluster, two of the isolates, B6 and B8, were identified as *Pseudomonas alcaligenes*. B10 is a related but yet unknown isolate. The other major cluster of seven organisms has several sub-clusters, of which B5 was identified as *Nocardia farcinica.*
- The isolates from Berhampur were characterised by SDS-PAGE Whole-cell Protein profiling and stained with Coomassie Blue R250. Phylogenetic relationship among them could not be determined due to the lack of sufficient bands.
- The isolates were microscopically examined for their ability to degrade Whatman filter paper No.2, a complex cellulosic substrate and degradation was observed.

Conclusion:

Off the microbial isolates, based on their CZ: CS ratio, Berhampur isolates were found to have a better average CZ: CS ratio as compared to the Bharuch isolates. The isolates obtained were identified as *Pseudomonas alcaligenes* based on 16 S rDNA sequencing. The other major isolates were identified as *Nocardia farcinica.* The results obtained so far will help in developing Consortia for Bio-Processing Agricultural Wastes and Bioremediation of Aquaculture Effluents

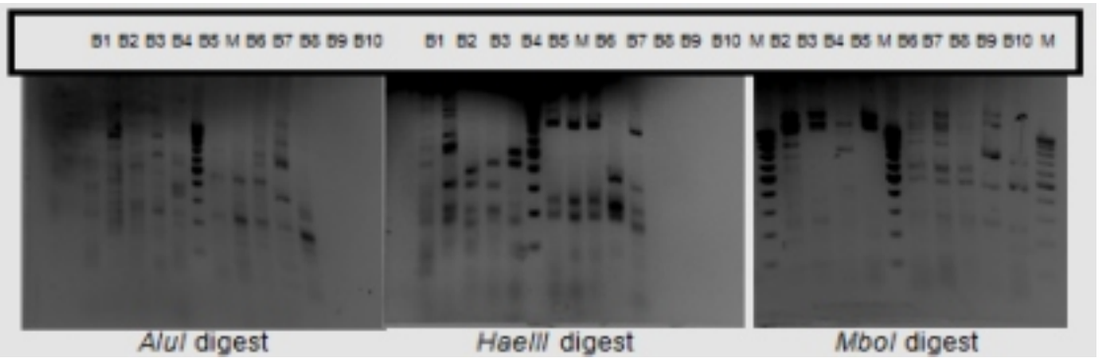


Fig 1. Amplified rDNA Restriction Analysis of the isolates from Bharuch

Microbial Bioremediation of Wastewater for Heavy metals

PI : P. K. Joshi
Co-PI : Khajanchi Lal
Central Soil Salinity Research Institute, Zarifa farm, Karnal-132001

Objectives:

- Isolation of microbes from sites polluted with heavy metals.
- Screening of microbes for tolerance to heavy metals.
- Monitoring of polluted sites for heavy metals.
- Microbial removal of heavy metals from wastewater under laboratory conditions.
- Removal of heavy metals from wastewater through immobilized efficient microbes.
- Development of technology for removal of heavy metals from wastewater through efficient microbes.
- Characterization of efficient microbes through biochemical tests and molecular techniques.
- Field testing of efficient microbes for removal of heavy metals from wastewater.

Significant Achievements:

- Fifty three bacterial isolates from sewage and sludge were screened for tolerance to Cr, Cd and Ni along with a few reference isolates from CSSRI, Karnal.
- Isolates B-10,B-11,B-12,B-15, B-18, B-19, B20, B-21, B-23, B-26 showed better tolerance and growth at 50 and 100 ppm of Cr in nutrient broth along with two reference isolates Cr12 and Cr23.
- Isolates B-4, B-5, B-11, B-12, B-20, B-21, B-22 along with reference isolates Ni10 and Ni12 showed tolerance and better growth at 50 and 100ppm of Ni in nutrient broth.
- Isolates B-2, B-11, B-20, B-39 and two reference isolates Cd6 and Cd10 showed better growth and tolerance to Cd at 50 and 100 ppm in nutrient broth.
- Two bacterial isolates B-11 and B-20 were found tolerant to four heavy metal such as Pb, Cd, Cr and Ni.
- Thirty fungal isolates were isolated from sewage, sludge and industrial wastewater and screened them for tolerance to 50 ppm of Pb and Cd.
- The maximum biomass of fungi and uptake of Pb was observed by *A. awamorii*, F2, F3 and FS16, FS29 respectively.
- The maximum biomass and uptake of Cd was observed by *A. awamorii*, FS7, FS9, FS38, FS39 and FS4, FS5, FS6, FS21 respectively.
- Wherever there was more biomass, there was less uptake of Pb and Cd and vice versa
- Heavy metal Cd was found more toxic to growth of fungi as compared to Pb at 50 ppm conc.
- There was higher concentration of heavy metals in samples of sludge than sewage of Karnal, Panipat and Sonepat districts of Haryana
- Thirty six bacterial and 14 fungal isolates were isolated from samples of industrial wastewater; sewage and sludge collected from samples of Industrial wastewater, sewage and sludge were collected from IMT, Manesar in Gurgaon, Faridabad and Yamunanagar.

Bioremediation of Effluents from Shrimp Farms

PI : S. V. Alavandi
Co-PI : T. C. Santiago, N. Kalaimani
Central Institute of Brackishwater Aquaculture, Chennai

Objectives:

- Characterization of efficient bacterial strains involved in nitrogen and sulfur cycles in brackishwater ponds of shrimp farming.
- Biochemical and physiological fingerprinting of promising consortia.
- Formulation of microbes/development of microbial consortia.
- DGGE analysis for genetic stability of biomass, formulation and *in vivo* evaluation of putative probionts.

Significant Achievements:

- A total of 148 isolates inclusive of 23 chemolithotrophic ammonia oxidizing bacteria (AOB), ten chemolithotrophic nitrite oxidizing bacteria (NOB), 22 heterotrophic nitrifying bacteria, and, 60 chemolithotrophic sulfur oxidizing bacteria (SOB) and 33 heterotrophic sulfur oxidizing bacteria have been isolated from brackishwater aquaculture pond sediments (Table 1).
- The heterotrophic nitrifying bacteria have been deposited with NBAIM culture collection and the 16S rDNA sequence data have been submitted to GeneBank.
- The heterotrophic bacteria isolated from the present study could be used as bioaugmentation probiotics for mitigation of ammonia and reduced sulfur compounds in aquaculture ponds.
- The chemolithotrophic AOB, NOB and SOB could be used as inocula for starting biofilters / bioreactors for ammonia removal from aquaculture wastes. These microbes can be also used for developing microbial biofilms or microbial mats for bioremediation of aquaculture wastes.
- A novel mixotrophic bacterium with dual property of NH_3 and thiosulfate oxidation has been isolated.

Conclusion:

The results of the present study revealed high prevalence and diversity of agarolytic bacteria from the aquaculture settings, estuary and coastal regions of south east coast of India. This study focused the presence of Vibrio species such as *Vibrio hepatarius*, *Vibrio fortis* and other bacterial species like *Photobacterium rosenbergii, Alteromonas macleodii* and *A.hispanica* which has not been associated with the agarolytic properties so far. This is the first report shows the presence of these bacterial species in the coastal regions of Indian south east coast. Pure culture of this algae has been cultivated and the identification as *Dunaliella sp* Seven archaebacterium has been isolated form salt pan and identified using 16S rRNA primer which is specific for archaebacterium.

Table: Bacteria involved in nitrogen and sulfur cycles isolated from shrimp culture ponds.

Bacterial Group	No. of Isolates
Chemolithotrophic Ammonia Oxidizing bacteria	23
Chemolithotrophic Nitrite Oxidizing bacteria	10
Heterotrophic Nitrifiers	22
Chemolithotrophic Sulfur Oxidizers	60
Heterotrophic Sulfur Oxidizers	33
Total number of isolates	148

Microbial and Shelf Life Studies During Canning of Vegetables

PI : Sudhir Singh
Co PI : Mathura Rai
Indian Institute of Vegetable Research, Varanasi

Objectives:

- Standardization of blanching treatment in vegetables.
- Optimization of steeping treatment, preservative and heat treatment for destruction of microorganisms.
- Shelf stability of heat-treated vegetables during storage.

Significant Achievements:

- The mean sensory score of onion bulb after steeping in acetic acid solution at pH 2-3 was carried out for flavor, body and texture, color and appearance and overall acceptability score on 9-point Hedonic scale. Judges felt sour taste in onion bulb, which was steeped at pH 2 and 2.25. However, flat flavor was observed during steeping at pH 3.0. However, the most acceptable preference was accorded for the onion bulb, which was steeped at pH 2.5 and 2.75.
- The microbial count in onion bulb during steeping at pH 2.5 varied from 2.3-3.3 x 10^2 cfu/ml, whereas the microbial load was in the range of 3.3-4.9 x 10^2 cfu/ml at pH 2.75 for steeping 1 to 7 days at 30°C with 1% brine solution (Table 2).. The increase in steeping period from 1 to 7 days at pH 2.5 and 2.75 resulted in increased reduction of microbial load from 79.4-85.6% and 69.4-79.4%, respectively
- The period of steeping from 1 to 7 days and acetic acid steeping at pH 2.5 and 2.75 with 2% brine solution significantly reduced the microbial count in onion bulb. The percent reduction in microbial load was of higher magnitude at pH 2.5 than at pH 2.75 at 30 °C.
- The acetic acid steeping at pH 2.5, 1% brine solution and varying levels of preservatives from 100-500 ppm significantly reduced the microbial load. The microbial load was decreased with increase the steeping period from 1 to 9 days. Similarly potassium metabisulfite treatment at 100-500 ppm to onion bulb reduced the microbial count as a result the percent reduction in microbial load was also increased.
- Minimum (79.4%) reduction in microbial load in onion bulb was obtained after steeping for 1 day at pH 2.75, 1% brine solution and 100 ppm potassium metabisulfite with microbial count of 3.3 x 10^2 cfu/ml. However, maximum (93.1%) reduction in microbial load occurred after 9 days of steeping and 300 ppm potassium metabisulfite treatment at pH 2.75.
- The microbial count in onion bulb at pH 2.5 declined with increase the steeping period from 1 to 9 days and with increase the level of preservative from 100 to 500 ppm of potassium metabisulfite. Maximum (0.2 x 10^2 cfu/ml)) decrease in microbial load was evident after 9 days of acetic acid steeping with 300 ppm of potassium metabisulfite treatment whereas minimum (3.1 x 10^2 cfu/ml) decrease in microbial load was obtained after 1 day steeping treatment of acetic acid at pH 2.5 with 100 ppm of potassium metabisulfite.
- The steeping of onion bulb and potassium metabisulfite treatment significantly reduced the microbial load and subsequently the percent reduction in microbial load was increased with increase the steeping period. Maximum (95.1%) reduction in microbial load occurred after 9 days of acetic acid steeping at pH 2.75 after treatment of 300 ppm potassium metabisulfite while minimum (79.4%) reduction in microbial load was obtained after 100 ppm of potassium metabisulfite treatment after steeping for 1 day in 2% brine solution at pH 2.75 in acetic acid solution.
- The steeping of onion bulb in 2% brine solution at pH 2.5 along with 300 ppm KMS resulted in reduction the microbial count. The microbial count decreased with increase the steeping period from 1 to 7 days. The extent of heat treatment during hurdle effect had also significant effect in reduction of microbial load. Minimum (20%) microbial reduction in onion bulb occurred in acetic acid steeping for 1 day in 2% brine solution, 300 ppm KMS and after heat treatment at 100 °C for 2 min. However, acetic acid steeping in 2% brine solution for 7 days with 300 ppm KMS and heat treatment at 100 °C for 2 min could reduce the microbial load to 99%. The heat treatment at 100°C for 2, 4 and 5 min also had significant effect in the reduction of D-value in onion bulb. Minimum reduction in D-value was obtained at 100 °C for 2 min. in hurdle effect of onion bulb and maximum D- value reduction occurred in onion bulb after heat treatment at 100°C/ 5 min.
- Microbial reduction in onion bulb with hurdle effect at pH 2.5 and 2.75 and heat treatment at 100 °C for 3 and 5 min decreased (98-99%) during storage of onion bulb from 15 to 60 days. The reduction in microbial load was in onion bulb decreased at pH 2.5 as compared to

pH 2.75. The reduction in D-value was maximum (1.58-1.0) in onion bulb at pH 2.5 with 2% brine solution, 300 ppm KMS and heat treatment at 100 °C for 3 min.

- Sensory score of acetic acid steeped onion bulb (pH 2.5), 2% brine solution, 300 ppm KMS and heat treatment at 100 °C for 3 min was preferred with maximum sensory score for flavour, body and texture, colour and appearance and overall acceptability score. However, the sensory score of onion bulb with hurdle effect at pH 2.75 and heat treatment at 100 °C for 5 min was least accepted to judges.
- The microbial count in onion paste at pH 2.5 and KMS preservative (100 – 300 ppm) declined with increase the steeping period from 1-7 days. The maximum (96.8%) decrease in microbial population was obtained in onion paste after 300 ppm of KMS and steeping for 7 days, while minimum (84.5%) decrease was attained in onion paste after steeping of 100 ppm KMS for 1 day.
- Maximum (99%) reduction in microbial population in onion paste was obtained at pH 2.5 with 300 ppm KMS preservative and steeping for 1-7 days. However, minimum (91.6 – 99.7%) reduction in microbial load was obtained with 100 ppm KMS at pH 2.5.
- Maximum (99%) reduction in microbial population in onion paste was obtained at pH 2.5 with 300 ppm KMS preservative and steeping for 1-7 days. However, minimum (91.6 – 99.7%) reduction in microbial load was obtained with 100 ppm KMS at pH 2.5.
- Microbial count in onion paste had declining trend with increase the steeping period from 1-7 days and KMS level from 100-300 ppm. Maximum (99.85) reduction in microbial population occurred after treating with 300 ppm of KMS for 7 days of steeping in onion paste, while minimum (91.6%) reduction in microbial population was obtained after 100 ppm of KMS and steeping for 1 day in onion paste.
- The microbial count in onion paste declined with heat treatment at 100 °C to 3 and 5 min with increase the steeping period from 1-7 days. Maximum (1.15 -0.69) decrease in D-value was obtained in onion paste after treatment with 2% brine solution, 300 PPM KMS at pH 2.5 and heat treatment at 100 °C for 5 min while minimum (1.15 – 1.30) decrease in D-value was attained in onion paste after heat treatment at 100 °C for 3 min in 2% brine solution at pH 2.75, 300 ppm KMS and steeping for 1-7 days.

