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Annual Report 2007-08 National Bureau of Agriculturally Important Microorganisms

भाकृअनुप
ICAR

NBAIM
रा.कृ.उ.सू.ब्यू.

NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT MICROORGANISMS

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो

Understanding and conserving our national heritage of agriculturally important microorganisms

Annual Report 2007-08

वार्षिक प्रतिवेदन 2007-08

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भारतीय कृषि अनुसंधान परिषद
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Understanding and conserving our national heritage of agriculturally important microorganisms

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July 2008

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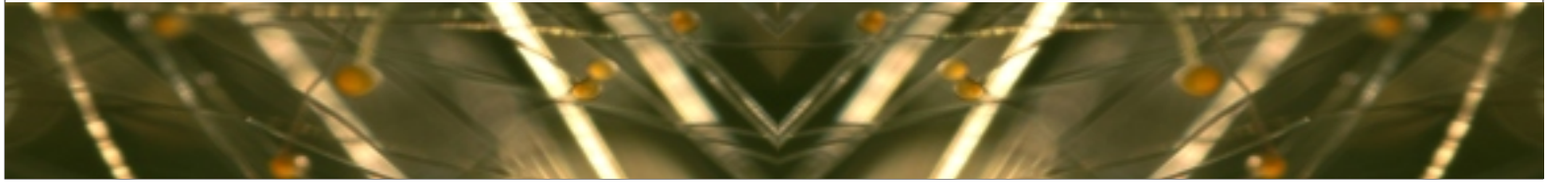


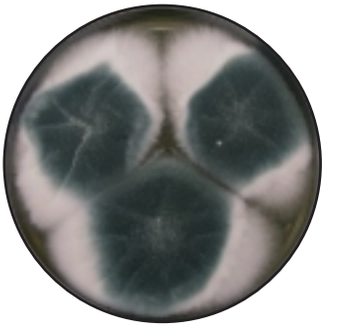
Executive Summary

- A network project on 'Application of Microorganisms in Agriculture and Allied Sectors' with NBAIM as the nodal center was approved for Xth five year plan. Besides the 6 theme areas earlier approved in the project, a new theme area on **"Microbial Genomic Resource Repository"** was added in the XI plan. In future following new perspectives will be included:
  - ✦ Mapping of microbial diversity through Geographical Information System;
  - ✦ Integration of bioinformatics and development of softwares for effective management of microbial holdings;
  - ✦ Development of "Quality Management System" based on OCDE and WFCC microbial resources guidelines;
  - ✦ Policies regarding the deposit of "Genetically Manipulated" AIMs, AIMs falling under IPR, patentable AIMs and its long term conservation;
  - ✦ Development of microbial gene bank;
  - ✦ Linkages with farmer, industry and academia;
- At present the Bureau has nine scientific and two technical staff.
- The Bureau has strengthened its linkages with other ICAR/ CSIR/ DBT institutes, State Agricultural Universities (SAUs), and International microbial resource centers.
- NBAIM is growing as a dynamic and vibrant organization and in future it will lead the national body for all Research & Development activities on microbial genetic resources with state of the art infrastructural facilities for identification, characterization and conservation of agriculturally important microorganisms.
- NBAIM has a well maintained National Microbial Repository. NBAIM is developing infrastructural facilities for microbial database and "Microbial Information Management System".
- A mid-term appraisal of the perspective plan has greatly contributed in analyzing the achievements made so far and also modifications required in certain items of the earlier plan. This was necessitated owing to the changing global scenario of microbes and its utilization. Thus certain new perspectives have emerged which now would be an integral part of the "microbial resource" programme. The recommendations of four Research Advisory Committee have also been duly considered for revising the perspective plan.
- Among the achievements made so far, within a short span of 4 years, since its establishment at Mau, the notable ones include collection of over 2800 accessions including many useful microbes.
- Sixteen special exploration mission have been undertaken in different states like Uttar Pradesh, Himachal Pradesh, Madhya Pradesh, Arunachal Pradesh, Assam, Rajasthan and Kerala for collection and isolation of AIMs.
- NBAIM maintains large number of cultures used as biofertilizers. It includes *Rhizobium, Azospirillum, Azotobacter*, PSM, *Bacillus, Pseudomonas* and *Gluconobacter*.
- Several microbes capable of disease suppression such as species of *Bacillus, Pseudomonas, Serratia, Trichoderma, Verticillium* are also conserved.
- Entomopathogenic microorganisms like species of *Metarhizium, Beauveria, Bacillus,* which are used to manage the insect pests are also maintained.
- Molecular fingerprints developed for large number of isolates of *Bacillus, Pseudomonas, Fusarium, Macrophomina* and *Serratia*.
- Novel gene sequences submitted to NCBI Gen Bank and accession numbers obtained.
- Bureau is accredited by the WFCC, OCED and BDA, India.
- Bureau has been recognized by BDA and ICAR to be a nodal agency for registration of microorganisms.
- A rapid molecular probe for the identification of *Bacillus* sp. have been developed based on RFLP pattern and sequencing of small region of 16S rDNA.
- Using housekeeping gene sequences a probe has been developed for the identification of *Fusarium*

at species level.

- Species specific primers and probe was developed for the identification of *Macrophomina phaseolina*.
- The "passport data" of each deposit has been developed. The evaluation of some agriculturally important microorganisms will be carried out as with time this Bureau will grow, in future.
- Augmentation of AIMs through repatriation from different national and international agencies was initiated and will be done in phase-wise manner.
- Repeatable and reliable protocols for cryopreservation of various agriculturally important microorganisms will be developed for *in vitro* repository, which would be useful for the long-term conservation of microbes. It is envisaged to carry out experiments to develop cryopreservation protocols in some of the most important genera that are useful for increasing crop productivity. Attempts will be made to cryopreserve existing microbial genetic resources of *in vitro* repository utilizing the developed protocols for its long-term conservation. Initiatives are to be taken for cryopreserving actinomycetes and other slow-growing microorganisms. These microbial species where cryopreservation is not possible, emphasis will be laid on lyophilization technique or mineral oil method of preservation. The viability of cryopreserved AIMs would be evaluated.
- Information on "Microbial Repository" would be integrated by the ARIS cell into a national database to get desired and reliable output in the form of reports. Linkages through electronic methods would be developed to integrate other "National Repository" of the country.
- The activities of human resource development will continue in various aspects of dissipation of knowledge on:
  - ✓ Study of biodiversity of AIMs;
  - ✓ Identification, preservation and conservation of AIMs;
  - ✓ New protocols and technologies;
  - ✓ Quality microbial management system with special emphasis on biosystematics;
  - ✓ DNA fingerprinting;
  - ✓ Molecular detection of microbes and on policy issues related to IPR.

Metarhizium sp.



Aspergillus fumigatus

Mandate

"To act as a nodal center at national and international level for acquisition and management of indigenous and exotic microbial resources for agriculture, and to carry out related research and human resources development for sustainable growth of agriculture."

Objectives

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

Bacillus sp.

Detailed Research Activities

Exploration and collection of AIMs

- From soils, plants, freshwater etc.-covering different agro-climatic regions of India
- Collection of AIMs from existing culture collection centers, institutions and universities.
- The Bureau will function as a repository for all the AIMs available in the country.
- Repatriation of cultures of Indian origin from different culture collections located at other countries, including international centers.

Identification, characterization and documentation of AIMs

- Morphological, physiological, biochemical, immunological and molecular characterization.
- Development of molecular markers and diagnostic tools.
- Database of the entire collection on electronic format for easy access of information.

Conservation, maintenance and utilization of AIMs

- Short-term and long-term conservation.
- Conservation of obligate parasites on host plants under controlled conditions.
- Build-up and exchange of exsiccate sets.
- Identification of AIMs for utilization as bio-fertilizers, bio-pesticides, growth promoters, bio-indicators and for bio-degradation, bio-remediation, bio-composting, food processing etc.
- Utilization of diagnostic tools.
- Utilization of molecular and immunological markers for diversity analysis.
- Information exchange.

Surveillance of indigenous/exotic AIMs

- Isolation and collection of exotic AIMs from different agro-climatic zones of India.
- Characterization of exotic AIMs on the basis of morphological, biochemical and molecular characters.
- Isolation and identification of bioactive compounds produced by exotic AIMs.
- Exploitation of AIMs for sustainable agriculture.

Microbial biodiversity and systematics

- Analysis of microbial diversity using different molecular methodology.
- Inter and intra species variation among microbial populations, its identification and quantification.
- Digitization of the microbial passport data.

Human resources development (HRD)

- Provide training to researchers in the field of molecular identification of AIMs; tool for microbial technology development and its implementation.
- Transfer of technology from laboratory to land.
- Training of scientists in the field of isolation, preservation and conservation of AIMs.
- Basic training regarding use of AIMs to students, teachers and farmers

Thrust Area During XIth Plan

Specific targets and monitoring during the XIth Plan

- Development of infrastructural facilities such as laboratories, library, cold rooms, culture collection units, cryopreservation unit, glasshouses, etc.
- Collaboration with other microbial resource centres (National and International).
- Repatriation of cultures.
- Study on microbial diversity of AIMs.

Characterization

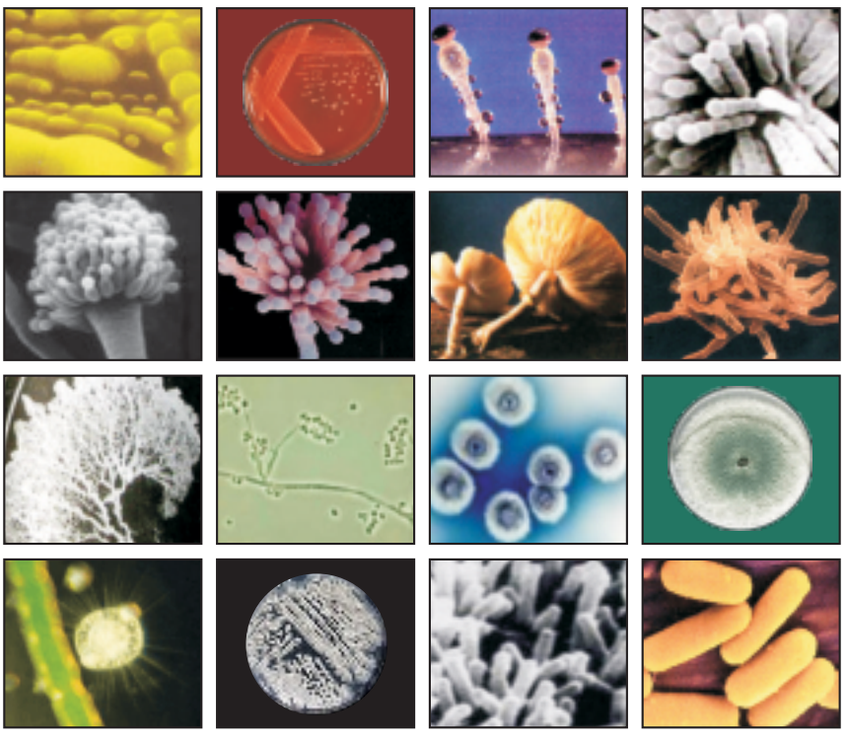
- Morphological, physiological, and biochemical.
- Molecular characterization based on prioritization with emphasis on IPR regimes.
- Development of molecular diagnostic tools.