Conclusion:

Acetic acid steeping (pH 2.5 – pH 2.75) and brine solution (1-2% sodium chloride) treatment for 1-6 days significantly reduced the microbial count in onion bulb and paste. However, potassium metabisulfite preservative at 300 ppm level during steeping with acetic acid at pH 2.5 for 1 day reduced the microbial count to 90%. Microbial reduction in onion bulb with hurdle effect at pH 2.5 and 2.75 and heat treatment at 100 °C for 3 and 5 min decreased (98-99%) during storage of onion bulb from 15 to 60 days. The reduction in D-value was maximum (1.58-1.0) in onion bulb at pH 2.5 with 2% brine solution, 300 ppm KMS and heat treatment at 100 °C for 3 min.

Fermented Products from Fruits, Vegetables and Cereals

PI : S. Gunasekaran
Co-PI : R. Murugesan; K. Vijila; S. Karthikeyan
Department of Agricultural Microbiology, TNAU, Coimbatore

Objectives:

- Isolation of microorganisms from fruits, vegetables and other agricultural wastes for the production of pigments, enzymes, and nutraceuticals
- Evaluation and screening of best strains for the production of enzymes (cellulase, amylase), pigments and nutraceuticals
- Selection of suitable cultures for rice wine fermentation

Significant Achievements:

- Forty isolates of LAB were obtained from different agro sources like soyabean, banana and maize. In screening of the LAB isolates for sucrose splitting ability, the two isolates LAB 18 and LAB 16 recorded highest values of 1.35 and 1.33 of glucose released per litre of crude extract.
- Purification of fructosyl oligosaccharide transferase enzyme system of the efficient isolates by ammonium sulphate precipitation showed that LAB 16 exhibited highest enzyme activity of 1.04 g of glucose released per hour at 20% saturation level.
- Fungal isolates exhibiting pectinolytic activity were isolated from citrus peel. Based on morphological and cultural characterization the fungal isolates were identified as *Aspergillus niger, Aspergillus flavus* and *Fusarium* sp., The fungal isolate *Aspergillus foetidus* (MTCC) recorded highest pectin esterase and polygalacturonase activity
- Five bacteria and four fungal cellulase producing microorganisms were isolated from agricultural wastes. The bacterial cultures are characterized as *Bacillus* sp and the fungal cultures as *Penicillium* sp, *Aspergillus* sp, *Fusarium* sp and *Streptomyces* sp
- A green pigment producing yeast culture was isolated and its stability fermentation
- Parameters (pH, Temp, C and N) for pigment production were studied.
- The dark brown color producing fungal culture was identified as *Epicoccum* sp., (TNAU-PF2)
- The Pink colour producing fungus was identified as *Fusarium* sp.,(TNAU-PF1)
- The red colour producing fungus was identified as *Penicillum* sp., (TNAU-PP1)
- The yellow pigment producing fungi has yet to be identified.
- The human pathogens were poisoned in media using swab and the pigment culture were spotted in plate and the inhibition was observed. The yellow pigment producing fungi inhibited only in *Staphylococcus aureus* (2.4cm). The yeast inhibits the growth of *Salmonella typhi* (3.4cm). The *Penicillium sp* (red pigment) inhibit the growth of *Cryptococcus* (2.0cm) and *Staphylococcus aureus* (3.2cm) and the *Epicoccum sp*. (brown pigment), culture inhibit the growth of *Cryptococcus* (2.1cm) and *Streptococcus* (2.1cm) but the *Fusarium* (pink pigment) did not inhibit any pathogens
- Alcohol content of rice varieties vary significantly according to the storage periods.
- Rice wine production was compared with microbial consortia of *Aspergillus oryzae* and *Rhizopus oryzae* with enzyme application. Compared to microbial consortia application, enzyme addition to the rice sample (fungal diastase and α-galactosidase) had enhanced the starch conversion. Also the time taken for alcohol conversion is also reduced. Within 15 days of time about 13.0 per cent of alcohol could be recovered

Conclusion:

Microbial isolates were obtained from different agro sources like soyabean, banana and maize with highest values of 1.35 and 1.33 of glucose. Enzyme fructosyl oligosaccharide transferase enzyme was purified. Fungal isolates exhibiting pectinolytic activity were isolated from citrus peel. The bacterial cultures are characterized as *Bacillus* sp and the fungal cultures as *Penicillium* sp, *Aspergillus* sp, *Fusarium* sp and *Streptomyces* sp. A green pigment producing yeast culture was isolated and its stability fermentation. Dark brown, pink and red color producing fungal culture was identified as *Epicoccum* sp., *Fusarium* sp. and *Penicillum* sp., respectively.

Alcohol content of rice varieties vary significantly according to the storage periods. Compared to microbial consortia application, enzyme addition to the rice sample (fungal diastase and α-galactosidase) had enhanced the starch conversion. Within 15 days of time about 13.0 per cent of alcohol could be recovered

Utilization of Mango Processing Waste for Obtaining Value Added Products through Fermentation

PI : Neelima Garg
Division of Post-harvest Management, Central Institute for Subtropical Horticulture, Lucknow

Objectives:

- Evaluate the potential use of different mango processing waste for production of pectinases, cellulases and amylases.
- Screening of micro organisms and optimisation of fermentation conditions.
- Molecular characterization of potent microbial isolates.
- Strain improvement using molecular tools for higher production of enzymes.
- Large scale production of desired end product and to work out the economic feasibility.

Significant achievements:

- Pectinolytic, amylolytic and cellulolytic microorganisms were isolated from pectin, starch and cellulose rich degrading organic matter respectively.
- Among forty one microbial isolates tested, after primary and secondary screening, highest pectinase production (0.099 mM/ml/min) was observed by *Aspergillus fumigatus*.
- Among five concentrations of mango peel viz. 1, 3, 5, 7 and 10 per cent tested, highest polygalacturonase production (specific activity 209.81U/gm) were observed at 5 per cent concentration. Addition of nitrogen as ammonium sulphate (1.5%) had a positive effect (213.61U/g) while addition of Phosphorus as O-Phosphoric acid had a negative effect on pectinase production. The maximum pectinase production was observed at 30^{0}C (216 U/g). Highest polygalacturonase (215.69 U/g), pectin methyl esterase (8.33 U/g) were observed at pH 5. Higher pectinase production (274.57U/g) was observed under aerated conditions (Fig 4)
- Solid state fermentation revealed a production of pectinase 274.51 U/g, with pectinase activity 245.05 U/g. pH 5 and temperature 30 °C were found optimum for pectinase activity (107.58 U and 178.03U respectively). Km and Vmax for pectinase 6.135 mg/ml and 0.121mM/min.
- Among forty six microbial isolates tested, after primary and secondary screening, highest cellulase production (0.010256µM/ml/min) was observed for C8 which was identified as *Bacillus sp*.
- Conditions have been optimized for cellulase production from mango peel using *Bacillus* isolate under submerged fermentation conditions. It has been observed that addition of nitrogen in the form of ammonium sulphate increases the production (0.025864 U/mg). After partial purification (precipitation) specific Activity increases to 0.102944U/mg. The maximum cellulase activity (0.014103µM/ml/min) was observed at 30 °C. Highest cellulase (0.015812 µM/ml/min) activity was observed at pH 5. The Km and Vmax value for cellulase was found 8.726 mg/ml and 17.805 mM/ml/min, respectively.
- Among the 27 amylolytic cultures, after primary and secondary screening isolate no. S-4, identified as *Pseudomonas sp*. showed maximum amylase production (0.036µM/ml/min) using mango kernel as substrate.

Conclusion:

Several pectinolytic, amylolytic and cellulolytic microorganisms were isolated from different fruit watses. Enzymes such as pectinase, amylase and cellulase were purified from these isolates and process parameters for their economic recovery were standardized for solid state fermentation. Molecular characterization of the selected isolates for strain improvement at genetic level will be carried out.

Theme 4: Microbial Management of Abiotic Stress

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

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Co-PI : Rajeev Kaushik
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Objectives:

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

Significant Achievements:

- Twenty four isolates reported earlier to be salt tolerant were analyzed for their growth kinetics using Nutrient broth. The generation time (gt) for each isolate at different salt concentration was calculated. In general with the increase in salt concentration, the gt increased for all isolates. Some of the isolates that could grow on plates up to 10% NaCl failed to grow in broth with 10% NaCl (Fig 1).
- In order to look for the expression or repression of certain proteins in the presence of salt stress, protein profiles were developed. The results revealed that certain proteins are expressed in presence of salt stress, while other proteins are repressed at high salt concentration. For certain proteins even the expression level was different at different salt concentrations (Fig 2).

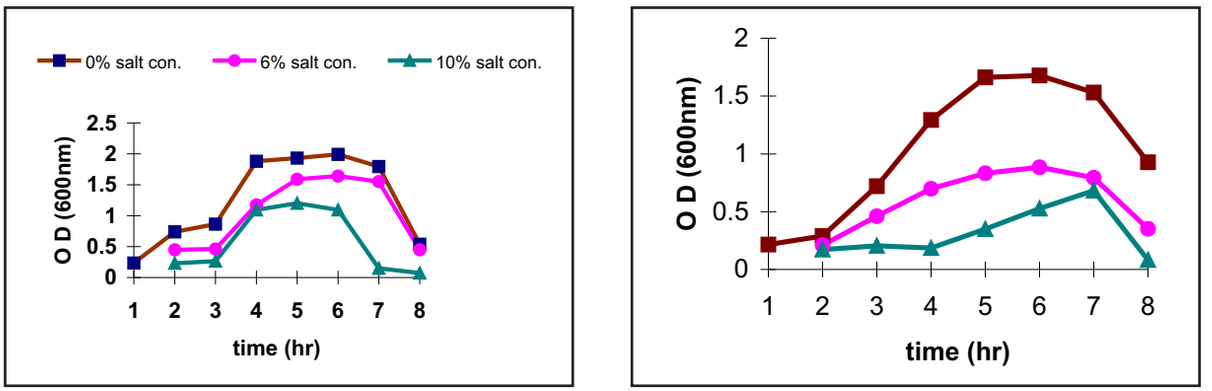

Fig 1: Growth kinetics of bacterial isolates 16 and 3 at different salt concentrations

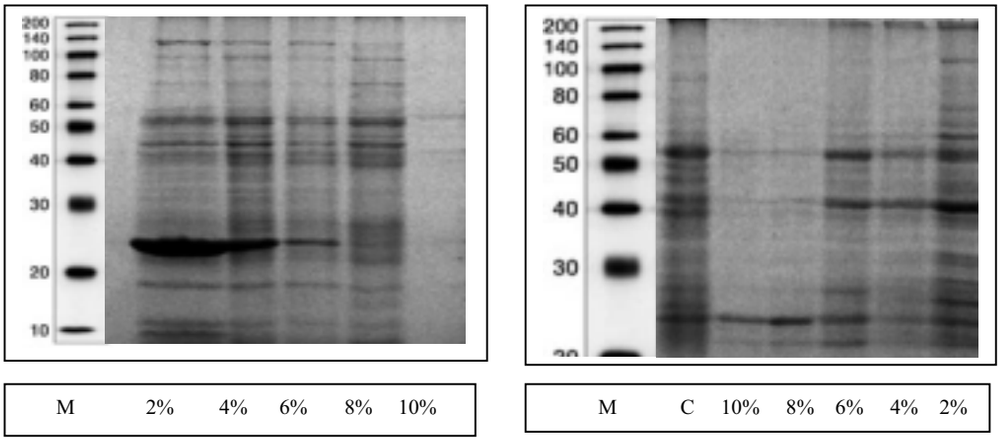

Fig 2: Protein profile of bacterial isolates grown at different salt concentration

- Nine isolates that performed better in the pot experiment were selected for field trial. A field experiment was conducted in RBD design with three replications and ten treatments including untreated control. The observations were made after 60, 90 DAS and at maturity. The grain yield and straw yield was recorded.
- The influence of different isolates on different plant parameters after 60 and 90 DAS are shown in table 1. Inoculations with isolate 44 produced significantly higher root dry weight whereas isolate N60 gave maximum shoot biomass both after 60 and 90DAS.
- Proline, reducing sugars (RS) and total soluble sugar (TSS) level was significantly higher for all treatments at 60 DAS as compared to 90DAS. Inoculation with isolate N60 induced significantly higher proline and TSS accumulation in plants where as isolate 44 resulted in higher accumulation of RS (Table 1).
- Grain yield and total biomass was significantly influenced by the inoculation. Isolate 121 was found to be the best and gave a 23 % increase in grain yield over control.

Conclusion:

Nine isolates that were tolerant to salt and could exhibit growth promoting attributes like production of IAA and solubilization of phosphorus at 6% salt concentration were evaluated in the field. Isolates N60 and 44 although could influence the vegetative parameters and physiological parameters but isolate 121 was found to gave the maximum grain yield. Inoculation with microorganisms can help to reduce the effect of salt stress and stimulate the growth and yield of wheat. These isolates that performed well will be utilized further to develop a consortium that can be recommended for inoculation in saline soils.

Table 1: Wheat (Variety K7903) growth and physiological parameters as influence by bacterial inoculating after 60 Days and 90 Days

Treatment	Plant length (cm)		Root Dry Wt (gm)		Shoot Dry Wt (gm)		Proline, µg ml^{-1}		RS, µg ml^{-1}		TSS, µg ml^{-1}	
	60 d	90 d	60 d	90 d	60 d	90 d	60 d	90 d	60 d	90 d	60 d	90 d
Control	42.16	59.5	1.52	3.47	4.14	16.63	0.722	0.913	160.05	87.52	128.88	121.41
18(N-12)	41.6	73.9	1.424	2.95	4.512	10.69	0.816	1.040	159.09	104.6	144.13	120.68
121(Azosp)	41.18	77.5	1.358	4.67	3.5	16.60	1.485	0.847	203.68	65	157.66	154.52
40(B-28)	46.2	60.5	1.338	4.01	4.084	16.70	1.179	0.764	187.22	85	166.59	142.03
24(B37)	49.93	73	1.994	4.14	4.594	23.50	0.845	0.630	151.06	88.48	120.15	114.59
47(B41)	51.6	82	1.598	3.57	4.768	15.83	0.966	0.840	230	52.77	250	174.48
8(N-60)	47.66	83	1.926	4.08	6.474	20.43	2.156	0.831	203.18	128.1	234.44	154.13
13(N72)	43.8	73	1.172	4	3.616	19.60	0.612	1.187	159.34	122.62	118.88	120.8
44(6A)	46.86	55	2.062	5.26	4.534	12.29	0.529	0.985	224.79	102.62	167.31	170.5
3(N48)	48.9	64.5	1.094	3.34	4.714	10.76	0.970	0.964	138.28	88.99	127.47	104.9

Table 2. Grain yield and Total biomass yield as influenced by bacterial inoculation

Treatment	Grain Yield Kg/ha	Total Biomass Kg/ha
Control	2239	5104
18 (N-12)	2416	4833
121 (Azospirillum)	3020	7291
40 (B-28)	3031	7500
24 (B37)	2906	7708
47 (B41)	2687	7187
8 (N-60)	2666	5729
13 (N72)	2281	6333
44 (6A)	2302	5104
3 (N48)	2958	7145

Isolation, inventorization and field assessment of agriculturally important microorganisms in the stress ecosystems of Karnataka

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Objectives:

- Isolation, enumeration, characterization and inventorization of AIMs (N_2 fixers, P-solubilizers, PGPRs, fluorescent pseudomonads, cellulose and lignin degraders) from saline/salt affected soils, water logged areas and dry tracts of Karnataka,
- Assessing the functional potential of each group of organisms for use in agriculture,
- Setting up the culture bank of the potential isolates under each group and deposit them with the NBAIM,
- Field testing of potentially efficient isolates/consortia of isolates (based on lab/ green house studies.

Significant Achievements:

- Soil, rhizosphere, root, leaf and decaying residue samples were collected from the salt affected and saline patches, waterlogged areas and dry areas of Bagalkot, Bijapur, Chitradurga, Gulbarga, Gadag, Mandya, Mysore and Kodagu districts and used for isolation of salt tolerant organisms of agricultural importance. The isolates were subjected to biochemical and physiological characterization in addition to their characterization with respect to salt tolerance, mechanisms of salt tolerance and functional properties under salt stress conditions.
- One hundred seventy five samples consisting of 83 rhizosphere soil samples, 5 non-rhizosphere soils, 75 root samples, 4 leaf samples and 8 decaying residue samples were collected from 77 locations of problematic soils covering 8 districts of Karnataka for isolation of agriculturally important microorganisms.
- One hundred seventy five samples consisting of 83 rhizosphere soil samples, 5 non-rhizosphere soils, 75 root samples, 4 leaf samples and 8 decaying residue samples collected from 77 locations of problematic soils covering 8 districts of Karnataka were used for isolation of agriculturally important microorganisms.
- The samples yielded 387 isolates consisting of 90 *Azotobacter*, 68 *Azospirillum* (all from endorhizosphere), 149 P-solubilizers (82 PSB and 59 PSF), 75 fluorescent pseudomonads, and 3 PPFMs (all form leaf samples) and 10 lignin degraders (Table 1a-1d).

Table 1a. Summary of isolates obtained from salt affected soils.

S. No.	Source	Azoto.	Azosp	PSB	PSF	Fluor. Pseud.	PPFM	Lignin Degr.	Total
1	Soil (NR)	1	--	2	--	--	--	--	3
2	Rhizosphere (RS)	28	--	28	30	33	--	--	119
3	Rhizoplane	30	--	19	20	23	--	--	92
4	Endorhizosphere	19	57	16	5	10	--	--	107
5	Leaf	--	--	--	--	--	3	--	3
6	Decomposed material	--	--	--	--	--	--	8	8
	Total	78	57	65	55	66	3	8	332

Table 1b. Summary of isolates obtained from arid soils.

S. No.	Source	Azoto.	Azosp	PSB	PSF	Fluor. Pseud.	PPF M	Lignin Degr.	Total
1	Soil (NR)	--	--	--	--	--	--	--	--
2	Rhizosphere (RS)	1	--	5	--	--	--	--	6
3	Rhizoplane	3	--	2	1	3	--	--	9
4	Endorhizosphere	1	5	2	--	--	--	--	8
5	Leaf	--	--	--	--	--	--	--	--
6	Decomposed material	--	--	--	--	--	--	--	--
Total		5	5	9	1	3	--	--	23

Table 1c. Summary of isolates obtained from water logged soils.

S. No.	Source	Azoto.	Azosp	PSB	PSF	Fluor. Pseud.	PPF M	Lignin Degr.	Total
1	Soil (NR)	--	--	--	--	--	--	--	--
2	Rhizosphere (RS)	1	--	6	1	3	--	--	11
3	Rhizoplane	4	--	--	2	3	--	--	9
4	Endorhizosphere	2	6	2	--	--	--	--	10
5	Leaf	--	--	--	--	--	--	--	--
6	Decomposed material	--	--	--	--	--	--	2	2
Total		7	6	8	3	6	--	2	32

Table 1d. Summary of isolates obtained from problematic soils from different regions.

S. No.	Sample collected from	Azoto.	Azosp	PSB	PSF	Fluor. Pseud.	PPF M	Lignin degr.	Total
1	Salt affected	78	57	65	55	66	03	08	332
2	Arid	05	05	09	01	03	--	--	23
3	Water-logged	07	06	08	03	06	--	02	32
Total		**90**	**68**	**82**	**59**	**75**	**03**	**10**	**387**

- The salt tolerant isolates obtained during the period include 81 *Azotobacter* (tolerating 1.5 – 5% NaCl), 10 *Azospirillum* (5% NaCl), 78 PSB (6 tolerating 15% NaCl, 17 at 12.5%, 32 at 10% and 23 at 5-7.5% NaCl), 53 PSF (1 at 6% NaCl, 3 1t 12.5%, 14 at 10% and 35 at 5-7.5% NaCl) and 63 fluorescent pseudomonads (1 at 12.5% NaCl, 25 at 105, 18 at 7.5% and 19 at 5% NaCl).
- Biochemical and physiological characterization of 53 fluorescent pseudomonads and 131 PSB isolates, *Azotobacter* and *Azospirillum* isolates tolerating different concentrations of NaCl has been completed and the identification of these isolates is in progress.
- Out of 90 isolates of *Azotobacter*, 13 were able to grow at 5% NaCl, 57 isolates at 3% NaCl and 81 at 1.5% NaCl in Norris N-free medium.
- Thirteen isolates showing tolerance to 5% NaCl were further examined for N_2 fixation and for proline and total sugar contents. Nine isolates showed higher N_2 fixation in control medium (without additional salt) whereas four isolates, viz., DR31R, S174E, S178E and S190S showed higher N_2 fixation in the presence of 5% NaCl (Fig 1). Isolate DR31R showed almost four-fold increase in N_2 fixation in the presence of 5% NaCl over control medium.
- While 3 of these isolates, viz., S174E, S178E and S190S recorded higher proline and total sugar (Table 2) at 5% NaCl, DR31R showed lower proline and total sugar at 5% NaCl. Most other isolates showed higher proline and total sugars in the presence of 5% NaCl.

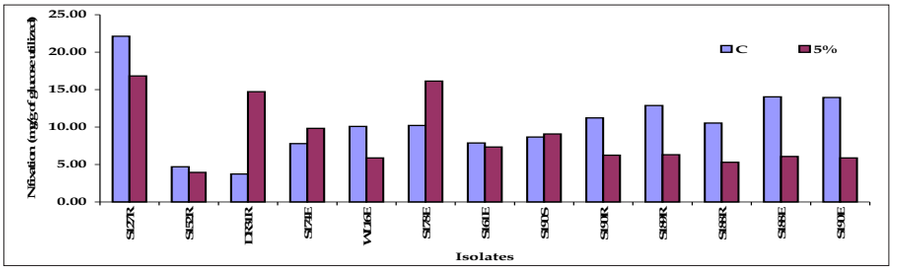

Fig 1. Nitrogen fixation by *Azotobacter* isolates

Table 2. Proline and total sugar production (µg/ml) by salt tolerant *Azotobacter* isolates

Isolates	Proline production (µg/ml)		Total sugar production (µg/ml)	
	Control medium	Medium with 5 % NaCl	Control medium	Medium with 5 % NaCl
S127R	0.13	0.59	0.55	0.46
S152R	0.03	0.43	0.54	1.14
DR31R	1.31	0.16	1.83	1.04
S174E	0.10	0.36	0.33	0.58
WL16E	0.36	0.56	0.19	0.17
S178E	0.13	0.62	0.29	1.29
S161E	0.23	0.92	0.26	1.57
S190S	0.75	2.66	0.47	0.84
S190R	0.62	0.23	0.28	2.04
S189R	1.11	0.26	0.75	0.28
S188R	2.26	0.36	0.64	1.03
S188E	0.10	0.39	2.18	2.72
S190E	1.64	0.13	0.44	0.70

- Thirty Eight *Azospirillum* isolates obtained during 2006-07 were screened for salt tolerance in Okon's medium with and without added N source. In Okon's medium with N source, 6 were able to grow at 5 % NaCl and two at 7.5 % NaCl. However, in N-free Okon's medium, 10 isolates were able to grow at 5 % NaCl. The isolate S22E was able to tolerate up to 7.5 % NaCl in Okon's medium with N source, and up to 5 % NaCl in N-free Okon's medium.

proline production but there was not much difference in the amount of proline produced by the isolates in the presence or absence of added NaCl.

- Seventy Eight P-solubilizing bacteria capable of growing at 5–15% NaCl in Pikovskaya's agar were isolated. Out of these, 6 isolates capable of growing at 15% NaCl and 20 isolates capable of growing at 10% NaCl were examined for their TCP solubilizing ability at different salt concentrations. Among 6 isolates

Table 3. Nitrogen fixation (mg/g malate) by salt tolerant *Azospirillum* isolates

Isolates	Control medium	Medium with 5 % NaCl
S22	7.3	4.2
S26	5.8	2.3
S40	8.1	5.78
S61	9.01	6.21
S63	4.26	3.02
S78	5.09	4.11
S83	4.01	2.12
DR8	4.88	2.16
DR18b	5.80	3.00
WL12	3.65	1.48

- Fourteen salt tolerant *Azospirillum* isolates were examined for their ability to fix N_2 which generally showed less N_2 fixation in the presence of added NaCl. The amount of N_2 fixed by the salt tolerant isolates ranged from 1.48 to 6.21 mg/g malate utilized in the presence of 5% NaCl as compared to 3.65 to 9.01 mg/g malate without additional NaCl (Table 3).
- These 14 salt tolerant isolates were also examined for

tolerating 15% NaCl, S122R recorded 2-3 fold increase in the amount of Pi released with increase in NaCl concentration up to 15%, whereas other 5 isolates showed decrease in the amount of Pi released with increase in NaCl concentration (fig 2). Similarly, among 20 isolates tolerating 10% NaCl, S138(1)S, WL19E, S160S and S175E showed higher TCP solubilization in the presence of 7.5 and 10% NaCl.

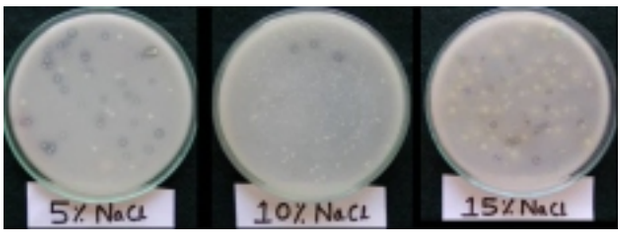

Fig 2. Salt tolerant PSB isolates showing TCP solubilization at 5-15% NaCl.