Documentation and inventorization

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

Utilization

- Build up and exchange of exsiccate sets.
- Identification of AIMs for utilization as bio-fertilizers, bio-pesticides, growth promoting microorganisms, bio-indicators and for bio-degradation, bio-remediation, bio-composting.



World of Agriculturally Important Microbes

Salient Achievements

Germplasm conservation

- The number of cultures in the long term repository of NBAIM culture collection is 2800.
- All the microbial data available at NBAIM have been digitized and put in retrievable format.

Major Externally Funded Research Projects Concluded

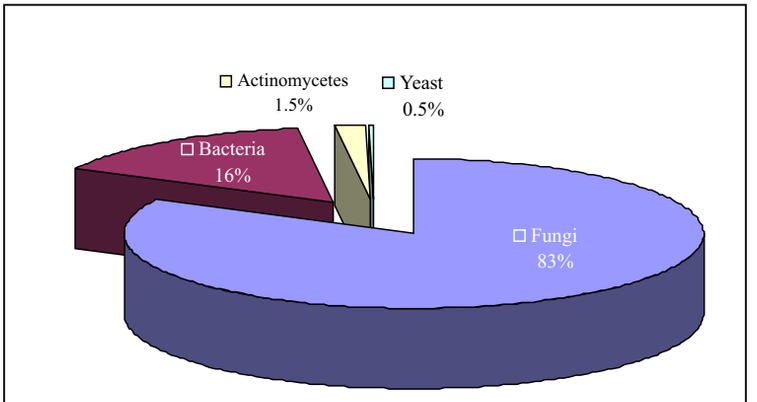
- "Diversity and Conservation of Agriculturally Important Microorganisms and their Potential as Biocontrol Agents" APCESS Project, ICAR, New Delhi.
- "Development of Sustainable Management Strategies for the Control of Parthenium weed in U.P using Biotechnological Approaches" funded by DBT, New Delhi.
- "Collection and Digitization of Agriculturally Important Microorganisms and their DNA Fingerprinting", APCESS Project, ICAR.
- "Development of Molecular Markers for the Identification and Characterization of *Fusarium* groups of Plant Pathogenic Fungi", ICAR Network Project, New Delhi.

Institute Projects

- Molecular and functional diversity of microorganisms isolated from extreme environments
- Assessment of genotypic diversity of *Bacillus, Bacillus*- derived genera and fluorescent Pseudomonads in Indo-Gangetic Plains
- Microbial diversity analysis of soils contaminated with industrial effluents in northern plains of Indo-Gangetic regions.
- Exploration, collection and identification of microbial pathogens from diseased rice plant samples from Indo Gangetic Plains of India
- Exploration, collection, biochemical, molecular and genetic characterization of actinomycetes in Indo-Gangetic Plains of India.
- Evaluation of rhizosphere fungi for plant growth promotion and induced resistance in some vegetable crops.
- Microbial management of soil borne plant pathogens in salt affected soils.
- Diversity analysis and utilization of some motile and non-motile actinomycetes from mangrove ecosystem of India
- Diversity analysis of plant growth promoting epiphytic and endophytic methylotrophic bacteria from different agro-ecological zones of India.

Culture Collection

- Biodiversity Authority of India (BDA) has recognized the NBAIM as National Repository.
- NBAIM follows strict quality control, biosafety standards and IPR issues in culture collection.
- Various types of microorganisms, including filamentous fungi, bacteria, actinomycetes and yeasts are maintained under long-term preservation. Each culture is preserved by at least two methods. Fungi are preserved either under mineral oil or by freeze-drying/ lyophilization. The bacteria, actinomycetes and yeast are preserved either by freeze-drying/ lyophilization or in glycerol at – 80° C. For short term storage, the cultures are maintained on slants in appropriate medium at 4°C.
- The NBAIM collection has wide diversity of fungi and includes more than 700 species belonging to 250 genera. Likewise the bacterial collection has more than 100 species belonging to 35 genera.



Diversity of microbes at NBAIM culture collection

Some useful microbes present in the repository are as follows:

Biocontrol Agents: *Trichoderma* spp. (*T. harzianum, T. viride, T. koningii, T. hametum*), *Paecilomyces lilacinus, Beauveria bassiana, Gliocladium vorens, Verticillium* spp.)

Plant growth Promoters: *Pseudomonas fluorescens, Rhizobium* spp., *Bradyrhizobium* spp., *Bacillus subtilis*

Potential Enzymes/ Antibiotics/ Toxins producers: *Fusarium pallidoroseum, F. oxysporum, Penicillium citronum, P. frequentens*, isolates of *Aspergillus*

Entomopathogenic: *Beauveria* spp., *Metarhizium* spp., *Paecilomyces* spp., *Verticillium* spp., *Nomuraea* spp.

Egg parasitic fungi: *Paecilomyces lilacinus, Verticillium chalamydosporium*

Bacteria possessing nematicidal and insecticidal properties: *Bacillus brevis, Paenibacillus alvei, Brevibacillus laterosporus*

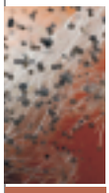
Biofertilizers: species of *Rhizobium, Azospirillum* and *Azotobacter*.

Some Important Bacterial Genera available at NBAIM Cultural Collection

Genera	Genera
Acacia	*Flavobacterium*
Acetobacter	*Lactobacillus*
Achromobacter	*Mesorhizobium*
Aeromonas	*Paenibacillus*
Aquitalea	*Pantoea*
Arthrobacter	*Pediococcus*
Azotobacter	*Peudomonas*
Bacillus	*Ralstonia*
Bradirhizobium	*Rhizobium*
Brevibacterium	*Sinorhizobium*
Candida	*Saccharomyces*
Cellulomonas	*Serratia*
Escherichia	*Staphylococcus*
Edwardsiella	*Stenotrophomonas*
Enterobacter	*Streptococcus*
Exiguobacterium	*Xanthomonas*

The Fungal Genera available at NBAIM Cultural Collection

Genera	Genera	Genera
Absidia	*Cylindrocarpon*	*Microxyphiella*
Achaetomiella	*Cylindrocladium*	*Moesziomyces*
Achaetomium	*Cytophaga*	*Monilinia*
Achlya	*Cytospora*	*Monoascus*
Acremoniella	*Dactylella*	*Monodictys*
Acremonium	*Datronia*	*Morchella*
Acrodictys	*Deightonella*	*Mortierella*
Acrophialophora	*Dichotomocladium*	*Mucor*
Actinomucor	*Dictyoarthrinium*	*Mycosphaerella*
Agaricus	*Diplodia*	*Mycotypha*
Agarwalia	*Dipodascus*	*Myrotheciopsis*
Allomyces	*Doassansia*	*Myrothecium*
Alternaria	*Drechslera*	*Narasimhania*
Amanita	*Dwayamala*	*Nattrassia*
Amblyosporium	*Echinocatena*	*Nectria*
Amorphotheca	*Ellurema*	*Nematoctonus*
Ampelomyces	*Elsinoë*	*Neocosmospora*
Aniptodera	*Emericella*	*Neosartorya*
Antromycopsis	*Emericellopsis*	*Neotestudina*
Aphanocladium	*Enterobacter*	*Neurospora*
Aplosporella	*Entyloma*	*Nigrospora*
Arcuadendron	*Ephelis*	*Nodulisporium*
Armillaria	*Epicoccum*	*Nomuorea*
Arthrinium	*Erwinia*	*Nomuraea*
Arthrobotrys	*Exophiala*	*Oidiodendron*
Arthroderma	*Exserohilum*	*Ophiostoma*
Ascobolu	*Fennellia*	*Pachleprium*
Ascochyta	*Flammulina*	*Paecilomyces*
Ascosphaera	*Fomes*	*Papulaspora*
Ascotricha	*Fusariella*	*Paracercospora*
Ashbya	*Fusarium*	*Paxillus*
Asordaria	*Gaeumannomyces*	*Pectinotrichum*
Aspergillus	*Ganoderma*	*Peltasterinostroma*
Aureobasidium	*Gelasinospora*	*Penicillifer*
Auricularia	*Geotrichum*	*Penicillium*
Bahusutrabeeja	*Gibberella*	*Perenniporia*
Bartalinia	*Gilbertella*	*Periconia*
Basidiobolus	*Gilmaniella*	*Pestalotiopsis*
Beauveria	*Glioc1adium*	*Ramichloridium*
Beltrania	*Gliocephalotrichum*	*Ramulispora*
Beltraniella	*Gliocladium*	*Rhinocladiella*
Beniowskia	*Gliomastix*	*Rhizoctonia*
Benjaminiella	*Gloeocercospora*	*Rhizomucor*
Bettsia	*Gloeophyllum*	*Rhizopus*
Bipolaris	*Gloeosporium*	*Sagenoma*
Blakesle	*Glomerella*	*Sarocladium*
Boothiella	*Gonatobotrys*	*Sclerotium*
Botryodiplodia	*Gonatobotryum*	*Scolecobasidium*
Botryosphaeria	*Gongronella*	*Scopulariopsis*
Botryotrichum	*Gonoderma*	*Sepedonium*



Botrytis	*Gonytrichum*	*Septoria*
Calonectria	*Graphium*	*Setosphaeria*
Cephaliophora	*Greeneria*	*Sordaria*
Cephalosporium	*Guignardia*	*Sirosporium*
Cephalotrichum	*Gymnascella*	*Spegazzinia*
Ceratocystis	*Helminthosporium*	*Sporothrix*
Cercospora	*Heterocephalum*	*Sporotrichum*
Ceriporia	*Heteroconium*	*Stachybotrys*
Chaetomella	*Hirsutella*	*Staphylotrichum*
Chaetomium	*Humicola*	*Stemphylium*
Chaetophona	*Hyalodendron*	*Stigmina*
Chalara	*Hymenochaete*	*Sutravarana*
Chlamydomyces	*Hymenopsis*	*Syncephalis*
Choanephora	*Hyphomucor*	*Thamnostylum*
Chondrostereum	*Hypocopra*	*Thermoascus*
Chrysosporium	*Hypoxylon*	*Thermomucor*
Ciliochorell	*Isaria*	*Thielavia*
Circinella	*Khuskia*	*Tiarosporella*
Cladobotryum	*Kirkomyces*	*Tolypocladium*
Cladosporium	*Laetiporus*	*Tolyposporium*
Claviceps	*Lagenidium*	*Trametes*
Clonostachys	*Lecythophora*	*Tricellula*
Cochliobolus	*Lentinula*	*Trichobotrys*
Cokeromyces	*Lentinus*	*Trichoderma*
Coleophoma	*Lenzites*	*Trichosporon*
Colletotrichum	*Lepiota*	*Trichothecium*
Conidiobolus	*Leptodontidium*	*Trichurus*
Coniella	*Linderina*	*Tritirachium*
Coniochaetidium	*Lophodermium*	*Ulocladium*
Coniothyrium	*Lophotrichus*	*Ulospora*
Coprinus	*Macrophomina*	*Utharomyces*
Coprinus	*Malbranchea*	*Venturia*
Coriolopsis	*Marasmius*	*Verticillium*
Coriolus	*Melanospora*	*Volutella*
Corynascus	*Memnoniella*	*Volvariella*
Corynespora	*Merimbla*	*Wardomyces*
Cryptosporiopsis	*Metarhizium*	*Wiesneriomyces*
Cunninghamella	*Microascus*	*Zopfiella*
Curvularia	*Microdochium*	*Zygorhynchus*
Cyclothyrium	*Microsporum*	*Zygosporium*

SIGNIFICANT RESEARCH ACHIEVEMENTS

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

PI : D. K. Arora, Director
Co-PI : A.K. Saxena, Principal Scientist
Rajeev Kaushik, Senior Scientist
A. B. Dash, Senior Scientist

Rationale

Extreme environments represent a unique ecosystem and may harbour novel microbial flora. Thermophiles from hot springs can be a source for enzymes that are active at high temperatures. They can also be used for decomposition process. Psychrophiles can be a source of anti freezing compounds. Halophiles and osmophiles can be a source of genes coding for osmolytes and can be used for the development of transgenic plants tolerant to salt and drought stress.

Objectives

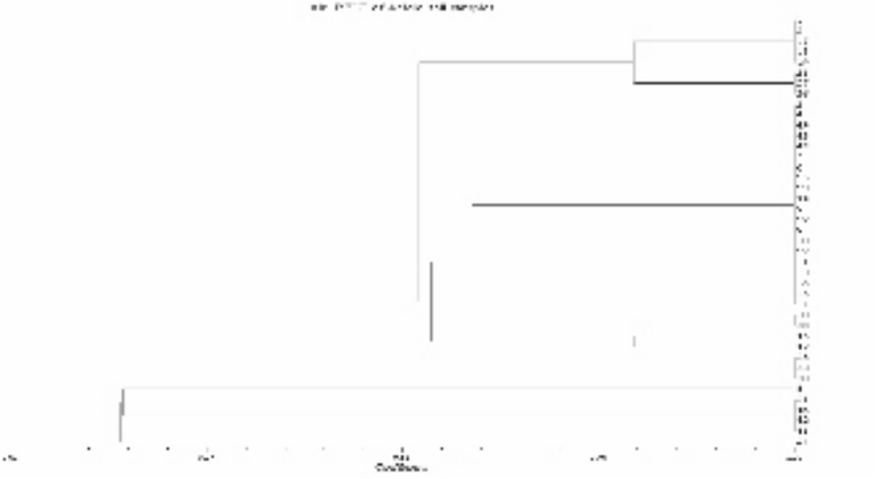
- To isolate, identify and characterize microbial strains from extreme environments (psychrophiles, thermophiles, halophiles and osmophiles).
- Molecular fingerprinting of the isolates.
- Characterization of novel microorganisms for their utilization in biodegradation of agricultural residues, bioremediation and mining genes for abiotic stress tolerance.

Diversity analysis of *Bacillus* in acidic soils of Kerala:

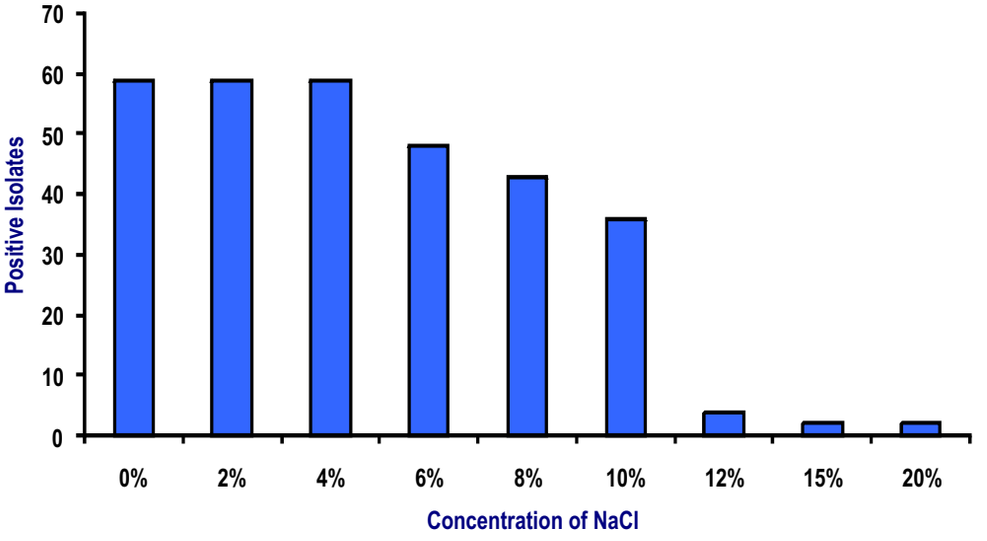
A total of 41 different morphotypes were selected for studies on functional attributes and molecular diversity. Of the 41 isolates, 28 and 22 were respectively positive for xylanase and cellulase activity. Of the biocontrol attributes, 7 isolates were positive for siderophore production, 17 produced ammonia whereas 18 produced HCN. As regards to the plant growth promoting attributes, 18 isolates exhibited P- solubilization activity whereas 23 were positive for IAA production.

Molecular characterization

PCR amplification of 16S rDNA gene, followed by RFLP analysis with *Alu*1 showed the presence of wide range of diversity among the isolates. Among the 41 isolates, 10 diffferent clusters were formed with similarity % ranging from 3 to 100%. The clustering of the isolates was irrespective of their geographical location.



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



Screening of isolates for salt tolerance

Isolation of bacteria from Sambhar salt lake (Rajasthan):

Soil, water and sediment samples were collected from Sambhar salt lake and processed for isolation of bacteria using different media, 59 isolates were obtained. Among the media used, the maximum population was obtained on NA medium amended with methyl red indicating the predominance of gram-positive bacteria in these samples. Of the eight samples analysed, the growth appeared on Jensen's medium only in four samples. Likewise, the population of fluorescent *Pseudomonas* was low in all the soil samples.

Screening of isolates for salt tolerance:

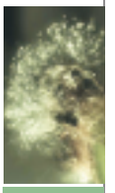
All isolates were screened for salt tolerance at graded concentration of NaCl ranging from 0 to 20%. All the 59 isolates were tolerant upto 4% NaCl wheras only 36 isolates showed growth upto 10% NaCl level. Two isolates were showed growth at 20% NaCl concentration.

DNA sequencing and Identification of some isolates:

PCR amplification of 16S rDNA was carried out and sequenced using ABI automated sequencer. Some of the isolates identified were: *Halomonas venusta*, *Natricola* sp., *Marinobacter alkaliphilus*, *Marinobacteria* sp. and *Bacillus thuringienses*.

Conclusions

Bacterial analysis of soil and water samples from two different extreme environments- acidic soils from Kerala and Sambhar salt lake (Rajasthan) led to the isolation of different types of microbes that could tolerate either low pH or high salt concentrations. There is a predominance of gram postive bacteria in Sambhar salt lake and the niche is not suitable for the growth and activity of nitrogen fixing bacteria. Bacteria capable of tolerating salt at 20% could have novel osmolytes and thus can be a source of novel genes that are responsible for tolerance to salt stress. Further work is in progress.



Project 2 : Assessment of Genotypic Diversity of *Bacillus, Bacillus*-derived Genera and Fluorescent Pseudomonads in Indo-Gangetic Plains

PI : A.K. Saxena, Principal Scientist
Co-PI : Rajeev Kaushik, Senior Scientist

Rationale

The genus *Bacillus* is a large, heterogeneous group of Gram positive, aerobic, endospore forming, rod shaped bacteria. Many species of *Bacillus* and fluorescent *Pseudomonas* are used as biocontrol agent and are effective against different soil-borne fungal diseases in vegetables, ornamental and agricultural plants. The distribution of *Bacillus* and *Pseudomonas* species is more pronounced in disease suppressive soils. In India, Indo-Gangetic plains are considered to be a fertile ecoregion with wheat-rice cropping system being most prevalent. However over the years there have been decline in the productivity in this region. Earlier there were no microbiological surveys carried out to look for the distribution of different species of *Bacillus* and fluorescent *Pseudomonas* that contribute significantly to crop productivity.

Objectives

1. Survey and collection of soil samples from Indo-gangetic plains.
2. Isolation of bacterial diversity from soil samples.
3. Biochemical characterization and identification of bacteria (*Bacillus, Bacillus*-derived genera and fluorescent Pseudomonads) from soil samples.
4. Molecular characterization of isolates and development of molecular probes for identification.

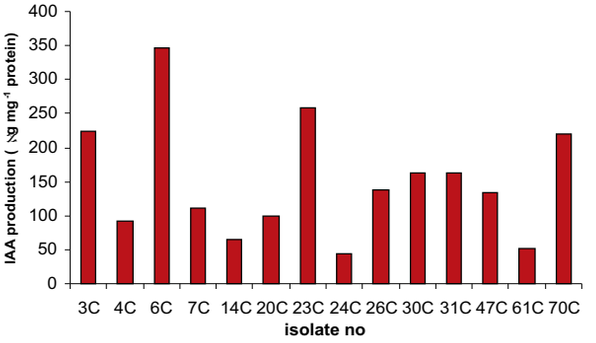
Research Achievements

Survey and collection of Soil Samples and isolation of *Bacillus* and fluorescent *Pseudomonas* :

Surveys were carried out to collect soil samples from different locations falling under Northern Indo Gangetic plains (IGP). It includes the districts of Northern Punjab *viz.,* Amritsar, Kapurthala, Jalandhar, Pagwara, Nawan Shahar, Ropar, and Mohali; villages falling in districts Lucknow, Sitapur, Bahraich, Allahabad, Kanpur and Varanasi of Uttar Pradesh. The soil samples were used for the isolation of *Bacillus* and fluorescent *Pseudomonas*. *Bacillus* obtained were isolated from Amritsar (Potato and Mustard), Kapurthala (Maize, Barley, Wheat and Berseem), Jalandhar (Mustard and Potato), Phagwara (Rice, Garlic, Jowar and Sugarcane), Nawansahar (Rice, Barley and Wheat), Ropar (Rice, Jowar and Wheat) and Mohali (Rice, Arhar, Maize and Wheat). A total of 24 soil samples were collected from the northern Indo-Gangetic plains and processed for the isolation of *Bacillus* and *Bacillus*-derived genera. Isolation of *Bacillus* species was carried out from the soil samples by enrichment technique. A total of 97 isolates were obtained and were purified on nutrient agar medium amended with methyl red.

Functional diversity among *Bacillus* isolates:

Functional diversity among *Bacillus* isolates was studied particularly with regards to plant growth promoting traits like production of IAA, siderophore and P-solubilization. Among the 97 isolates, only 14 were found to produce IAA, 20 were able to produce siderophore and 5 could solubilize phosphorus. The results clearly indicated that the soils do have population of *Bacillus* isolates in high numbers but the isolates/strains have lost the ability to express plant growth promoting attributes. Among the 14 isolates that produced IAA, maximum was produced by isolate 6C (346 µg mg^{-1} protein).



Production of IAA by *Bacillus* isolates obtained from different districts of Punjab

Isolation of fluorescent *Pseudomonas*:

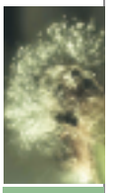
Fluorescent *Pseudomonas* were isolated using King's B medium from samples collected from different locations in Uttar Pradesh (Lucknow, Bahraich, Mau, Varanasi, Allahabad, Kanpur and Sitapur), Punjab (Amritsar, Kapurthala, Jalandhar, Phagwara, Nawansahar, Ropar and Mohali). The cultures showed green, yellow and blue coloured water-soluble fluorescent pigments. A total of 57 isolates were obtained from soil samples collected from districts of Lucknow, Sitapur and Bahraich, 41 from Punjab and 39 from districts Mau, Allahabad, Kanpur and Varanasi.