- As for the proline production by the isolates tolerating 15% NaCl, the isolate S122R which recorded 2-3 fold increase in the amount of Pi released with increase in NaCl concentration did not show proline production up to 7.5% NaCl but thereafter showed increase in proline with increasing salt concentration. Other 5 isolates showed higher proline at 7.5 – 12.5% NaCl.
- All 31 P-solubilizing fungi isolated from the problematic soils were examined for growth and P-solubilization on Pikovskaya's agar in the presence of different concentrations of NaCl. Out of 31 isolates, 30 were able to grow at 5% NaCl, 28 at 10 % NaCl, 20 at 12.5 % NaCl and 3 at 15 % NaCl. None were able to grow at 17.5 and 20 % NaCl. However, as far as P-solubilization was concerned, only 3 isolates viz., WL9S, DR12(b)R and S114S were able to form zones of P-solubilization on Pikovskaya's agar containing 5 % NaCl. These three isolates were further examined for solubilization of TCP in Pikovskaya's broth containing 5 % NaCl. While all three isolates showed reduction in P-solubilization in the presence of 5 % NaCl, the extent of reduction was greater in DR12(b)R and S114S as compared to WL9S which showed considerable amount of Pi release even at 5 % NaCl.
- Fifty-three isolates of fluorescent pseudomonads isolated during 2006-07 were examined for growth and P-solubilization in Pikovskaya's agar during the reporting period. While all 53 were able to grow on Pikovskaya's agar medium, only 26 were able to form zone of solubilization. Out of these 26 isolates, 19 were able to form zone of solubilization at 5 % NaCl, 12 at 7.5 % NaCl and none at 10 % NaCl and above (Table 16 and Plate 2).
- Twelve isolates which showed zone of solubilization on Pikovskaya's agar containing 7.5 % NaCl were also examined for the amount of Pi released from TCP in the presence of 5 and 7.5 % NaCl. Out of 12 isolates, S4(1)S showed continuous increase in TCP solubilization with increase in NaCl concentration whereas 5 isolates showed continuous decrease in TCP solubilization with increase in NaCl concentration (Table 17). Of the remaining 6 isolates, 3 showed higher solubilization of TCP only at 5 % NaCl whereas other 3 isolates showed higher solubilization of TCP both at 5 and 7.5 % NaCl over the medium without added NaCl.
- Thirty-four isolates of salt tolerant fluorescent pseudomonads were examined for proline production at different concentrations of NaCl added to King's B medium. The isolates showed variation to produce proline at different salt concentrations, but many isolates showed higher proline in the presence of salt (Table 17). Among the 3 isolates which showed salt tolerance up to 17.5 % NaCl, two isolates showed higher proline production up to 12.5 % NaCl. Similarly, two isolates which showed salt tolerance up to 15 % NaCl, showed continuous increase in proline production.
- Growth chamber experiments have been initiated to study the inoculation effect of salt tolerant isolates of each group on growth of sorghum and wheat plants in soil with graded levels of EC. The experiments are in progress.

Conclusion:

The survey work has been completed by collecting 175 samples of soil, rhizosphere soil, roots, leaves and decaying residue collected from 77 locations of problematic soils covering 8 districts of Karnataka. The samples yielded 285 salt tolerant organisms consisting of 81 *Azotobacter* (tolerating 1.5–5% NaCl), 10 *Azospirillum* (5% NaCl), 78 PSB (5-15% NaCl), 53 PSF (5-15% NaCl) and 63 fluorescent pseudomonads (5-12.5% NaCl). 4 *Azotobacter* isolates showed higher N_2 fixation at 5% NaCl; isolate DR31R showed four-fold increase in N_2 fixation at 5% NaCl over control. Ten *Azospirillum* isolates showed N_2 fixation at 5% NaCl. Among 6 PSB isolates tolerating 15% NaCl, S122R recorded 2-3 fold increase in the amount of Pi released with increase in NaCl concentration up to 15%. Twenty six fluorescent pseudomonads possessed P-solubilizing ability at 5 - 7.5 % NaCl. Isolate S4 (1) S showed continuous increase in TCP solubilization with increase in NaCl up to 7.5 %, whereas 3 showed higher solubilization of TCP only at 5 % NaCl. The salt tolerance mechanisms in the isolates appear to be proline production and sugar accumulation.

Development of microorganism consortium to alleviate abiotic stresses like drought, high temperature and salinity in millets

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Co-PI : S. K. Yadav
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Objectives:

- To isolate microorganisms from rhizotic zones of millets grown under stress conditions of salt, drought and extreme temperatures
- Biochemical characterizations and testing of these organisms for their response to drought, high temperature and salt stress under *in vitro* conditions.
- Evaluation of promising strains on millet crops (pearl millet and finger millet) in pot culture conditions.
- Development of consortium of microorganisms and field evaluation for improvement of growth and yield of millets under the above mentioned stresses.

Significant Achievements:

- Ninty five isolates of *Pseudomonas* and thirty-four of *Bacillus* were tested for their survival ability at different levels of drought and temperature stress. The number of strains tested and those survived at different levels of drought and higher temperatures are given below. With regard to temperature, there was a gradual decrease in the number of strains survived with increasing temperatures and at 50 °C ten strains of *Pseudomonas* and nine strains of *Bacillus* survived out of 95 and 34 tested, respectively. Where as under drought conditions 8 *Pseudomonas* and 8 *Bacillus* were survived at 25% and 15% PEG.
- Out of the tolerant strains of *Pseudomonas* and *Bacillus* found in the above experiments, the EPS production (grown on under normal medium without any additional carbon source) was detected (Fig. 1) and quantified for all the tolerant strains of *Pseudomonas* and *Bacillus* under drought and temperatures stresses. The EPS production enhanced significantly under stress conditions in all the strains.
- Inoculation with abiotic stress tolerant *Pseudomonas* strain helped in better survival and growth of sorghum seedlings at ET. Even at this high temperature, seeds in all treatments germinated successfully. However, in control (un-inoculated) treatment, the growth of the seedlings was stunted and by two weeks all seedlings died, while the treated (inoculated) seedlings continued to grow up to 15 days. The seedlings continued to be healthy and showed marked growth with strain P6 and P7 (Fig 2a). Treated seedlings produced significantly higher root and shoot length and seedling biomass (Fig 2b). The electron micrographs of the inoculated roots with the best strains P6 has been prepared for further evidence (Fig 3) The results indicates that stress tolerant *Pseudomonas* helping sorghum seedlings to grow successfully under elevated temperatures

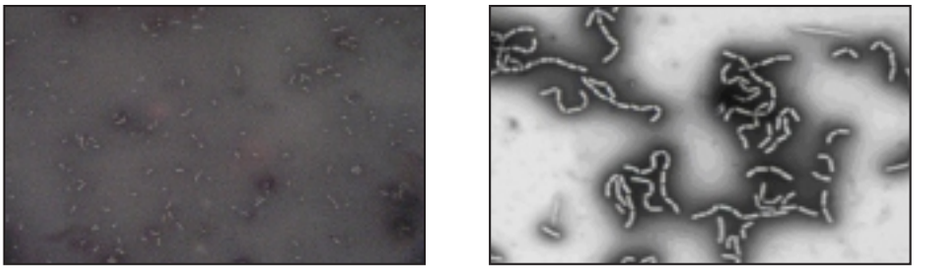
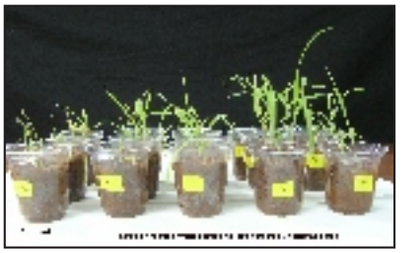


Fig 1. Negative staining for EPS detection left (*Pseudomonas*) and right (*Bacillus*).



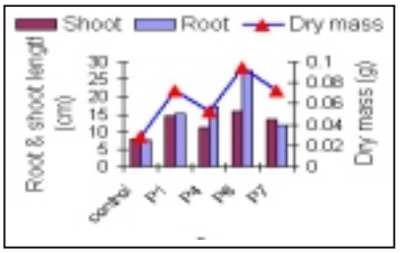


Fig 2a &b: Effect of seed treatment with stress tolerant *Pseudomonas* strains on growth and survival of sorghum seedlings at elevated temperatures of 50 °C.

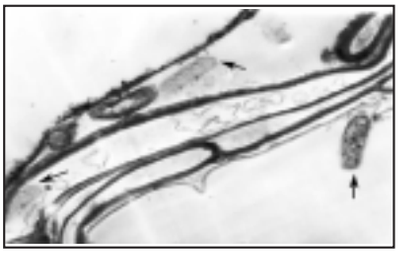


Fig 3. Root section of sorghum seedlings under TEM inoculated with *P. putida* (P_6), showing the presence of bacterial cells in side the roots

- Similar experiment was carried out with the best strain P6, wherein sorghum seedlings were grown in non-sterile soil with control and inoculated treatments. The results indicate that strain P6 showed better growth of sorghum seedling under elevated temperature even in non sterile conditions by over coming the activities of other soil microorganisms
- Another preliminary paper cup experiment was carried out wherein Pearl millet seedlings were grown in sterile soil with control and inoculated treatments (with 10 strains of Pseudomonas separately). In control (uninoculated) treatment, the growth of the seedlings was stunted and all the seedlings died four days after transfer to elevated temperature, while the treated (inoculated) seedlings continued to grow up to 15 days. The seedlings continued to be healthy and showed marked growth with strain P6 and P7 and P46.

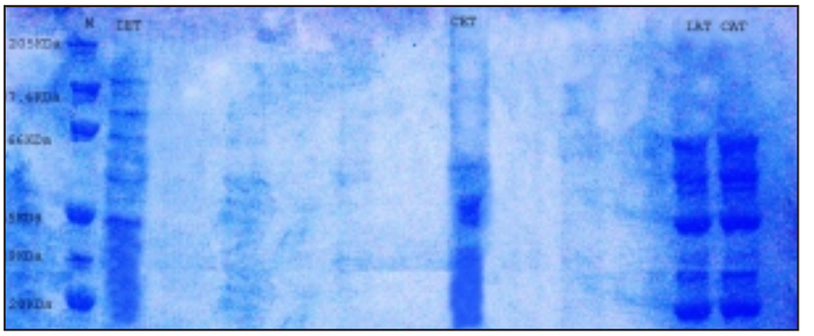


Fig 4. High molecular weight proteins induced in leaves of sorghum seedlings grown at elevated temperatures and inoculated with *P. putida* (P_6). (CET= Control at elevated temperature; CAT= Control at ambient temperature; IET= Inoculated at elevated temperature; IAT= Inoculated at ambient temperature; M= Marker)

Treated seedlings produced significantly higher root and shoot length and seedling biomass.

- Inoculation helped in maintenance of normal protein and carbohydrate metabolism in seedlings exposed to ET. Total proteins and carbohydrate levels decreased due to high temperature exposure but inoculation helped the plants to maintain normal levels as that of seedlings grown at ambient conditions. Free proline was higher in inoculated plants exposed to ET, which was not detected at ambient conditions.
- Three high molecular weight proteins between 100-60Kda were detected in leaves of inoculated seedlings, which were absent in the control plants (Fig 4). Such new proteins were also absent in the seedlings grown at ambient condition. Inoculation also reduced the membrane injury as measured by electrolyte leakage.
- The activity of antioxidant enzymes like SOD, APX and POX were significantly lower in inoculated plants exposed to ET as compared to control. Activity of these enzymes was insignificant in plants grown at ambient conditions
- Soil samples from different locations collected were analyzed for the presence and distribution of AM fungi. A total of 12 soil samples were analyzed. It was observed that all soil samples harbored at least three genera of mycorrhiza, out of which *Glomus* species was found more frequently with more number of species.
- Genus Entropospora was observed only in Andhra Pradesh and Rajasthan soils, similarly Scutellospora was observed in soils from Andhra Pradesh, Gujarat and Madhya Pradesh Number of spores was as high as 150 per 50g of soil sample taken from Andhra Pradesh. Interestingly it was observed that at least two genera of *Glomus* was observed in all soils analyzed

Conclusion:

From millet growing regions of 11 states covering arid/semi arid regions, 129 strains of AIMS were isolated and characterized. Promising isolates of drought and high temperature stress (beyond 25% PEG and 50°C) were identified. Preliminary studies indicate that the EPS production enhanced significantly under stress conditions in all the strains. Seed bacterization with stress tolerant strains of *Pseudomonas* (strain P6) helped sorghum and pearl millet seedlings to survive at 50°C up to 21 days. The strain P6 was characterized and identified as *Pseudomonas putida*. Seed inoculation also induced synthesis of a novel high molecular weight protein. Role of this protein in offering protection to seedlings against abiotic stresses is being investigated. Less electrolyte leakage in inoculated plants suggested protection of membrane integrity of cell by bacterium. Inoculation also reduced the oxidative stress in seedlings exposed to high temperature (50 °C) as evidenced by significantly lower anti oxidative enzyme activity in treated seedlings. The electron micrograph of the sorghum roots inoculated with P6 strain indicated the entry of the organism inside the roots.

Development of a bacterial consortium to alleviate cold stress

PI : Pankaj Kumar Mishra
Co-PI : G. Selvakumar
Vivekananda Parvatiya Krishi Anusandhan Sansthan (ICAR), Almora, Uttarakhand

Objectives:

- To isolate cold tolerant bacteria from the rhizosphere of various hill crops and screen them under *invitro* cold conditions for their PGPR activity.
- To develop comprehensive biomarkers for the quality control and field detection of the elite PGPR isolates.
- To develop a consortium of elite bacterial isolates to alleviate the effect of cold stress.
- To evaluate the performance of consortium on selected *rabi* crops under pot culture conditions.