Functional diversity among fluorescent *Pseudomonas* isolates:

Functional diversity analysis clearly indicated that soil samples from Punjab and Lucknow had very low percentage of isolates producing IAA and siderophore; and solubilizing phosphorus. In contrast the isolates obtained from districts of Mau, Varanasi, Allahabad and Kanpur showed good production of all the traits. Out of 39 isolates, 28 were positive for IAA, 39 for siderophore and 27 for P-solubilization.

Conclusions

The diversity analysis of the two most important PGPRs, i.e *Bacillus* and *Pseudomonas* in northern IGP indicated that the population of both the groups is high in these areas. But the percentage of isolates expressing the PGP traits is very low in areas like Punjab and Lucknow where chemical fertilizers are used in moderately good quantity by the farmers. This study on functional diversity is in progress but still gives some microbiological reasons for the reduction in productivity under rice- wheat cropping system in IGP.



Project 3 : Microbial Diversity Analysis of Soils Contaminated with Industrial Effluent in Northern Plains of Indo-Gangetic Regions

PI : Rajeev Kaushik, Senior Scientist
Co-PI : A.K. Saxena, Principal Scientist

Rationale

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

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

Objectives:

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

Research Achievements

Survey of the farmer's field:

Survey of the farmer's fields, which are being irrigated with distillery and paper mill effluent for over 20 years in succession, was carried out in the month of October 2007 and January 2008 for collection of soil samples from rice and wheat fields respectively. Survey was carried out in the districts of Uttar Pradesh (*viz.* Gazhiabad, Meerut, Jyotiba Phule Nagar and Rampur) and Uttranchal (Udham Singh Nagar). The agricultural fields irrigated with different industrial effluents were surveyed for collection of soil and plant samples (*Viz.* Daurala Sugar Works, Daurala, Meerut; Simbhaoli Distilleries, Simbhaoli, Gazhiabad; Jubilant Organosys, Gajraula, Jyotiba Phule Nagar; Rampur distillery, Rampur and Century Paper mills, Lalkuan, Udham Singh Nagar). 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 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.

Changes on soil biological properties as a result of distillery effluent irrigation:

The short-term changes in soil microbial biomass are useful indicator for understanding the long-term productivity of soil. It is also frequently used as an early indicator of changes in soil physio-chemical properties resulting from soil management and environmental stresses in agricultural ecosystems. The long term irrigation of agricultural fields with anaerobically digested molasses based distillery effluent in Gajraula, Western Uttar Pradesh, caused significant increase in microbial biomass carbon to

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

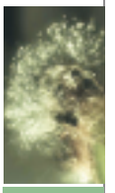
Carbon Source	Control Soil	DEIF Soil	CEIF Soil
Glycogen	0.31	2.52	2.04
α- D – Lactose	0.29	3.07	0.20
β-Methyl-D- Glucoside	1.60	3.21	1.84
D- Mannitol	3.63	2.09	1.55
D- Galactonic Acid Lactone	2.84	0.00	0.82
l- Arginine	0.62	1.74	1.41

microbial biomass nitrogen ratio (C_{mic} : N_{mic} ratio) from 3.72 to 6.48 in *kharif* season and from 4.34 to 7.62 in *Rabi* season. It has been reported that C_{mic} : N_{mic} is affected by soil properties such as application of organic effluents, pH, N- fertilization etc. The C_{mic} : N_{mic} ratio is often used to describe the structure and the state of the microbial community. A high C_{mic}:N_{mic} ratio of 7 - 12 indicates that the microbial biomass contains a higher proportion of fungi, whereas a low value of 3 – 6 suggests that bacteria predominate in the microbial population. In the present study the C_{mic}:N_{mic} ratio varied between 3.72 to 7.62 in effluent irrigated soils. The increase in C_{mic} : N_{mic} ratio suggests shift in bacterial community structure.

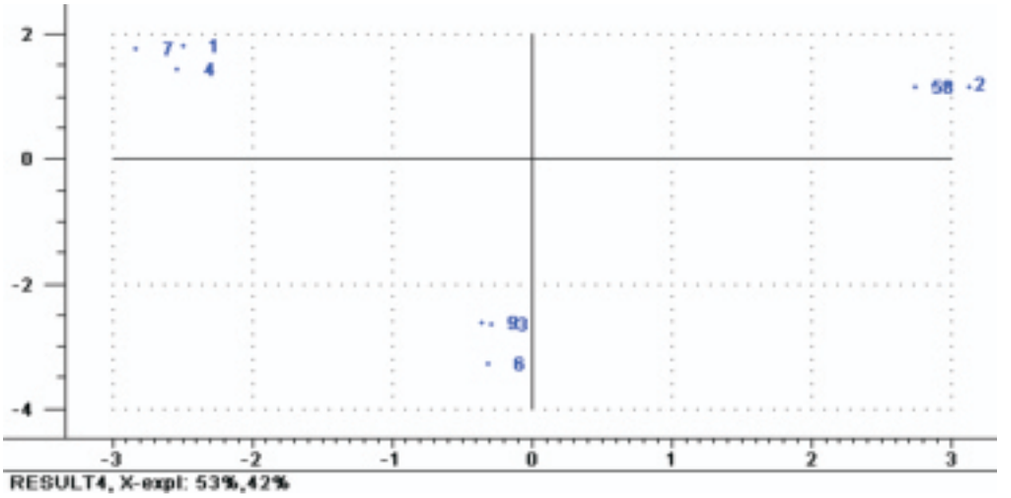
Long term application of distillery effluent also showed increase in soil organic carbon (SOC) over the period of 20 years. The percentage of C_{mic} in total soil OC showed significant variation in effluent irrigated fields of rice and wheat (1.46 – 2.16 and 2.18 – 3.43) respectively, whereas, in control soil it was 1.18 and 1.51, respectively. Although, the C_{mic} constitutes only 1–3% of SOC but it gives an estimation of the quantity of carbon in microbial biomass, quality of organic matter in soil, its availability and soil health. Increase in soil C_{mic} to SOC ratio up to 2 to more than 3 due to application of effluent in rice and wheat indicates that soil health may deteriorate if the practice of effluent application in regular manner is not stopped.

Community Level Physiological Profiling:

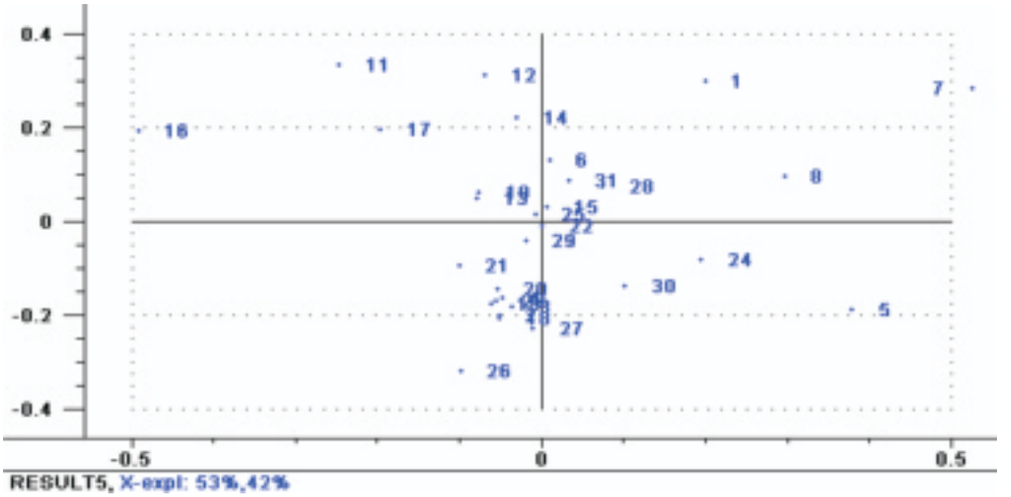
Carbon is a key factor governing microbial growth in soil, and functional aspects related to substrate utilisation could provide important information beyond that afforded by taxonomic level investigations or structural investigations based on rRNA analysis. The functional diversity of microorganisms,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



Three kinds of soil samples from pulp and paper mill effluent irrigated soils (control soil, diluted effluent irrigated field soil and concentrated effluent irrigated field soil samples) were used for community level physiological profiling in three replications. The data obtained was analyzed using Principal Component. Analysis and results are shown in Figures. The first principal components accounted for about 95% of variance. An ordination diagram from the CLPP results produced clear separation of the control as well as treated soil samples. Significant effect of pulp and paper mill effluent application on soil metabolic profiling was observed in 32 hrs of incubation. An ordination diagram from 31 C source utilization pattern also showed clear separation of different C utilization. C source Glycogen, α- D – Lactose, β-Methyl-D- Glucoside, D- Mannitol, D- Galactonic Acid Lactone and l- Arginine in particular showed more variation than other types of carbons.



PCA of all data (n=3 samples) of three types of soil samples (sample No. 1, 7 and 4 represent control soil; sample No. 2, 5 and 8 represent diluted effluent irrigated field soil sample; sample no. 3, 6 and 9 represents concentrated effluent irrigated field soil samples



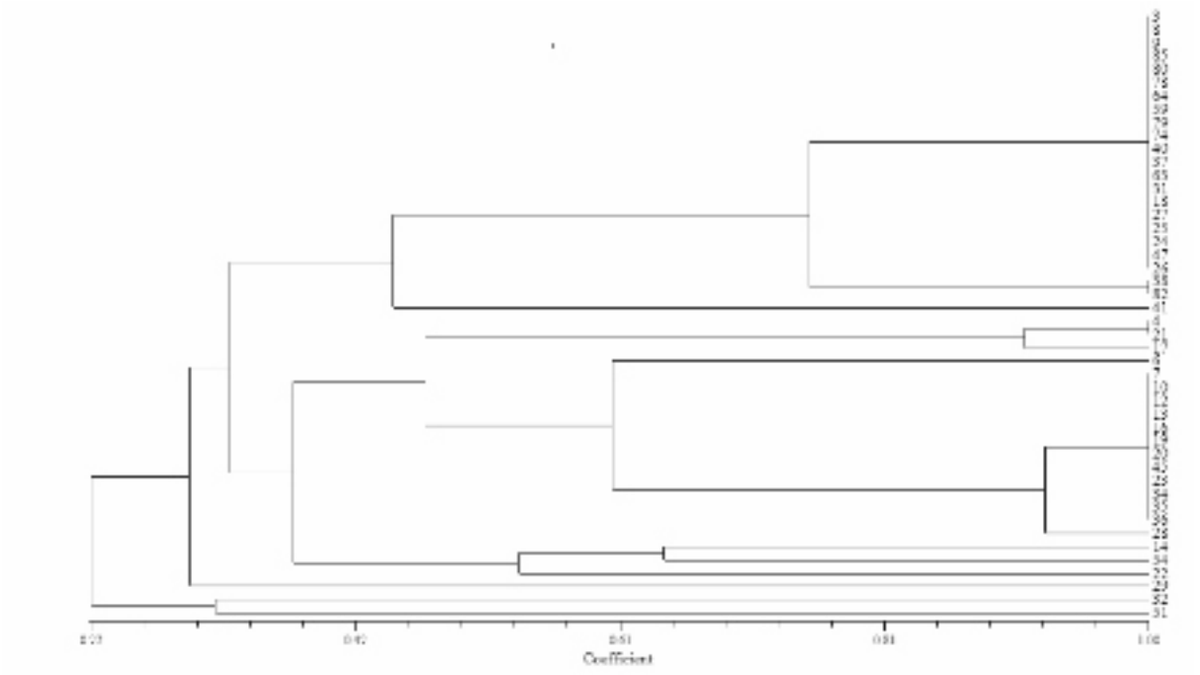
Ordination diagram of 31 carbon source

Isolation of *Bacillus* from soils treated with paper mill effluent (Lalkuan)

Fourteen samples collected from paper mill effluent treated soils were analyzed for the counts of *Bacillus*. The *Bacillus* count in the samples varied from 6.4 to 283 x 10^4 cfu g^{-1} soil. In general, there is no influence on the population of *Bacillus* due to irrigation of soil samples with paper mill effluent. A total of 51 isolates based on colony morphology were picked and further characterized for production of xylanases and cellulase activity.

Molecular characterization of *Bacillus* isolates

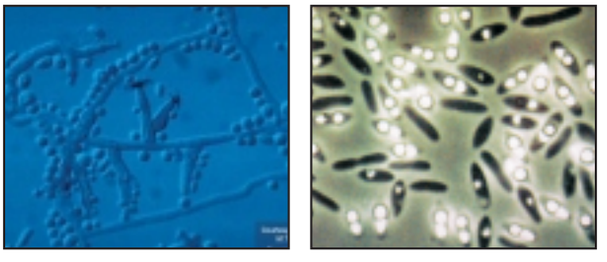
PCR amplification of 16S rDNA was carried out employing the genomic DNA isolated from all the *Bacillus* isolates obtained from paper effluent treated soils. RFLP analysis was carried out using three restriction enzymes. The isolates showed similarity between 22 to 100%. Twenty isolates showed 100% homology among themselves. Another cluster of 12 isolates showed 100% homology (fig 3). The results revealed limited diversity among the *Bacillus* isolates indicating the enrichment of only certain groups in soils having long history of irrigation with paper mill effluent.



Combined dendrogram showing similarity among *Bacillus* isolates obtained from paper industry effluent treated soil samples based on 16SrDNA –RFLP with restriction endonucleases *Alu*I, *Hae*III, *Msp*I.

Conclusions

The irrigation of agricultural soils with effluent from the paper mill and distillery has a significant influence on the soil physicochemical properties and microbial diversity. The CLPP analysis using BIOLOG ECO plates clearly indicated that the microbial population in the control fields is different from the population in the effluent irrigated field based on the C utilization pattern. The results could be further authenticated by using more number of soil samples for the PCA analysis. The diversity of the genus *Bacillus* is not influenced by paper mill effluent treatment.



Project 4: Evaluation of Rhizosphere Fungi for Plant Growth Promotion and Induced Resistance in some Vegetable Crops

PI : Alok K. Srivastava, Senior Scientist
Co-PI : Sudheer Kumar, Senior Scientist

Rationale

Growing of cucurbitaceous and solanaceous vegetables in IGP ecosystem constitute a distinct type of farming in India. The cropping pattern in these areas is based on rainfed subsistence farming and wholesome indigenous vegetables grown in the lower Indo-Gangetic plains of India. The direct effects of the rhizosphere fungi on plant growth and development are crucially important for agricultural uses and for understanding the roles of these fungi in natural and managed ecosystems. Root colonization by *Trichoderma* and other fungal strains frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients. *Trichoderma* strains that produce cytokinin-like molecules, e.g. zeatyn and gibberellin GA3 or GA3-related, have been recently detected. The controlled production of these compounds could improve biofertilization. Together with the synthesis or stimulation of phytohormone production, most of the rhizosphere fungi acidify their surrounding environment by secreting organic acids and able to solubilize phosphates, micronutrients and mineral cations including iron, manganese and magnesium.

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

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

Objectives

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

Significant Achievements

- Soil and water samples from Kanpur and Lucknow area was obtained from the survey conducted at different locations. Soil samples were taken from the cultivated, uncultivated, barren field, forest rhizosphere etc. pH and texture of the samples were determined and a total of 40 fungal isolates were purified from the samples. The isolates were characterized on the basis of morphology and growth characteristics and finally 15 isolates were maintained on acidified potato dextrose agar (APDA).

- Plant parts viz: leaves, root and rhizosphere soil from different vegetable crops were collected from the field and examined for the isolation of fungi as endophyte. The samples were washed thoroughly with water then surface sterilized by the NaOCl and 70% methanol. The surface sterilized plant parts were crushed in sterile buffer saline and aliquots (0.2 ml) plated on different fungal media. Six distinct fungal isolates growing as endophyte were obtained and maintained.
- Besides the isolates of IGP, 15 fungi, 4 bacteria and 2 actinomycetes isolates from NBAIM culture collection were evaluated for growth suppression of plant pathogenic fungi *R. solani* by dual culture on APDA plates. Out of 22 fungal isolates, 9 isolates showing inhibition zone >15mm were selected for further study in field soil.
- Interaction studies between *Trichoderma* spp. and *R. solani* was carried out to look for molecular signalling between the pathogen and antagonistic agent. Strips of freshly grown pure culture of *T. harzianum* and *R. solani* were taken out, placed 5 cm apart in the petri plates and incubated at 28±2^0 C. The actively growing mycelia of *Trichoderma* were collected from the zone of interaction at different intervals i.e. before contact, at the time of contact and 24 h after contact till 5 days, weighed and ground in liquid nitrogen. The powder was homogenized in cold PBS (1:2 w/v; 2 min at 4 ^{0}C). The homogenate was centrifuged twice (10000 *g*; 20 min at 4 ^{0}C), supernatant was collected and stored at -20 ^{0}C. The supernatant served as crude enzyme preparation (CEP). Proteins were obtained during the interaction of *T. harzianum* with *R. solani* on dual cultures. The activity was recorded at 24 h before contact, 0 h (just before contact), 24 h, 72 h, 96h an d 120 h after contact.

Conclusions

Overall, the findings demonstrate that some fungal isolates selected in this study have the potential to suppress the growth of *R. solani* in direct confrontation assay. *Trichoderma* strain showed enhanced secretion of chitinases during interaction with *R. solani.* Further work is in progress to elucidate the role of hydrolytic enzymes in signaling during antagonistic interactions and to utilize them as biocontrol agent under greenhouse.

Macrophomina phaseolina

Fusarium roseum

Project 5 : Microbial Management of Soil Borne Plant Pathogens in Salt Affected Soils

PI : Sudheer Kumar, Senior Scientist
Co-PI : Mahesh Yandigeri, Scientist

Rationale

There is an increasing interest in the exploitation of fungi and bacteria as biocontrol agents (BCAs) for the management of diseases of agricultural crops worldwide to replace/minimize the uses of harmful chemical pesticides. The excessive dependence on chemical pesticides leads to the development of resistance in plant pathogens and occurrence of residues in food chain and also because of the increased demand for organically grown agricultural products, BCAs have become an important alternative disease control agents.

Biocontrol agents are applied in agricultural soils with certain pH characteristic, salinity levels, moisture levels and different temperature. The most commonly used antagonistic fungi are *Trichoderma* species. The increased salt (NaCl) concentration decreases the antagonistic potential of *Trichoderma* spp. One of the most crucial boundary to use *Trichoderma* strains as biocontrol agent is their low salt tolerance. Thus the research programme is formulated to select the salt tolerant strain of different biocontrol agents including *Trichoderma* spp., and its mechanism of antagonism.

Objectives

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

Significant Achievements

Soil samples were collected from salt affected soil of IGP region viz. Kanpur, Unnao, Lucknow, Allahbad, Mau, Varanasi, and Ghazipur. The samples having high pH or salinity were used to isolate different fungi and bacteria. The different selective and semi-selective media were used for isolation and a total 44 fungi and 130 bacteria were isolated and purified. These fungal and bacterial isolates were screened against locally important soil borne plant pathogens i.e. *Rhizoctonia solani*, *Fusarium udum* and *Sclerotium rolfsii*. Out of 44 fungal isolates, 5 found potential antagonist against *Rhizoctonia solani*, 8 against *Fusarium udum* and 5 against *Sclerotium rolfsii,* respectively in dual plate method on Potato Dextrose Medium. The maximum mycelial growth inhibition was recorded upto 75%.

Among bacteria, 40 belong to *Pseudomonas* and remaining 90 to *Bacillus* and other genera. Thirteen isolates showed potential antagonist against *Rhizoctonia solani*, 20 against *Fusarium udum* and 23 against *Sclerotium rolfsii*. The bacterial isolates showed more than 2mm zone of inhibition were selected for further study. On the basis of preliminary screening the secondary metabolite / culture filtrate of 36 bacterial isolates were evaluated for growth inhibition of pathogens using well diffusion method. Out of 36, the culture filtrate of 8 isolates inhibited the mycelial growth of *Rhizoctonia solani* and 11 of *Fusarium udum* in well diffusion test. The size of zone of inhibition ranged from 7 to 16 mm. No bacterial isolate found promising against *Sclerotium rolfsii* in well diffusion test.

These selected bacterial isolates were also evaluated for production of volatile compound for growth inhibition. The sealed plate method was used to evaluate volatile compound production. Out of 36 bacterial isolates 13 were found effective in growth inhibition of all the test pathogens and some bacterial isolates showed maximum upto 98% growth inhibition over control.

The fungal isolates found promising in preliminary screening were screened for salt tolerance on semi solid and broth medium at different salt concentrations (0%, 2%, 4% and 6% of NaCl). The screening was done on the basis of growth, sporulation and dry weight of the mycelium grown in broth culture of fungal isolates. In general, the growth reduces as increasing of salt concentration but some fungal isolates given stable growth upto 6% of NaCl. The growth kinetics of potential bacterial antagonists at different salt conc. is under progress.

Conclusion:

In order to select salt tolerant biocontrol agent that can work better under salt affected soils, total 44 fungal and 130 bacterial isolates were screened against locally important plant pathogen *viz*. *Rhizoctonia solani, Fusarium udum, Sclerotium rolfsii*. Out of these, 8 fungal and 13 bacterial isolates found promising under *in vitro* condition on dual plate, secondary metabolite / culture filtrate and volatile compound screening. The selected fungal isolates have been screened for salt tolerance and some isolates showed stable growth upto 6% of NaCl. The bacterial isolates will be evaluated for salt tolerance and disease management.

Project 6 : Exploration, Collection, Biochemical, Molecular and Genetic Characterization of Actinomycetes from Indogangetic Plain of India.

PI : Anurag Chaurasia

Rationale

Indogangetic plain is a major crop production region of the country and has rich biodiversity, hence this project has been formulated to isolate, utilize and conserve actinomycetes diversity of indogangetic plain which will be exploited for agricultural productivity and human welfare.

Objectives:

- Survey and collection of soil/plant/water samples from Indogangetic plain of India.
- Isolation of actinomycetes diversity from experimental samples.
- Biochemical, molecular and genetic characterization of identified isolates.
- Application of useful isolates for agricultural productivity and human welfare.

Significant Achievements

Actinomycetes were isolated from soil, water and plant samples. Sixty eight actinomycetes strains were isolated from soil samples collected from Lucknow, Bahiraich, Kanpur, Allahabad and Lal Kuan (Pantnagar) area of Indo-Gangetic Plain. From Ganga water of Varanasi (sewage discharge site, funeral site and normal fresh site) diverse, unique and location specific actinomycetes were isolated.