Significant Achievements:

- Six elite isolates were deposited at NBAIM, Mau and GenBank accession number obtained for all the deposited isolates.
- Multiple antibiotic resistance markers have been determined for identification of elite isolates, this will facilitate the environmental monitoring of the consortium produced using these strains and also help in the detection of these isolates when released into the rhizosphere/ phyllosphere/ phylloplane.
- Most of the isolates showed increase in Chl a, Chl b, total chlorophyll content and physiologically available iron/ total iron content. Isolate NARs1 and PGERs 17 showed increase nutrient content while others are not contributing much.
- Decrease in Na^+/ K^+ ratio was observed in wheat plants inoculated with the cold tolerant isolates, which is critical to the plant's ability to tolerate stress conditions.
- Most of the cold tolerant bacterial cultures are having low ice nucleation activity (Table 1). Therefore, these cultures increase the supercooling temperature of the medium and can be exploited for the development of consortium to alleviate cold.

Conclusion:

Twelve elite cold tolerant isolates having multiple PGP activities at 4° and 15°C have been selected from a collection of 447 cold tolerant bacterial isolates. Multiple antibiotic resistance markers were identified of elite isolates, this will facilitate the environmental monitoring of the consortium produced using these strains and also help in the detection of these isolates when released into the rhizosphere/ phyllosphere/ phylloplane. Eight elite isolates were identified based on their 16S rRNA sequences *viz., Pseudomonas* sp strain PPERs23, *Pseudomonas* sp strain PGERs17, *Pseudomonas* sp strainPCRs4, *Pseudomonas* sp strain NARs9, *Pseudomonas putida* PGRs4, *Pseudomonas* sp strain NARs1, *Pseudomonas lurida* NPRp15 and *Pseudomonas putida* PBRs5. Six elite isolates were deposited at NBAIM, Mau and GenBank accession number obtained for all the deposited isolates. Selected elite isolates showed increase in Chl a, Chl b, total chlorophyll content and physiologically available iron/ total iron content in wheat. Decrease in Na^+/ K^+ ratio was observed in wheat plants inoculated with the cold tolerant isolates, which is critical to the plant's ability to tolerate stress conditions. Elite cold tolerant bacterial cultures having ice nucleation activity in the range of -6.12 to -9.83. It is further proposed to determine the cold alleviation potential of these isolates in conjunction with plant growth promoting activities and standardize the consortium production technology.

Table 1. Ice nucleation activity by cold tolerant bacterial cultures in vitro at -10°C

Treatments	Ice nucleation activity (log ice nuclei CFU^{-1})			
	Time (minutes)			
	6	12	18	24
Control	-6.81	-6.66	-6.06	-5.96
Pseudomonas sp. PPERs23	-7.19	-7.16	-7.08	-7.04
Pseudomonas sp.PGERs17	-7.51	-7.03	-6.66	-6.39
Pseudomonas sp. PCRs4	-7.78	-7.61	-7.54	-7.30
Pseudomonas sp.NARs9	-6.12	-6.18	-6.10	-6.08
Pseudomonas putida PGRs4	-8.51	-8.16	-8.12	-8.09
*Pseudomonas sp.*NARs1	-8.34	-7.74	-7.52	-7.12
Pseudomonas lurida NPRp15	-9.83	-9.83	-8.68	-8.53
PPRs4	-8.01	-8.19	-8.39	-8.13
NPRs3	-9.50	-9.50	-9.20	-8.14

Theme 5 : Microbial Genomics

Structural Genomics of *Mesorhizobium ciceri* Ca 181)

PI: N. K. Singh.
Co-PI: Aqbal Singh, Saravjeet Kaur, J. C. Padaria, Kanika, Rithu Rai
National Research Center on Plant Biotechnology, IARI, New Delhi

Objectives:

- Complete Genoe sequencingof *Mesorizobium ciceri* strain Ca181 with genome size of 8Mb.

Significant Achievements:

- Genomic DNA from *Mesorhizobium ciceri* Ca181 was isolated, nebulised and after size selection , 2-4kb DNA was eluted from gel. After polishing, this DNA was used for library construction in plasmid vector pUC19 (2.97kb). The insert size was checked from randomly picked clones by plasmid DNA isolation (fig 1)

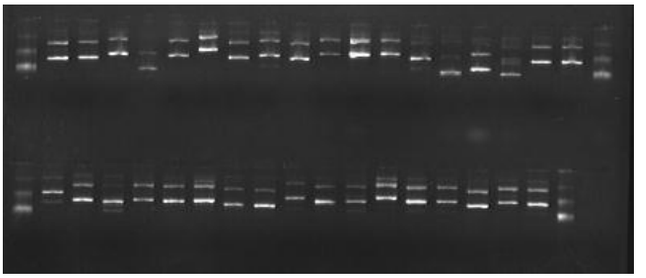


Fig 3. Plasmid DNA of randomly picked clones for quantification Lane 1& 20 (upper & lower lane) : Plasmid DNA of pUC19 Lanes 2-19 (Upper and lower lanes): Plasmid DNA of randomly picked clones

- *Sequencing of plasmid clones*: 1400 clones have been sequenced till now through forward and reverse sequencing. 2800 sequences have been compared with other organisms through BLAST. Maximum matches were with *Agrobacterium tumefaciens*, *Rhizobium* sp., *Sinorhizobium* sp. and *Mesorhizobium* sp. (table 1). Around 396 sequences were found to be unique.
- *Sequence assembly and annotation*: The genome assembly was done by using software PHRED, PHRAP and CONSED. Total 24 (12forward and 12reverse) sequenced plates were used for the assembly of the microbial genome. In the actual genome assembly as shown in table 2 to 5, total 2102 (F, R) electrogram sequenced data (ESD) reads were used whose range of read length is 300-400. After the assembly, total 325 contigs were formed and 13 singletons were left, the largest contig size is 2611 bp which was formed by the alignment of 10 reads. In the assembly there were 5 contigs with size above 2 Kb and 169 contigs with size below 1kb. The sequence of 5 contigs with size above 2 Kb and their respective matches with database are given below. Out of the five contigs, three had maximum match with *Agrobacterium tumefaciens* and two contigs did not match with available database.
- *Construction of Large insert cosmid library* Protocol for isolation of high molecular weight genomic DNA of M. ciceri strain Ca181 for cosmid library construction using modified standard phenol-chloroform method was standardized. The isolated DNA was run on 0.7% agarose gel as well as on pulse field gel.
- PCR amplification for 16s rDNA of *Mesorhizobium ciceri* Ca181: Using primer pair 41f (5' GCTCAAGATTGAACGCTGGCG 3') AND 1488r 5'GTTACCTTGTTACGACTTCACC 3') 16S rDNA region of *M. ciceri* Ca181 was done. The amplified 1.5 Kb fragment was purified and cloned in pGEMT Easy vector system (Promega). Ten random clones were picked and plasmid DNA was isolated. The isolated plasmid DNA was sequenced. The BLAST analysis showed top match with *Agrobacterium tumefaciens*

Table 1. Summary of BLAST results for *Mesorhizobium ciceri* plasmid library

Plate ID	Samples matching with databases	A. tumefaciens	R.etli	R.leguminosarum	Sinorhizobium sp	Mesorhizobium sp	Others	Vectors sequences	Unique sequences	Total
03z	50	35	2	2	1	0	2	8	31	81
03y	65	37	3	4	0	0	7	14	18	83
04z	58	46	2	0	1	2	1	7	16	74
04y	76	51	1	1	2	1	3	17	16	92
05z	59	47	0	0	1	0	3	8	39	88
05y	73	53	1	0	0	1	3	15	18	91
06z	68	55	1	0	0	0	5	7	12	80
06y	83	63	2	0	1	1	7	9	8	91
07z	69	54	1	0	0	1	4	9	9	88
07y	81	53	0	1	2	0	7	18	12	93
08z	81	56	1	2	1	3	7	11	11	92
08y	80	59	1	0	0	0	12	8	10	90
09z	68	55	0	0	0	0	5	8	16	84
09y	86	60	0	0	0	1	5	20	6	92
10z	74	54	3	1	2	0	2	12	15	89
10y	79	53	3	0	2	0	8	13	11	90
11z	59	44	1	1	0	0	5	8	28	87
11y	67	47	1	0	0	0	4	15	20	87
12z	73	54	0	2	2	0	2	13	14	87
12y	80	54	0	2	1	0	9	14	11	91
14z	57	45	0	0	1	2	0	9	31	88
14y	74	41	0	1	0	1	17	14	15	89
15z	75	58	2	0	1	0	10	8	13	88
15y	81	54	1	1	2	1	7	14	6	87
Total	**1716**	**1228**	**26**	**18**	**20**	**14**	**143**	**279**	**386**	**2102**

Table 2. Summary of sequence assembly and annotation

1	Number of plates used in assembly	12 (F,R) = total 24 plates
2	Total number of reads used in assembly	2102 (F,R)
3	Total number of contigs formed after assembly	325
4	Number of reads which do not align	13
5	Reads above 2 Kb	5
6	Reads below 1 Kb	169
7	Largest contig contain	10 reads (2611 b)
8	Range of read length	300-400

Table 3.Summary of BLAST results of contig 1 of *Mesorhizobium ciceri* Ca181

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AE007869.2	*Agrobacterium tumefaciens* str. C58 circular chromosome, complete sequence	**1919**	1919	90%	0.0	85%
BA000012.4	*Mesorhizobium loti* MAFF303099 DNA, complete genome	**279**	279	21%	2e-71	78%
CP000267.1	*Rhodoferax ferrireducens* DSM 15236, complete genome	**89.8**	89.8	4%	4e-14	86%
AE016825.1	*Chromobacterium violaceum* ATCC 12472, complete genome	**86.1**	162	5%	5e-13	90%
CP000269.1	*Janthinobacterium* sp. Marseille, complete genome	**80.5**	80.5	4%	2e-11	83%
AE012344.1	*Xanthomonas campestris* pv. campestris str. ATCC 33913, section 252 of 460 of the complete genome	**76.8**	76.8	3%	3e-10	86%

Table 4. Summary of BLAST results of contig 1 of *Mesorhizobium ciceri* Ca181

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AE007870.2	*Agrobacterium tumefaciens* str. C58 linear chromosome, complete sequence	**1279**	1279	49%	0.0	87%
AM236085.1	*Rhizobium leguminosarum* bv. viciae plasmid pRL11 complete genome, strain 3841	**1077**	1077	48%	0.0	84%
BA000040.2	*Bradyrhizobium japonicum* USDA 110 DNA, complete genome	**368**	368	34%	5e-98	76%
AP009384.1	*Azorhizobium caulinodans* ORS 571 DNA, complete genome	**329**	329	24%	3e-86	77%
CP000283.1	*Rhodopseudomonas palustris* BisB5, complete genome	**309**	309	25%	3e-80	76%
CP000133.1	*Rhizobium e tli* CFN 42, complete genome	**113**	113	9%	3e-21	77%

Table 5. Summary of BLAST results of contig 4 of *Mesorhizobium ciceri* Ca181

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AE007872.2	*Agrobacterium tumefaciens* str. C58 plasmid At, complete sequence	**1029**	1029	60%	0.0	81%

Structural Genomics of *Mesorhizobium ciceri* Ca 181)

PI : Major Singh.
Indian Institute of Vegetable Research, Varanasi

Objectives:

- Complete Genoe sequencingof *Mesorizobium ciceri* strain Ca181 with genome size of 8Mb.

Significant Achievements:

- It is difficult to isolate high molecular weight Genomic DNA due to the gum production by the *Mesorhizobium*. Therefore, Guanidium thiocyanate and SDS method of DNA isolation were tested. Yield of genomic DNA isolated by Guanidium thiocyanate method was better than that of SDS method. Moreover, DNA isolated by Guanidium thiocyanate method was more suitable for further enzymatic reactions.
- Since the Guanidium thiocyanate method gives better result over SDS method, Guanidium thiocyanate method is further standardized to reduce the gum the content and to yield more pure DNA.
- After the Genomic DNA was digested to make clonable fragments of 2-5 Kb. Enzymes generating blunt end as well as cohesive end were used. *Eco*RI, *Hind*III, *Taq*I, *Sma*I and ScaI restriction enzymes were tested. Digestion using EcoRI, HindIII, SmaI enzymes separately and double digestion using SmaI+ScaI results in clonable DNA fragments.
- The Sheared DNA between 2-5 Kb was eluted using Qiagen elution kit (fig 1) and was further used for ligation.

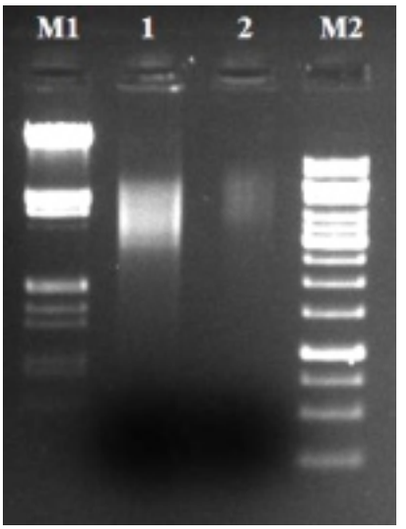


Fig 1. Eluted genomic DNA after digestion. M1, λ marker (EcoRI-Hind III digest); M2, 1 Kb DNA Ladder; 1-2, Eluted Genomic DNA after digestion

- *Isolation, digestion and elution of pUC19 DNA (vector DNA)*: Plasmid pUC19 was used as a vector for transformation of cloning of the genomic DNA. For this, the pUC19 was isolated from *E. coli* cells cloned by intact *pUC*19. The plasmid DNA was isolated by miniprep-alkaline lysis method. Then the *pUC*19 is digested to linearize. *Eco*RI and *Sma*I restriction enzymes used separately for the digestion (fig 2).

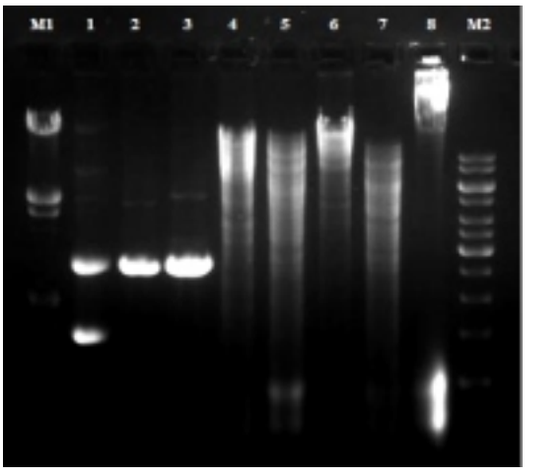


Fig 2. Digested pUC19 and genomic DNA. M1, λ marker (EcoRI-Hind III digest); 1, Intact (circular) pUC19; 2, pUC19 digested with EcoRI; 3, pUC19 digested with SmaI; 4, Genomic DNA digested with EcoRI restriction enzyme; 5, Genomic DNA digested with SmaI; 6, Genomic DNA digested with ScaI; 7, Double digested Genomic DNA SmaI+ScaI restriction enzymes; 8, Genomic DNA; M2, 1 Kb DNA ladder.

- Ligation protocol was standardize in terms of amount of Plasmid and genomic DNA, concentration of T4 DNA Ligase, PEG concentration. After ligation, the ligation product was precipitated and dissolved in water.
- Electroporation of ligation product (transformation) and cloning of transformants Ligation product was electroporated in XL1 Blue strain of *E.coli* (fig 3).

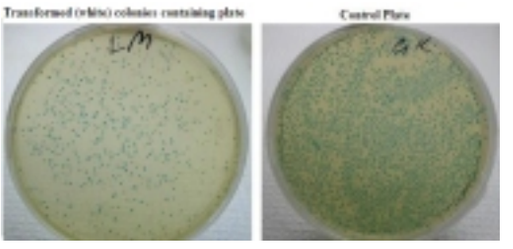


Fig 3. Plates containing transformed (white) colonies and control plate.

- Plasmid DNA is isolated by manual methods from the white colonies were isolated and transformation is confirmed (fig 4).
- Library preparation and insert size determination: Genomic DNA library preparation is started and 500 Clones are prepared. Plasmid DNA of 200 clones is isolated and insert of desired size is confirmed in 128 clones (fig 5).

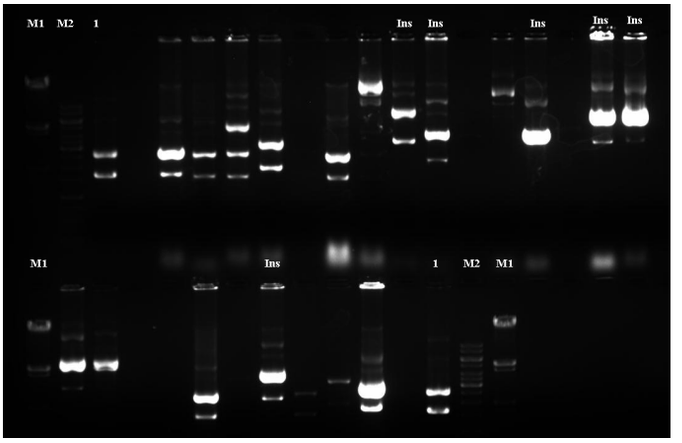


Fig 4. Confirmation of transformation. M1, λ marker (EcoRI-Hind III digest);
1, Intact (circular) pUC19; Ins, Vector+Insert of desired size; M2, 1 Kb DNA ladder.

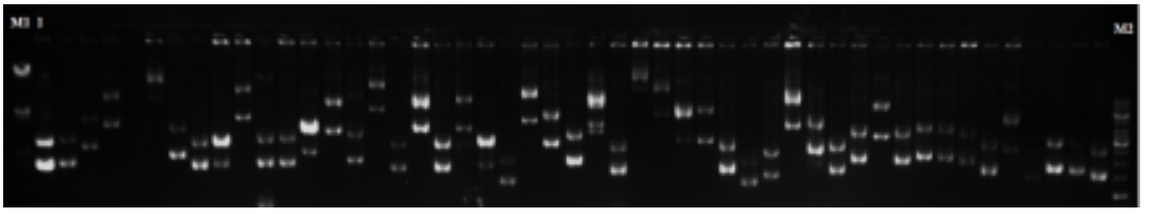


Fig 5. Plasmid DNA from clones of the library. M1, λ marker (EcoRI-Hind III digest);
1, Intact (circular) pUC19; M2, 1 Kb DNA ladder.

Genome analysis of the nitrogen fixing symbiotic bacterium *Mesorizobium ciceri*

PI : K. V. Bhatt
Co-PI : A. B. Gaikwad, Mukesh kuar Rana, Rakes Singh, Sunil Archak
School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu

Objectives:

- Complete Genoe sequencingof *Mesorizobium ciceri* with genome size of 8Mb.

Significant achievements

- A summary of some of the putative genes identified through BLAST search is presented in Table. Over 100 functional regions were identified through the analysis. The putative gene sequences included ATP-dependent helicase, probable sulfate uptake ABC transporter, heavy-metal transporting P-type ATPase, ammonium transporter etc.

Table: Putative genes identified in the genome

95 bp at 5' side: L -lactate dehydrogenase 36 bp at 3' side: quaternary ammonium compound-resistance protein	polygalacturonase-like protein ABC transporter, substrate binding protein (sugar)	58 bp at 5' side: riboflavin synthase subunit alpha 124 bp at 3' side: RhtB family transporter
Diguanylate cyclase	dihydrodipicolinate synthase	adenine deaminase
Uroporphyrinogen decarboxylase	Phosphoribosylanthranilate isomerase	94 bp at 3' side: RNA methyltransferase
TRAP dicarboxylate transporter	Permease protein	ribonuclease D
Lactose operon repressor	ATP-dependent helicase HrpB	exodeoxyribonuclease III
Succinate semialdehyde dehydrogenase	S-adenosyl-methyltransferase MraW	putative malate dehydrogenase
Sulfate adenylyl transferase subunit 2	35 bp at 5' side: methyltransferase 51 bp at 3' side: pseudoazurin	probable sulfate uptake ABC transporter
exodeoxyribonuclease VII large subunit	extracellular solute-binding protein, family 1	penicillin-insensitive murein endopeptidase
dehydrogenase	beta-D-galactosidase	mannonate dehydratase
methyl-accepting chemotaxis protein	glucose-1-phosphate adenylyltransferase	phospho-2-dehydro-3-deoxyheptonate aldolase
2-dehydro-3-deoxyphosphoheptonate aldolase	bifunctional phosphoribosylaminoimidazolecarboxamide form...	probable drug resistance transporter protein, Bcr/CflA
flaB	flagella associated protein	lipoprotein
oxidoreductase	ADP-glucose pyrophosphorylase	acetyltransferase
cobaltochelatase, CobS subunit	putative d-galactose 1-dehydrogenase protein	polysaccharide export protein
N-(5'-phosphoribosyl) anthranilate isomerase tryptophan synthase subunit beta	ABC transporter, membrane spanning protein (trehalose/maleate) ABC transporter, nucleotide binding/ATPase protein	F0F1 ATP synthase subunit epsilon F0F1 ATP synthase subunit beta
ATP-dependent DNA helicase	flagellin domain protein	NADH dehydrogenase

Xylose isomerase domain protein TIM barrel	3-deoxy-D-arabino-heptulosonate 7-phosphate synthase	putative glycine/D -amino acid oxidases
glycosyltransferase	MFS permease	cell division protein FtsZ
penicillin binding protein	uracil transport protein	phosphopentomutase
ABC transporter, membrane spanning protein (putrescine)	transglutaminase family protein cysteine peptidase BTLCP	protein of unknown function DUF861 cupin_3
sarcosine oxidase delta subunit sarcosine oxidase alpha subunit	trehalose utilization-related protein trehalose utilization-related protein	alpha-glucosidase 2-dehydro-3-deoxygluconokinase
cobyrinic acid synthase	protein of unknown function DUF1037	beta (1 -->2) glucan export ATP -binding protein
Uridinekinase: Disease resistance protein:ATP/GTP-binding	cobalamin biosynthesis protein molecular chaperone, DNAJ family	DNA polymerase III subuni ts gamma and tau
iron compound ABC transporter, ATP -binding protein, putative	38 bp at 5' side: two component response regulator 164 bp at 3' side: cell division protein FtsZ	ABC transporter, membrane spanning protein molybdopterin converting factor, large subunit
surfeit 1cytochrome o ubiquinol oxidase subunit IV	acyl-CoA thioesterase II nitrogen regulatory protein PII	formamidopyrimidine-DNA glycosylase
iron(iii)-transport atp-binding protein	phosphoribosylaminoimidazole carboxylase ATPase subunit	beta (1 -->2) glucan biosynthesis protein
heavy-metal transporting P-type ATPase	Ubiquinone biosynthesis protein ubiB	hippurate hydrolase
Sun protein	Aminotransferase class-III	O-acetylhomoserine sulfhydrylase
beta alanine-pyruvate transaminase	Polyhydroxyalkanoate depolymerase, intracellular	O-acetylhomoserine aminocarboxypropyltransferase
Putative alpha -glycosylase protein	inositol monophosphatase family protein	Apolipoprotein N-acyltransferase
Intracellular PHB depolymerase	aminopeptidase N	D-galactarate dehydratase
LexA repressor	D-fructose-6-phosphate amidotransferase	Periplasmic mannitol-binding protein
Twin-arginine translocation pathway signal	Peptidase S24 protein	Extensin family protein
Ammonium transporter	beta-1,2-glucan export protein ndvA	

Structural Genomics of *Mesorhizobium ciceri* Ca 181)

PI : A. K. Saxena
Co-PI : D. K. Arora; Rajeev Kaushik
National Bureau of Agriculturally Important Microorganisms, Mau, Uttar Pradesh

Objectives:

- Complete Genoe sequencingof *Mesorizobium ciceri* with genome size of 8Mb.

Significant Achievements:

- The authenticity of the *Mesorhizobium ciceri* strain ca 181 was checked using BIOLOG microbial identification system and it showed 99% similarity with the *Rhizobium radiobacter*. The BIOLOG database does not have *Mesorhizobium ciceri* in it therefore it gave resemblance with the closest genera and species (fig 1).
- 16S rDNA of *M. ciceri* was amplified using universal primer pair 27f (5'AGAGTTTGATCCCTCAG-3') & 1520 r(5'AAGGAGCTGATCCAGCCGCA-3')
- The amplified fragment of ≈1.5kb length was purified, sequenced and BLAST searched at NCBI database. Results showed high similarity with *Mesorhizobium* loti strains, *Bradyrhizobium sp. and Agrobacterium.*
- In order to prepare genomic DNA library the DNA was isolated and nebulized for the formation of 2-4 kb short fragments. Nebulized gDNA was purified and ends of the fragments were repaired to form the blunt end fragments. Ligation of gDNA (2.0-4.0 kb) was done in the vector pUC19. Ligated DNA was transferred to *E.coli* strain by electroporation. The transformants were scored on Luria Agar plates with X-Gal-IPTG-Amp.
- Plasmid DNA from the transformants was isolated and digested with *sma* I enzyme (Fig 2). The digested fragment was amplified using T7 and M13 universal primers.
- The amplified fragments were sequenced (fig 3).

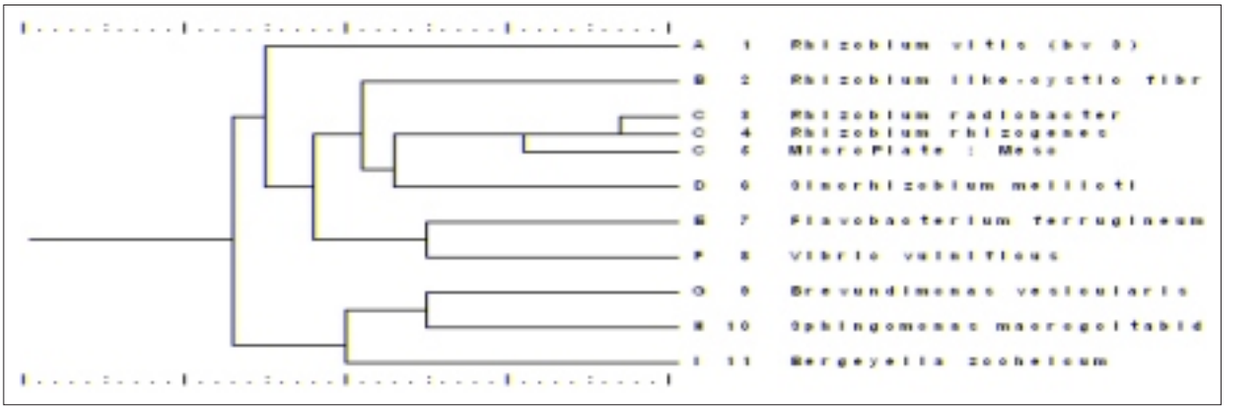


Fig 1. Dendrogram obtained by BIOLOG microbial identification system. The *M. ciceri* at No. 6 resembling with *R. radiobacter* at No. 3