Endophytic Actinomycetes

From root samples of rice, water caltrop, tomato, banana, wheat and rhizome of turmeric plant, endophytic actinomycetes were isolated. These isolates were identified as *Streptomycetes* species based on morphological chrateristics. From the wheat root samples few Actinoplanes were also isolated. Pleomorphic actinomycetes which shows bacterial, actinomycetes and fungal morphology in same life cycle was isolated from water caltrop roots. Interestingly no actinomycetes could be isolated from pond water samples of caltrop plant.

Conclusion:

Soil samples show more diversity of actinomycetes compared to water samples. Even in water samples diversity changes with respect to location and time. Least diversity was observed in the case of endophytic actinomycetes population. Healthy resistant plants show different diversity pattern of endophytic actinomycetes compared to disease infested plant, it is because of presence of specific endophytic actinomycetes which work as antibiotic factory and produces bio-active compounds with biocontrol and PGPR potential.

Project 7: Diversity Analysis and Utilization of Some Motile and Non-motile Actinomycetes from Mangrove Ecosystems of India

PI : Mahesh Yandigeri, Scientist
Co-PI : D. K. Arora, Director

Rationale

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

Objectives:

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

Significant achievements:

Survey and soil sample collection was carried out in Sunderbans (Mangrove ecosystem) of West Bengal for the isolation of motile and non-motile actinomycetes using enrichment culture technique. From eight soil samples 21 distinct morphotypes of non-motile actinomycetes were isolated using starch casein agar, actinomycetes isolation agar and streptomyces agar. For isolation of motile actinomycetes 'Rehydration and Centrifugation' method was used. Morphological characters such as colour of aerial mycelium, colour of substrate mycelium, colour of soluble pigments produced, single colony morphology were recorded. Based on growth pattern (fast, moderate and slow), and sporulation three categories of actinomycetes were classified *viz.,* six moderate to fast growers, 11 moderate growers and five slow growers. The isolates were subjected for antagonistic activities against root rot pathogen *Rhizoctonia solani.* Isolate numbers 15 and 21 were able to show moderate antagonism towards *R. solani.* Isolates were also tested for their ability to tolerate salt stress with a view to utilize their potential under saline soils as bio-inoculants with known potential. Seven actinomycetes isolates were able to tolerate up to 10% NaCl till 10 days of incubation.

From the soil samples collected from Pulicat salt lake and Chennai (Tamil Nadu), 11 morphologically distinct actinomycetes were isolated. Five moderate growers, four moderate to fast growers and two fast growing actinomycetes were isolated. Twenty one different morphotypes of actinomycetes were obtained from Mangroves of Gujarat (Surat, Bharuch and Navasari) and are maintained in pure form.

Conclusion

Out of the three mangrove ecosystems *viz.*, Sunderbans of West Bengal, mangroves of Tamilnadu (Pulicat salt Lake) and mangroves of Gujarat a total of 53 actinomycetes were isolated. Structural diversity of actinomycetes based on the morphotypes was observed to be more in West Bengal mangrove ecosystem in comparison to mangroves of Tamil Nadu and Gujarat. Two actinomycete isolates from Sunderbans were found to be antagonistic against *R. solani*. Three isolates were found to be pigment producers. Seven isolates were found to have salinity tolerance up to 10% NaCl. These isolates may have potential in alleviation of salt stresses and biotechnological applications. The isolates will be further studied for the functional diversity. The huge database developed from the study will aid in identification of the isolates.

Project 8. Diversity Analysis of Plant Growth Promoting Epiphytic and Endophytic Methylotrophic Bacteria from Different Agro-ecological Zones of India

PI : Kamlesh Kumar Meena, Scientist
Co-PI : Mahesh Yandigeri, Scientist

Rationale

Bacteria of the genus *Methylobacterium* are facultative methylotrophs classified as α-proteobacteria, and because of their distinctive pink pigmentation, they are sometimes referred to as pink-pigmented facultative methylotrophic bacteria (PPFMs). They are strict aerobes, Gram-negative rods, able to grow on methanol and methylamine and several other C_2, C_3 and C_4 carbon compounds. PPFMs are characterized by the capability to grow on one-carbon compounds. They are easily isolated on a methanol based mineral medium. PPFMs are commonly found in association within plants and have been hypothesized to potentially dominate the phyllosphere bacterial population. The diversity of such microbes is unseen national as well as international resource that deserves greater attention. The ability of PPFMs to utilize and grow on various carbon compounds will be helpful in minimizing the hazardous environmental impacts. The PPFMs offer the associative symbiotic life with crop plants by utilizing the methanol emitting through the leaves and in return provide cytokinins to the plants. Thus these microorganisms have diverse roles to play with crop production and enhancement of crop yield. Recently published data suggest that the degree of the plant-*Methylobacterium* association varies from strong, or symbiotic to loose or epiphytic, a range that also includes the intermediate endophytic association. *Methylobacterium*, as plant symbiont, thus impart beneficial effects on plant growth through direct or indirect mechanisms, which include production of phytohormones or enzymes that modulate plant growth, secretion of compounds involved in biocontrol, or disease suppression

Bacteria of the genus *Methylobacterium* were, furthermore, found to nodulate legumes of the genus *Crotalaria* indicating strong plant bacteria interaction. As for the PPFMs population on crop plants and their dynamics, available data are very less or negligible in respect to Indian scenario. Therefore, this project is being formulated to study the rhizospheric, phyllospheric and endophytic diversity of PPFMs and other non-pigmented *Methylobacterium* with respect to different plant in different ecological regions of India, to evaluate their potential to improve the crop productivity and biocontrol, to know about the diversity of this bacterium in India with respect to plant of different regions.

Objectives:

- Isolation and characterization of pigmented and non-pigmented methylotrophic bacteria from epiphytic and endophytic regions of different major crops growing in agro-ecological regions of India
- Assessment of PGP, biocontrol potential and nodulation efficiency of selected isolates and their utilization to improve crop production
- Development of molecular probes for rapid identification of *Methylobacterium*.

Significant Achievements

Survey and collection of soil samples:

Two surveys were carried out in different regions of Mau district in Uttar Pradesh and in the mangrove ecosystem of Gujrat for collection of soil, plant and water samples. From the samples Pink Pigmented Facultative Methylotrophs (PPFMs) were isolated using selective medium - Ammonium Mineral Salt medium (AMS). The colonies of the methylotrophs were selected on the basis of their color and purified by repeated streaking method.

Isolation of PPFMs

From Mau district in Uttar Pradesh isolations were made from the rhizosphere and phyllosphere of rice and sugarcane crop. The population of PPFMs was more in the phyllosphere as compared to rhizosphere of both the crops. Among the crops, rice phyllosphere supported a population of 155 x 10^2 g^{-1} leaf as compared to sugarcane that supported 145× x 10^2 g^{-1} leaf.

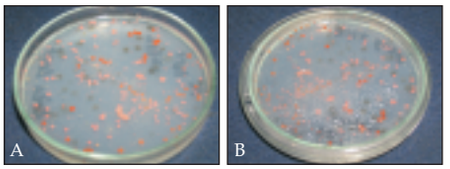
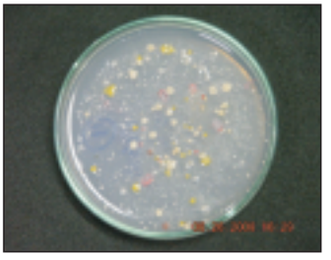


Plate count of PPFMs on Ammonium Mineral Salt Medium from A. Rhizosphere of rice and B. Phyllosphere of rice

Isolations were also made from the plant and soil samples collected from mangrove ecosystem of Gujarat. In general, among the 18 samples collected, the population of PPFMs varied from 15 to 290 cfu g^{-1} soil. Maximum cfu of 290 x 10^2 PPFM g^{-1} soil was obtained from the rhizospheric samples of Bilimora-Navsari followed by Olpad-Surat (286 x 10^2 PPFM g^{-1} soil). The plates spread with dilutions showed non-pigmented as well as yellow pigmented colonies besides the pink pigmented colonies.



Population count of methylotroph in phyllosphere of lady finger

Isolations were also made by directly imprinting the leaves of the plants on the medium. The population of the PPFMs was higher near the midrib and veins. It is reported that the leaves of the plants releases methanol that helps in the proliferation of these microbes in the phyllosphere. All these isolates are in the process of identification and characterization both at biochemical and molecular level.

Conclusions:

The population of methylotrophs was greater in the phyllosphere as compared to the rhizosphere for both crops *i.e.,* rice and sugarcane. Phyllosphere seems to be an ideal niche for the proliferation of methylotrophs as it has been reported that leaves exude methanol which is the chief C-source for the methylotrophs. The methylotrophs were found to be diverse phenotypically in terms of colony colour ranging from pink, yellow and whitish. Population count was higher in case of water samples from Danti Village, Gulf of the Khambat, Gujarat followed by water sample collected from the Naramda river basin near the Bharuch District of Gujarat. Methylotrophs having non-pigmented colonies were isolated from rhizosphere of the *Salicornia* (a salt tolerant plant having a very good medicinal value, generally found in extreme regions like drought affected soils of the Rajasthan, in marsh and salty mangroves ecosystem of the country). This could have wider application as these methylotrophs may be contributing to the growth of *Salicornia* under extreme environments. These unique methyotrophs are in the process of characterization.

Exploration, Collection and Identification of Agriculturally Important Microorganisms (AIMs) Collected from Diseased Plant materials and Soils from Different Agro-climatic Regions of Indo-Gangetic Plains (IGP).

PI : A. B. Dash

Rationale

In the Indo Gangetic Plain (IGP) numbers of fungal, bacterial and actionomycetes are noticed on different crop plants. Some of the disease appears in a severe form due to the emergence of different races of pathogens. So the collection, identification and characterization of different strains of the pathogens from different Agroclimatic regions of Indo-Gangetic Plain are absolutely necessary. Apart from pathogens isolation of different microorganisms from rhizosphere is done for future use to promote plant growth, as bio-fertilizer, bio-remediation, as bio-control agents and to produce broad range of insecticidal toxins.

Significant Achievements:

Survey was undertaken during October, 2007 at Kanpur Urban and its adjacent villages like; Karmi-mizala Mau, Gahera, Bhauti, Mangauli, Chachandi, Ramnagar, Chaubepur, Hradaypur, etc. and Kanpur to Allahabad on the way villages like; Gudgudiyapur, Aown, Jainpur, Fatehpur, Thariyaon, Kathahan, Kalahanpur, Sirathu, Saini, Kamasin, Tedimore and Muratganj.

A total number of 26 fungal flora were isolated from the diseased rice plants and discoloured seeds. The following fungi were identified based on their diseased symptoms and morphological characters.

Rhizoctonia solani – causes sheath blight disease of rice (4 isolates). The lesions are greenish grey, irregular with dark brown margin and appears on the sheath of older plants like snake scales. Small sclerotia are formed on the lesion and are loosely attached to the surface. The hyphae are colourless when young, becoming yellowish brown when older with infrequent septations. The hyphae sometimes give rise to short, swollen, much branched cells. The sclerotia are brown, globose but flattened below and loosely connected by mycelial threads.