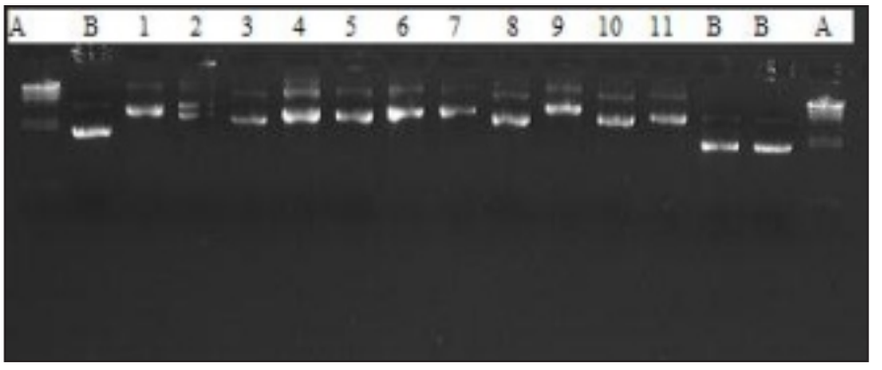


Fig 2. Quantification of clones DNA for sequencing; Lane A. Lamda DNA *Eco R1-HindIII* Marker, Lane-B.pUC 19,1-11

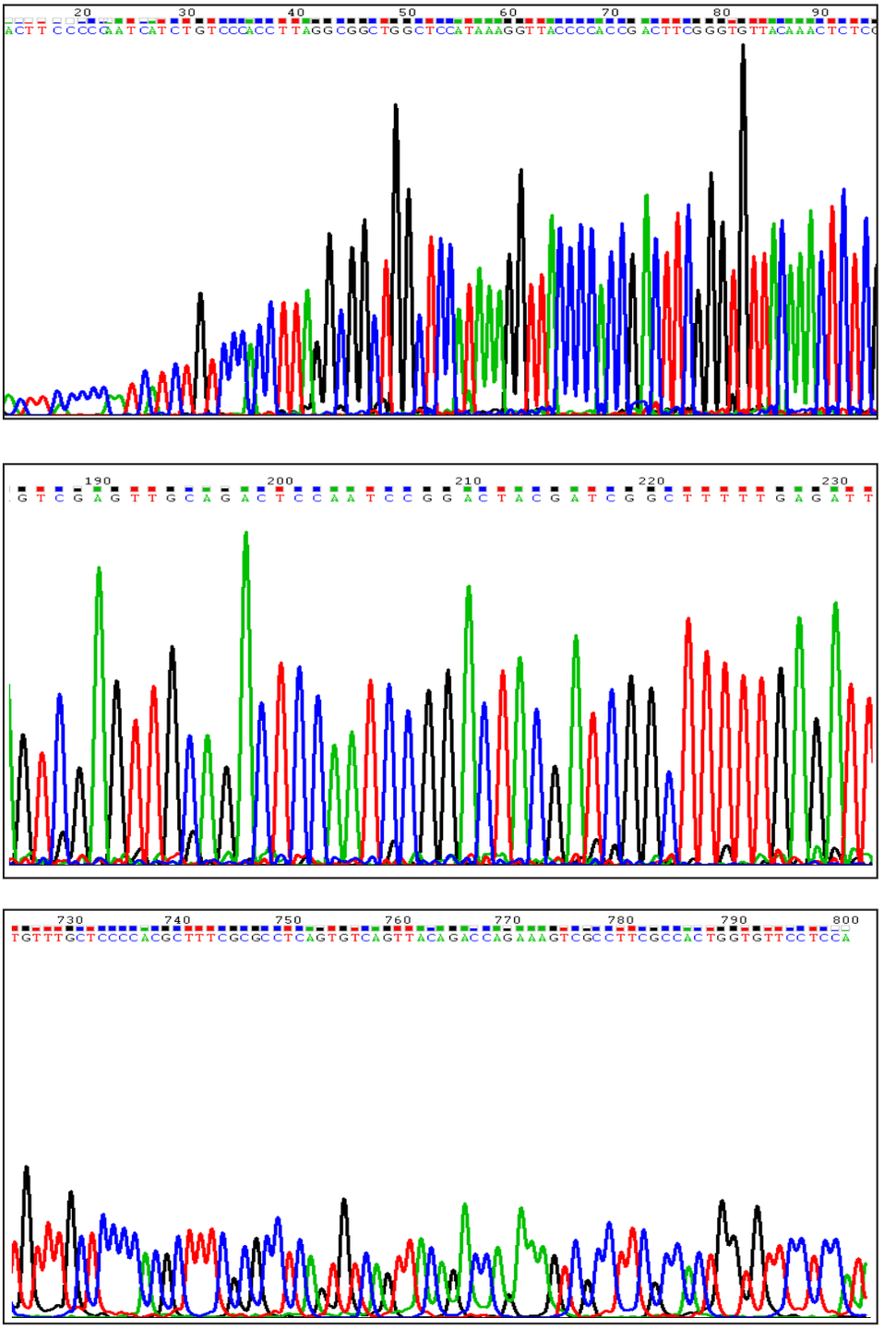


Fig 3. Sequence of a clone upto 800 bp

Conclusion:

The genomic library of small fragments (2-4 Kb) of *Mesorhizobium* strain was constructed in *puc* 19 vector. The clones were generated and are in the process of sequencing. The size of genomic DNA is about 8 Mb and the complete genome sequencing will be achieved in the stipulated time frame. The BLAST search of the sequences will lead to the identification of some novel genes involved in nitrogen fixation, assimilation, nodulation and competitiveness.

Functional Genomic Analysis of Plant Growth Promoting Rhizobacteria (PGPR) Fluorescent *Pseudomonads*

PI : P. Gunasekaran
Co-PI : K. Manoharan
School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu

Objectives:

- Selection and characterization of PGPR fluorescent Pseudomonads. Genome wide comparison of the PGPR fluorescent Pseudomonads isolated from different ecological niche.
- Gene expression profiling of the PGPR fluorescent Pseudomonads growing in the rice rhizosphere and root environments.
- Identification of novel genes involved in the rhizosphere colonization using *in vivo* expression technology.
- Characterization of bacterial genes that are upregulated / downregulated during the rhizosphere colonization.

Significant achievements

- Transposon induced mutant library consisting of 3900 mutants was constructed from *Pseudomonas putida* S11.
- Sixty one auxotrophic mutants were identified by screening.
- Nine 8 HQ tolerant mutants and twenty six 8 HQ sensitive mutants were obtained in the preliminary screening.
- Upon further screening, five 8HQ hypertolerant mutants and six 8HQ hyper sensitive mutants were obtained.
- Tn*5* transposon insertion site in HQ tolerant mutant was mapped by genome walking technique.
- The site of insertion of Tn*5* in HQ tolerant mutant was found to be in the *PqqF* gene involved in the biosynthesis of coenzyme PQQ

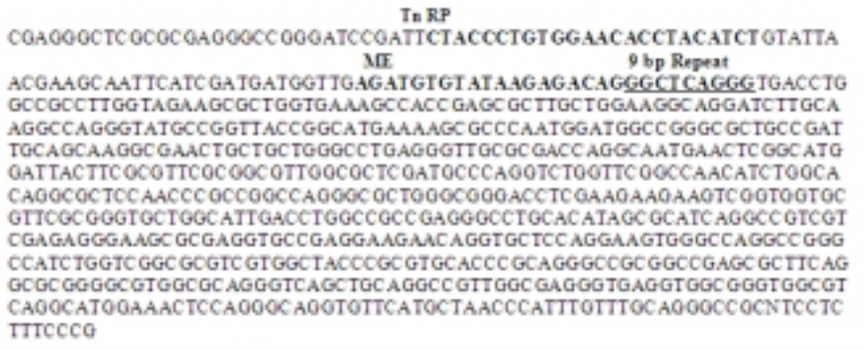


Fig 1. Sequence of the genome of 8HQ tolerant mutant of *P. putida* S11 amplified with the Tn*5* specific primer

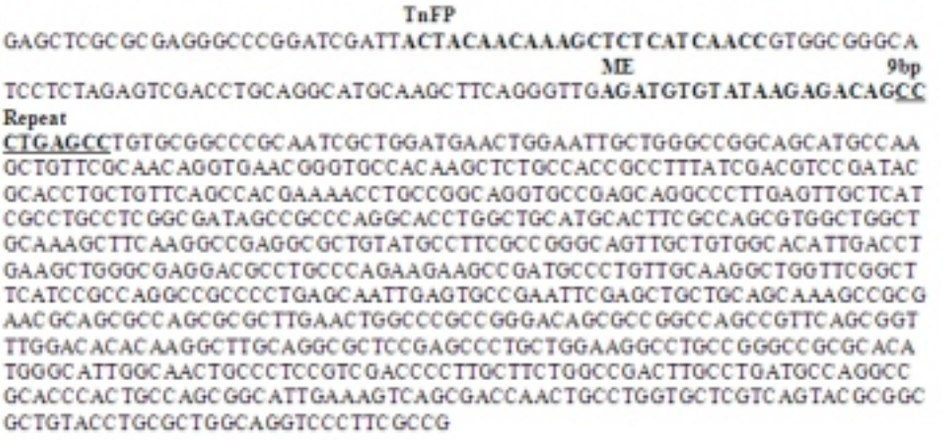


Fig 2. Sequence of the genome of 8HQ tolerant mutant of *P. putida* S11 amplified with the Tn*5* specific primer

Mining for genes involved in production of fungicidal compounds in *Anabaena* strains

PI : Radha Prasanna
Co-PI : N. K. Singh
Division of Microbiology, IARI, New Delhi

Objectives:

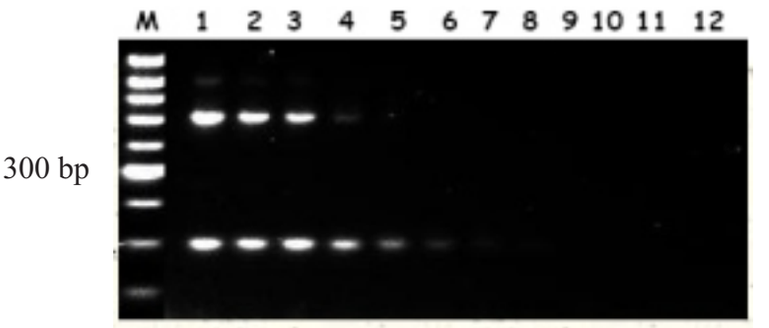
- To utilize PCR based techniques and sequencing tools to identify genes involved in the production of the fungicidal compound(s) in a selected set of *Anabaena* strains.
- To sequence the genes involved in the production of the fungicidal compounds.
- To validate the fungicidal effect of the genes by transformation of non toxic strain.
- To develop delevery and expression systems for enhanced production of fungicidal compounds.

Significant Achievements:

- Partial purification and partioning of culture filtrates using organic solvents was carried out for a set of 35 *Anabaena* strains - for analyzing the chemical nature of the culture filtrate. Among the 35 *Anabaena* strains, three strains showed inhibition zone in aqueous phase only indicative of enzyme being responsible for fungicidal activity and the four strains showed inhibition in the solvent phase of culture filtrate (s) perhaps, reflective of compound(s).
- Microscopic analyses of cyanobacterial metabolite-fungal interactions revealed abnormalities in the hyphal structure of *Fusarium moniliforme*.
- Optimized protocols for determination of MIC values for selected culture filtrates and their ethyl acetate extracts/aqueous phase fractions using microtitre plate assays
- Useful sets of degenerate oligonuceotides were identified towards the partial/complete sequences of fungicidal compounds and hydrolytic enzymes. Amplification patterns revealed the presence of expected size of PCR products in several strains. From the set of 35 *Anabaena* strains, cloned reaction products of two strains - RP8 and RP9 were sequenced and BLAST-N and Clustal W analyses revealed significant similarities with chi IS gene in *Streptomyces* sp.

Conclusion:

Screening of available collection of 80 *Anabaena* strains from diverse ecologies of India for fungicidal activity against one or more selected phytopathogenic fungi (*Fusarium moniliforme, F. solani, Alternaria solani, Pythium* sps. and *Macrophomina phaseolina*) in disc diffusion assays led to the selection of 35 promising isolates. Chemical nature responsible for fungicidal activity reveled the role of enzyme for fungicidal activity in the selected strains. Cyanobacterial strains exhibiting activity of hydrolytic enzymes- chitosanase, FPase and xylanase was reported for the first time. Useful sets of degenerate oligonucleotides were identified towards the partial/complete sequences of cyanotoxins, fungicidal compounds and hydrolytic enzymes. From the set of 35 *Anabaena* strains, cloned reaction products of two strains - RP8 and RP9 were sequenced and BLAST-N and Clustal W analyses revealed significant similarities with *chi* IS gene in *Streptomyces* sp.



Gradient PCR of 2a3b primers with strain RP10 (Lane 1-12). M is the 100bp ladder

Lane 1: 52.1 °C; Lane 2: 53.1 °C; Lane 3: 54.2 °C; Lane 4: 55.3 °C; Lane 5: 56.4 °C; Lane 6: 57.5 °C

Genomic Studies of Uncultivated N_2-fixing Communities from Uttarakhand

PI: Reeta Goel
Department of Microbiology, GBPUAT, Pantnagar, Uttarakhand

Objective:

- Identification of targets/isolates from different agroclimatic regions.
- Metagenomics for *csp* and *nif*.
- Isolation of full-length nitrogen fixing genes from a large number of isolates.
- Characterization of the above genes by sequencing.
- Sequence alignment and identification of different alternate forms of the above mentioned target genes.

Significant Achievements:

- In toto 17 cloned sequences are already in public domain through NCBI database (Table 1).
- More than 100 eubacterial clones are characterized out of five agroclimatic region of Uttarakhand (Table 2).
- However, 22 clones have shown more than 85% similarity with prominent nitrogen fixing community (*Mesorhizobium* sp., *Azorhizobium* sp., *Nitrobacter* sp., *Arthrobacter* sp., *Frankia* sp., *Nostoc* sp., etc.) (Fig 1), respectively.
- Phylogenetic relatedness among nif H community of Pantnagar, Badrinath, Almora and Chamoli soils is calculated (Fig. 2).