Ustilago virens – causes False smut disease(2 isolates). Green and Yellow balls found in some individual grains of the panicle. The fungus transforms individual grains of the panicle into greenish spore balls of velvety appearance. The balls are smooth, yellow and are covered by a membrane. The mycelium are tightly woven together with host tissue. Chlamydospores formed on the spore balls are borne laterally on minute sterigmata on radial hyphae. Chlamydospores germinate in culture by germ tubes which become septate and form conidiophores bearing conidia at the tapering apex. These conidia are ovoid and very minute.

Helminthosporium oryzae – causes Brown spot disease (6 isolates – 4 isolates from leaves and 2 isolates from discoloured grains). Leaf spotting is the most common and readily observed symptom. Spots are brown, round to oval, 2-5 mm length. Rice plants are commonly susceptible to this disease at the flowering stage. Black oval spots appears on the grain. Abundant branching and anastomosing, dark brown hyphae; Sporophores arises as lateral branches from hyphae, usually branched, septated, brown to black conidia (4-13 septa), slightly curved, widest at the middle and rounded at both the ends.

Sclerotium oryzae – causes Stem rot disease of rice (2 isolates). The main symptoms of the disease are the excessive production of late tillers; which becomes discoloured and rotten. The first symptom is the appearance of small, black, irregular lesions on the outer leaf sheath. Numerous sclerotia are found in the host tissue, which are black and shiny and visible in naked eye as black spots. Hyphae white to olivaceous, septate, profusely branched. In culture mycelium white at first, later it becomes black. Sclerotia black, smooth and covered with white mycelium. Conidiophores dark coloured, septate, erect. Conidia form singly onn pointed sterigmata, 3-septate and curved.

Pyricularia oryzae - causes Blast disease of rice (4 isolates – 3 isolates from leaf and one isolate from discoloured seed).The fungus attacks all aerial parts of rice plants in all stages of growth. Leaves and neck of the panicle are commonly affected. On the leaves the lesions appear as distinct large, indefinite, spindle shaped, grey centre and brown margin. Spindle shaped spots appears on the grains. Conidiophores

long, slender, mostly simple, rounded shaped, little thickened at the base and septate at that part. Conidia obclavate, tapering at the apex, 2 septate and attach at the broader end.

Fusarium moniliforme – causes foot rot disease of rice plants (3 isolates; one isolate from discoloured sheath and 2 isolates from discoloured seeds). Leaves and leaf sheath drying out. Lower nodes discoloured above water levels and with adventitious roots. Plants taller than normal. Colonies broadly spreading yellow to rosy white in colour. In the initial stage micro conidia produced in chains and remains attached, with the conidiophore one to two celled, spindled shaped. Macro conidia straight, tapering at both ends and three to five septate . Chlamydospore lacking.

Sarocladium oryzae – causes Sheath rot disease of rice (one isolate). Oblong or irregular lesions with brown margins and grey centres appear on leaf sheath, especially on the sheath covering the panicle. The panicle remain within the sheath or only partially emerge. Whitish powdery fungal growth inside the rotten sheath. Mycelium white, branched and septate. Conidiophores arising from the mycelium, slightly thicker than the vegetative hyphae, 3-4 branches in a whorl.

Curvularia lunata – 2 isolates from discoloured seeds. Black tipped grains. Colonies broadly spreading brown to black and velvety. Mycelium septate, richly branched. Conidiophores dark brown septate, erect and unbranched. Conidia borne at the apex, four celled and tapering towards both the ends. Conidia dark brown, curved and borne in clusters of 2 or 3.

Penicillium spp. – 3 isolates from discoloured grains. Green or yellowish grains. Colonies growing rapidly in medium, Vegetative hyphae creeping, densely . Interwoven, separate, much branched and green in colour. Conidiophores erect, unbranched, septate and with brush like tips. Conidia bearing cells (phialida) borne directly on the apex of the conidiophores and in some secondary conidiophores are borne on the main conidiophores. Conidia borne in chains which typically form a brush like head and are spherical.

Fusarium solani – 2 isolates from discoloured grains. Grains with whitish powdery growth. Colonies broadly spreading on PDA medium, hyphae brownish – white in colour and leathery type. Micro conidia scattered. Macro conidia spindle shaped, slightly curved and 3-5 septate. Chlamydospores globes one celled.

Aspergillus flavus – 2 isolates from discoloured grains. Green or yellowish grains

Colonies growing rapidly in PDA medium and is green in colour. Conidiophores arise separately from the substratum and varying in length and diameter. Conidia produce in chains, pyriform (pear shaped) and green in colour. This species produce sclerotia. Cleisothecia (closed fruit body) not found.

Aspergillus niger – 2 isolates from discoloured grains. Colonies rapidly growing with abundant mycelium in PDA medium. Conidiophores arise directly from the substratum, smooth, septate varying greatly in length and diameter. Conidia are blackish – brown in color varying in size, produced in chains and are globose (spherical)

Conclusion

Out of the samples collected in the survey 41 pathogenic and saparophytic species of different general were identified and maintained in culture collection. Few other isolates are in the process of identification. Besides these isolates other 60 fungal cultures were isolated from different soil samples and 15 were identified.

Network project on 'Application of Microorganisms in Agriculture and Allied Sectors'

Theme: Microbial Diversity and Identification

Sub Project I: Diagnostic Kit for the Identification of Soil Microbes (*Bacillus* and *Pseudomonas*)

PI : D. K. Arora, Director
Co-PI : A. K. Saxena, Principal Scientist
SRFs : Sachi Vardhan, Subhash Yadav

Rationale

The genus *Bacillus* is a large, heterogeneous group of Gram +ve, aerobic, endospore forming, rod shaped bacteria. Several approaches based on phenotypic or genotypic characters have been proposed to classify *Bacillus* sp. Further characterization at the genotypic and phenotypic levels of selected *Bacillus* species have led to the creation of several new genera like *Alicyclobacillus, Paenibacillus, Brevibacillus, Virgibacillus, Geobacillus, Filobacillus, Jeotgalibacillus, Aneurinibacillus, Gracibacillus* and *Marinibacillus*. There are more than 200 species of *Bacillus* and it is difficult to identify the species of *Bacillus* on morphological, cultural and biochemical methods. Diagnostics based on molecular techniques could be employed to distinguish *Bacillus* species and *Bacillus* derived genera.

Objectives

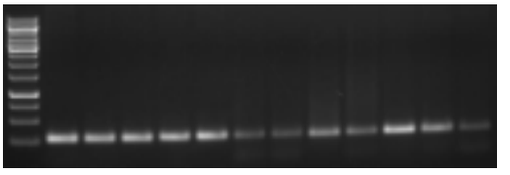
1. To isolate and characterize the species of *Bacillus* and *Pseudomonas.*
2. To develop rapid diagnostic kits.

Significant Achievements

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'
265R1 - 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.



PCR Amplification of 220 bp fragement in *Bacillus* strains by designed primers 265F1 and 265R1.

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 from 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 could give the identity of the species.

16S rDNA Sequences submitted to NCBI GenBank with Accession numbers

EU430985	*Bacillus oleronius*
EU430986	*Paucisalibacillus globulus*
EU430987	*Bacillus stearothermophilus*
EU430989	*Bacillus circulans*
EU 439990	*Bacillus pumilus*
EU430993	*Bacillus fusiformis*

Conclusion:

Based on the results of the present investigation we suggest a simple approach for identification of *Bacillus 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.

Sub- Project 2 : Diversity Analysis of Microbes in Extreme Conditions

PI : D. K. Arora
Co-PI : A. K. Saxena; Rajeev Kaushik, Alok K Srivastava, Sudheer Kumar, Mahesh Yandigeri
SRFs : Harmesh Sahay, Atul K. Singh, Surinder Kaur, Anupma P.D., S. Reddy, Arvind Yadav

Rationale

Microbial life in extreme environment has been studied intensively focusing attention on the diversity of the organisms and molecular and regulatory mechanisms involved. The microbial products obtainable from extremophiles such as proteins, enzymes (extremozymes) and compatible solutes are of great interest to biotechnology. This field of research has also attracted attention because of its impact on the possible existence of life on other planets.

Objectives

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

Isolation of microorganisms from Manikaran thermal springs, Vashisht hot sulphur springs and screening for temperature tolerance.

Five different media were employed for the isolation. From Manikaran and Vashisht thermal springs, 77 bacteria were obtained. Out of 77 isolates, 60 could grow at temperature of 45°C, 30 at 55 °C and 12 cultures at 65 °C. These 12 cultures were further screened up to a temperature of 90^{0}C. Nine cultures retained more than 50% growth even when exposed to 90 °C for 10 min.

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

Isolation, Screening, and Selection of Xylanase-Producing alkalo-tolerant thermophilic bacteria

One hundred ten alkalo-tolerant thermophilic bacteria were isolated from 17 samples (water and sediment) collected from Manikaran, Vashisht and Rajgiri thermal springs. Of 110 isolates, 70 showed the production of xylanases as indicated by the appearance of yellow clearing zone around the colonies after Congo red staining. These isolates were further screened for growth and production of xylanases at different temperature ranging from 40 to 75 °C. Eleven isolates that showed growth and xylanase production at temperatures >50 °C were selected for quantitative estimation in modified Reese mineral liquid medium containing wheat bran. All the isolates were found to produce >75 IU ml^{-1} xylanases. Significantly higher xylanase activity was produced by isolate R-9 (410.0 IU ml^{-1}) followed by H-7 (102.5 IU ml^{-1}) and H-9 (79.4 IU ml^{-1}) and was statistically superior to other isolates. These three isolates were further selected for cultural, morphological and molecular characterization. Further they were identified based on carbon utilization pattern and DNA sequencing of 16 S rDNA.

Cultural and morphological characteristics.

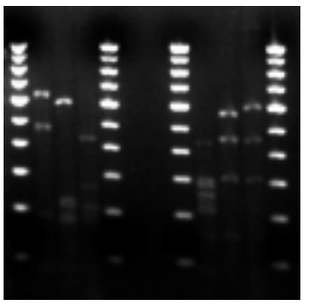
Morphological observations of these isolates showed typical growth characteristics of bacteria on solid media, i.e. ability to form a smooth, protective endospore allowing the organism to tolerate extreme environmental conditions, bigger-sized colonies characterized with peculiar brownish colour colony. The microscopic observation showed that the isolates possessed the typical rod with endospore, characteristic of *Bacillus* sp. The isolates were found to be oxidase and catalase positive.

Carbon utilization pattern

Using Biolog microbial identification system based on utilization of 95 carbon sources, three bacterial isolates (H7, H9 and R9) were identified as *Bacillus subtilis*, *Bacillus cereus/B. thuringiensis*, and *Paenibacillus* sp.

PCR-RFLP analysis of 16S rDNA

To look for the species variation among the morphotypes selected, PCR amplification of 16S rDNA followed by RFLP analysis with two restriction endonucleases was carried out. When 16S rDNA amplicons were digested with restriction enzymes, different profiles containing between 4 and 7 fragments ranging in size from 86 to 700 base pairs were seen with three isolates. The variations in restriction profile (size and number of the bands) of the PCR products from three isolates by *Alu*I and *Hae*III indicated that the isolates were genetically different from each other.



RFLP analysis of 16S rRNA fragments of three selected isolates with *Alu* I and *Hae*III. Lane 1,5: 100-bp DNA marker; lane 2: isolate H-7; lane 3: isolate H-9: lane 4, isolate RXA-9.

Analysis of 16S rRNA gene sequences of three bacterial isolates.