Table 1: Clones submitted to NCBI database

Soil sample collection site at Uttarakhand	Clones	Accession No.
CHAMOLI	CNI	EU446289
	CN2	EU446290
	CN3	EU446291
	CN4	EU446292
PANTNAGAR	PN1	EU446293
	PN4	EU446294
	PN3	EU446295
	PN2	EU446296
	PN5	EU446297
BHAWALI	S1	DQ517500
	S2	EF092429
	S3	EF092430
	S4	EF092431
PITHORAGARH	S5	DQ517501
	S6	EF092432
	S7	EF092433

Table 2: Selected positive clones and their respective location used in Fig. 1

Clones	Soil samples location at Uttarakhand
GE7,GE6;GE3,GE2	Glacier
BE10,BE3,BE1	Badrinaath
RE13,RE10,RE8,RE1	Ranichauri
PE6x,PE5 ,PE3,PE2	Pantnagar
CE12,CE10,CE8,CE2,	Chamoli
CE9,CE13,Ce11,Ce14	

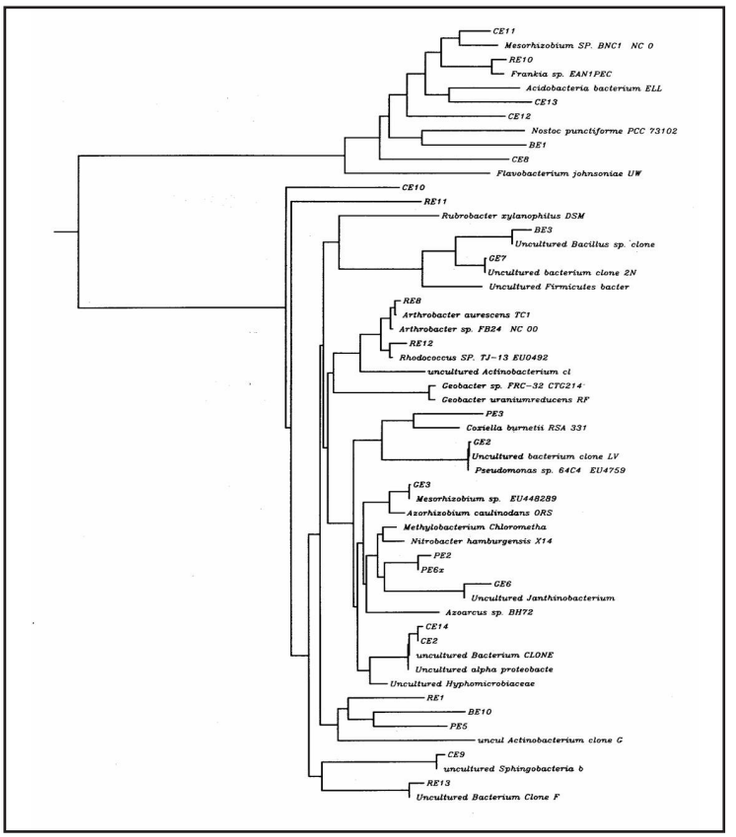


Fig. 1: Sequence relationship among metagenome sequences amplified from soil DNA using universal eubacterial 16s rRNA gene specific primers, (partial gene sequences were amplified). The obtained metagenome sequences are analyzed through NCBI-Blast for homology analysis. Homologous sequences were retrieved from NCBI database and aligned, using clustal W(1.83) program. Phylogenetic tree is constructed using neighbor-joining method.

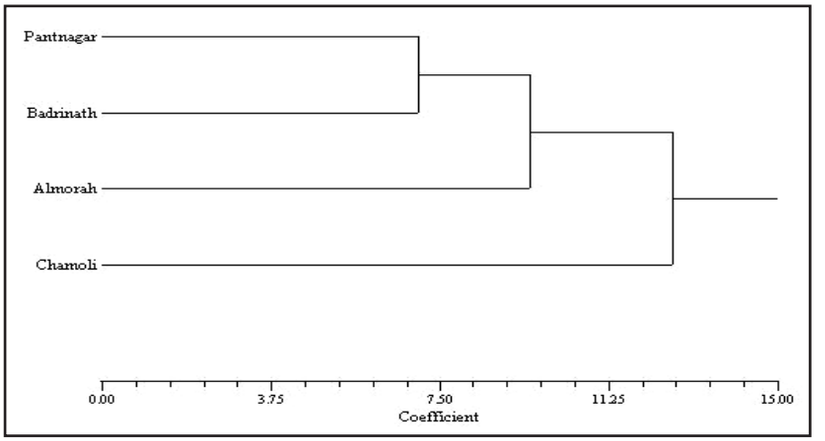


Fig. 2: Phylogenetic tree showing the relatedness among the nif H community of different soils DNA. In each case amplified soil DNA was restricted with *Hae* III, *Alu* I, *Msp* I and *pas* I enzymes and tree is constructed using RFLP pattern generated, respectively.

Conclusion:

More than 100 eubacterial clones are characterized out of Glacier, Badrinath, Ranichauri, Pantnagar and Chamoli region of Uttarakhand. However, 22 clones have shown more than 85% similarity with prominent nitrogen fixing community (*Mesorhizobium* sp., *Azorhizobium* sp., *Nitrobacter* sp., *Arthrobacter* sp., *Frankia* sp., *Nostoc* sp., etc.) respectively. Further, Phylogenetic relatedness among nif H community of Pantnagar, Badrinath, Almora and Chamoli soils is calculated based on their RFLP pattern. It was surprising to note that Pantnagar and Badrinath community is closer unlikely to Chamoli and Almora region. Moreover, in total 43 cloned sequences are already in public domain through NCBI database.

Theme: Human Resource Development

Human Resource Development

D. K. Arora
NBAIM, Mau

Objectives:

- To train scientists/researchers/technicians/ farmers for the exploration and application of microorganism in agriculture

Significant Achievements:

- In the year 2007-08 following two trainings organized:

1. "DNA Sequencing and microbial identification module of agriculturally Important Microorganisms' from September 17-21, 2007
2. "Molecular and serological detection of plant viruses from February 24-March 1, 2008

Introduction to National Training on 'DNA Sequencing and Microbial Identification Module of Agriculturally Important Microorganisms'

The small size of microbes belies their powerful influence. They surround and inhabit us, and every creature on earth depends on them for life. In order to understand and exploit microbes, human beings must inventory and understand the vast repertoire of microbial activities. Because a creature's capabilities are reflected in and dictated by the DNA it carries, a microorganism's complete—or genomic—DNA sequence provides a blueprint for its biochemical and behavioral endowment.

Microbes live everywhere, including in deep-sea vents miles under the ocean, boiling hot springs, glaciers, and even rock, as well as more familiar settings such as soil, ponds, and animal intestines. Their ability to survive in diverse environments reflects an astounding array of biochemical aptitude. They churn out the nitrogen that plants need for growth and emit gasses that create and maintain the critical composition of the earth's atmosphere. In our bodies, they help digest food, ward off attempts by harmful organisms to invade, provoke misdirected immune responses against our own tissues, and even shape the development of our immune systems.

Humans have co-opted microbial talents to enhance the quality of our lives. For example, many large-scale industrial processes depend on microbes. In addition, we exploit them for making food and medicines and put them to work cleaning up our sewage and industrial waste. Microbes can dramatically influence ecosystems because many possess unique abilities to recycle both organic and inorganic substances. As the world's human population increases and natural resources diminish, environmental challenges grow. Humans are trying to harness the vast metabolic potential of microorganisms to apply microbial solutions to some of these ecological problems.

Microbes are not only vital to our bodies, forests, factories, and all of life. They also represent a record of evolution. Because all life descended from tiny, single-celled creatures, microbes open a window into antiquity. By peering inside these simple organisms, we learn about our origins and those of every living thing. A comparison of new genome sequences with those previously known shows us aspects of biology unique to an organism, as well as features that are shared with others. Elucidating the patterns of particular genes (and the capabilities they confer) among microorganisms improves our understanding of how different creatures are related. This information is critical for attempts to study, capitalize on, and interfere with the activities of microorganisms. Discovering which microbes contain which genes reveals the capabilities demanded by particular types of environments or lifestyles, for example, and points to the deviations that allow organisms to adapt to unusual habitats or behaviors. It also provides the basis for predictions about what capacities a new microorganism might possess, based on the activities of its genetic neighbors.

Despite their importance, we know strikingly little about microbes. They compose greater than 50% of the living matter on this planet, yet we have identified far less

than one percent of all the predicted microbial groups. The microbial world, therefore, represents a vast reservoir of untapped knowledge and potential utility. Microbes offer a view into the capabilities of living things that far exceeds the capabilities that will be revealed by, for example, the human genome sequence alone. The amount of DNA carried by a member of the human species is equivalent to the DNA cache of approximately 1,000 microbes, and there are far more than 10 times this number of different microbes in the world around us. Microbial genome sequencing, therefore, offers an extremely efficient way to generate a picture of the tremendous biological diversity that living creatures possess, particularly because microbes inhabit such an array of bizarre and extreme environments. To profit from this natural biological archive, we need to embark upon a coordinated, large-scale effort to uncover and interpret a comprehensive set of microbial genome sequences. Already governmental, academic, and private agencies are deciphering the genetic code of many microbes. Since the debut of whole genome sequencing in the mid-1990s, the genomes of 29 microbes and three chromosomes from two lower eukaryotic parasites have been sequenced; more than 100 other sequencing projects are under way. Yet, inevitably for an enterprise that is advancing rapidly and generating an explosion of valuable information, problems have arisen. Organization and dissemination of technology and data have been haphazard. The absence of a broad discussion regarding priorities and goals has contributed to the lack of coordination and absence of generally accepted standards. Sequencing technology has matured at an unexpectedly rapid pace, making quite feasible goals that would not have been imaginable just a few years ago. But, funding is inadequate for the kind of large-scale, integrated effort that should be undertaken.

This training programme was organized with the aim of directing attention toward the challenges that face this new and promising field in the hope of productively interceding while the enterprise is still young and malleable. Now is the time to conduct discussions that include people with a broad range of interests and expertise about current and foreseeable challenges. Already, federal agencies have begun to ask for guidance on these issues. It is essential that the sequencing efforts accelerate as rapidly and as smoothly as possible. With timely, well-aimed interventions, it should be possible to transform the vision of a large-scale microbial genome sequencing effort into reality, and to maximize the effort's efficiency and economy.

Introduction to National Training on 'Molecular and Serological Detection of Plant Viruses'

Viruses are very small (submicroscopic) infectious particles (virions) composed of a protein coat and a nucleic acid core. They carry genetic information encoded in their nucleic acid, which typically specifies two or more proteins. Translation of the genome (to produce proteins) or transcription and replication (to produce more nucleic acid) takes place within the host cell and uses some of the host's biochemical "machinery". Viruses do not capture or store free energy and are not functionally active outside their host. They are therefore parasites (and usually pathogens) but are not usually regarded as genuine microorganisms. Most viruses are restricted to a particular type of host. Some infect bacteria, and are known as bacteriophages, whereas others are known that infect algae, protozoa, fungi (mycoviruses), invertebrates, vertebrates or vascular plants. However, some viruses that are transmitted between vertebrate or plant hosts by feeding insects (vectors) can replicate within both their host and their vector.

Plant viruses are generally identified by particle morphology, host range and the serological properties of the coat protein. Cross-reactivity of antisera raised against viruses from different groups has frequently been used for classification and for the establishment of taxonomic relationships. However, nucleic acid sequence data are accumulating rapidly and allow more accurate relationships to be established between the individual members of virus groups than serological methods do. Identification of a virus by sequencing parts of its genome is often done if extensive serological analysis cannot provide conclusive data about the nature of the virus. This approach requires the purification and isolation of the virus particles and the subsequent cloning of parts of the virus genome. However, with novel molecular tools like virus group specific PCR, sequence data from new viruses can be obtained, even without the need to purify a virus or to clone parts of its genome. Sequences obtained by such methods can reveal close and distant relationships between new and existing viruses from a single group as will be demonstrated for the group of potyviruses which is the main group of plant viruses occurring in bulbous crops. Characterization of new potyviruses appeared to be possible even in case of mixed infections. For the identification of new potyviruses several general PCR primer sets have been developed which allow the amplification of the complete 3'-end of the potyvirus genome including the coat protein cistron and part of the

replicase Nlb cistron. For several other groups of plant viruses similar PCR primer sets are available.

Once identification of a virus has been accomplished by nucleic acid sequencing, specific PCR primer sets can be designed for very sensitive detection of the virus. However, to compete with current serological methods for mass detection of plant viruses, the development of fast and reliable PCR protocols including automated sample preparation is a major challenge to overcome. Alternatively, the molecular approach provides new possibilities to the development of very specific and sensitive antisera against individual viruses or (sub) groups of viruses. The expression of parts of a virus coat protein as recombinant fusion products can provide insight in the location of epitopes on the coat protein's amino acid sequence recognized by existing virus specific and virus (sub) group specific antisera. Then, virus specific epitopes can be isolated from group specific epitopes in recombinant fusion products for production of pure antigens in bacteria, yeast or other cell systems. Such antigens will provide an infinite source for the production of a new generation of virus specific antisera.

The training program has the following theme areas to address:

1. Molecular detection of some important plant viruses
2. Serological detection of some important plant viruses
3. Bioinformatics in characterization of important plant viruses