Three selected isolates were sequenced and the sequence data was analysed by BLAST and the nearest match from GenBank data was reported. Sequences were deposited in the GenBank. DNA sequencing and phylogenetic analysis revealed that all the isolates obtained from Manikaran thermal springs showed 97 to 100% similarity with the sequences within the GenBank. The closest phylogenetic neighbours according to the 16S rRNA gene sequence data for the three isolates H-7, H-9, and R-9 were *B. ehimensis, B.cerus, B.subtilis*, respectively.

Microbial Diversity analysis in Rajgiri thermal springs

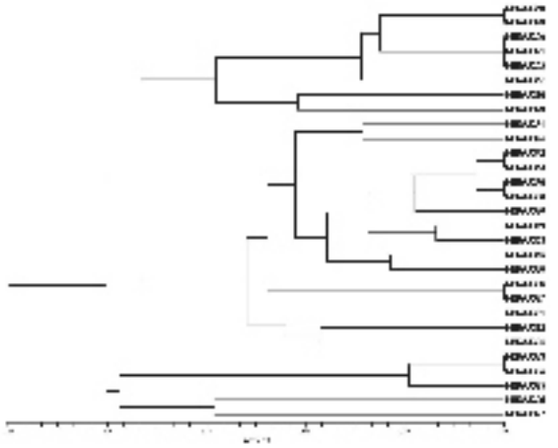
Exotic niches harbor population of microorganisms that are a source of several commercially important products like enzymes, sugars and antibiotics. Thermal springs represent extreme niches that have maintained some degree of pristine quality and their biotechnological potential has remained unrealized. Rajgiri thermal springs are situated at the foot of the Vaibhava hill, and are located about 100 kms from Patna, the capital city of Bihar. The hot springs are filled with water coming from the seven streams or Saptadhara and believed to have a medicinal value. The hottest of the springs is the Brahmakund with a temperature of 45 °C. Tourists use the hot water of Rajgiri springs for drinking and bathing purposes. No reports are available on the bacterial diversity in these thermal springs. The present study was aimed to decipher the genotypic diversity of the *Bacillus* isolates from thermal springs of Rajgiri, Gaya, Bihar. In the present study, the isolates obtained from thermal springs were grouped based on RFLP analysis of PCR amplified 16S rDNA and 16-23S IGS rDNA and the representative isolates were sequenced to reveal their identity. However the results obtained were surprising and contrary to the aims of the study. The isolates obtained did not belonged to the genus *Bacillus* but to different genera that are supposed to be opportunistic human pathogens. This report opens up a debatable issue on how safe the thermal springs of Rajgiri are?

The analysis of the three sediment and five water samples indicated that the water and sediment samples were acidic with pH ranging from 5.5 to 6.0. The total viable count of aerobic bacteria in the samples varied from 8 to 20 x 10^3 cfu g^{-1} or ml^{-1}. After heat treatment, water and sediment samples were diluted and plated on nutrient agar with methyl red. A total of 29 isolates were selected on the basis of different colony morphology. All the isolates were found to be Gram negative except for NBRAJG 76, 80, 82 and 88. None of the isolates showed the presence of endospore and majority of them were found to be oxidase negative. All these results gave an indication that the cultures isolated did not belong to the genus *Bacillus.*

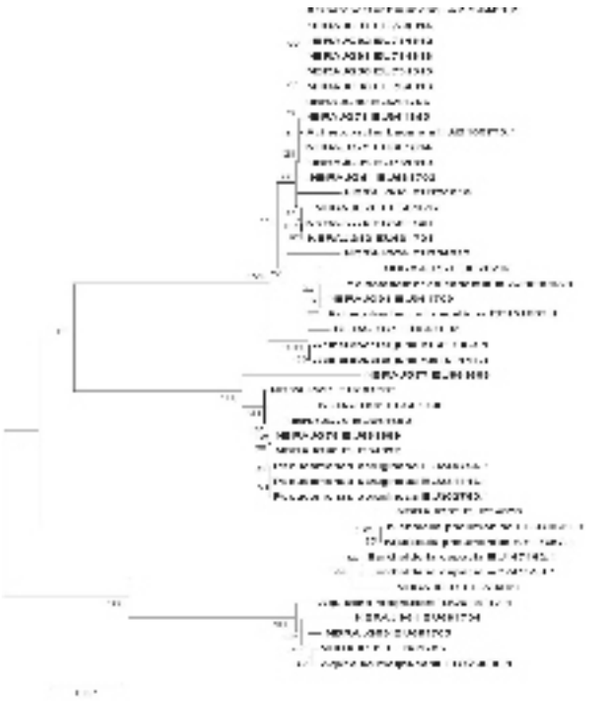
To look for the species variation among the morphotypes selected, PCR amplification of 16 S rDNA and 16S-23S IGS rDNA followed by RFLP analysis with four restriction endonucleases was carried out. When 16S rDNA or 16S – 23S IGS rDNA amplicons were digested with restriction enzymes, different profiles containing between 4 and 7 fragments ranging in size from 86 to 700 base pairs were seen with different isolates. Of the two gene sequences, 16-23S rDNA was more discriminatory and could distinguish isolates otherwise not differentiated by 16S rDNA-RFLP analysis. A combined dendrogram was constructed to determine the percent similarity among the isolates. At a level of 20 % similarity, all the isolates could be grouped into two major clusters A and B. Cluster A was further subdivided into two (A1 and A2) at a level of 26% similarity. The sub cluster A1 had isolates NBRAJG - 70, 78, 90, 91, 92, 77, 89 and 98. The sub cluster A2 was the largest and included 16 isolates. Among them there was 100% similarity between isolates NBRAJG-72 and 73, NBRAJG- 76 and 88 and NBRAJG – 86 and 87. Cluster B was also subdivided into two small sub clusters B1 and B2 at 22% similarity level. The sub cluster B1 included three isolates of which NBRAJG 83 and 84 showed 100% homology among themselves. The other subcluster B2 contains only two isolates NBRAJG-96 and 97.

All the 29 isolates were sequenced and the sequence data was analysed by BLAST and the nearest match from GenBank data was reported. Sequences were deposited in the GenBank. DNA sequencing and phylogenetic analysis revealed that all the isolates obtained from Rajgiri thermal springs showed 97 to 100% similarity with the sequences within the GenBank. Among the isolates, majority showed sequence similarity to genus *Acinetobacter* (59%) with *A. baumannii* as the major species. There were 2 isolates that showed 99% sequence similarity to *A. calcoaceticus* and one each to *A. baylyi* and *A. junii*. Six isolates (21%) showed sequence similarity to

Pseudomonas aeruginosa while three isolates were closer to *Aquitalea maqnusonii*. In addition there were one isolate each of *Burkholderia cepacia* and *Klebsiella pneumoniae*.



Phylogenetic relationship of the bacterial isolates on the basis of RFLP of 16S and 16-23S rDNA.



Unrooted phylogenetic tree based on a comparison of the 16S ribosomal DNA sequences of Rajgiri thermal springs isolates and some of their closest phylogenetic relatives. The phylogenetic tree was constructed on the aligned datasets using Neighbour Joining (NJ) method using the program MEGA 4.0.2. The numbers on the tree indicates the percentages of bootstrap sampling derived from 1000 random samples. Isolates characterized in the present study are indicated with initials NBRAJG.

Accession number

All the sequences were submitted to NCBI GenBank and accession numbers were assigned: EU661692 (NBRAJG 70), EU661693 (NBRAJG 71), EU661694 (NBRAJG 72), EU661695 (NBRAJG 73), EU661696 (NBRAJG 75), EU661697 (NBRAJG 76), EU661698 (NBRAJG 77), EU661699 (NBRAJG 78), EU661700 (NBRAJG 79), EU661701 (NBRAJG 80), EU661702 (NBRAJG 81), EU661703 (NBRAJG 83), EU661704 (NBRAJG 84), EU661705 (NBRAJG 85), EU661706 (NBRAJG 89), EU661707 (NBRAJG 91), EU661708 (NBRAJG 92), EU661709 (NBRAJG 93), EU734812 (NBRAJG 82), EU734813 (NBRAJG 86), EU734814 (NBRAJG 87), EU734815 (NBRAJG 88), EU734816 (NBRAJG 98), EU734817 (NBRAJG 74), EU734818 (NBRAJG 94), EU734819 (NBRAJG 96), EU734820 (NBRAJG 95), EU734821 (NBRAJG 97), EU734822 (NBRAJG 90).

Conclusions

The results of the present investigation were in contrast to the objective and led to the isolation of species that were identified as *Acinetobacter baumanii*, *A. junii*, *A. baylyi*, *A. calcoaceticus*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Aquitalea maqnusonii* and *Klebsiella pneumoniae*. All the species are Gram –ve, aerobic and are reported to be opportunistic human pathogens except *Aquitalea maqnusonii*. *Acinetobacter* is a group of bacteria commonly found in soil and water. It can also be found on the skin of healthy people, especially healthcare personnel. While there are many types or "species" of *Acinetobacter* and all can cause human disease, *Acinetobacter baumannii* accounts for about 80% of reported infections. The organism can survive for months on clothing and bedclothes, bed rails, ventilators and other surfaces in the environment, including sinks and doorknobs, making nosocomial transmission extremely difficult to control. Three isolates obtained from Rajgiri thermal springs were closer to *Aquitalea maqnusonii.* It is a Gram-negative, rod-shaped, non-spore-forming betaproteobacterium and belongs to the family Neisseriaceae. There is only one report describing gen. nov., sp. nov. *Aquitalea magnusonii* isolated from humic-lake samples collected from northern Wisconsin, USA but there are about ten 16S rRNA sequences submitted in NCBI GenBank of genus *Aquitalea* with no species epithet. Of the three isolates obtained in the present study, two (NBRAJG 83 and 85) showed 98% homology with

the type strain whereas NBRAJG 84 showed only 96% homology. Since *A. maqnusonii* is the only validly described species in the genus *Aquitalea*, there is a possibility that this isolate could be a novel species of *Aquitalea* and needs further characterization. One isolates each of *Bukholderia cepacia* (NBRAJG 97) and *Klebsiella pneumoniae* (NBRAJG 95) were obtained from the thermal springs. *Burkholderia cepacia* is unique in the way that it is versatile in its uses as plant pathogen, human pathogen, bioremediation agent, and biocontrol agent. *B. cepacia* is inherently resistant to multiple antibiotics, can metabolize diverse substrates, and is found in soil and in moist environments. The organism has a particular predilection for the lung in patients with cystic fibrosis (CF) and has emerged as an important opportunistic human pathogen in hospitalized and immunocompromised. *Klebsiella* is among the five gram-negative pathogens most commonly encountered in hospital-acquired infections, and *Klebsiella pneumoniae* is the most frequently occurring species, accounting for 75 to 86% of *Klebsiella* species reported. Six isolates showed the production of fluorescent pigment in King's B medium and through sequencing of 16S rDNA were identified as *Pseudomonas aeruginosa*. This organism is not only an opportunistic human pathogen but is also an opportunistic pathogen of plants. The results of the present investigation indicated the existence of opportunist human pathogen in the thermal springs of Rajgiri. These water springs are frequently used by tourists and local people for bathing and healing wounds. Because natural springs are popular tourist attractions, health authorities should be aware of possible hazards and provide tactful measures and guidelines to ensure safety without causing undue alarm to foreign and Indian tourists. Although the isolates identified are opportunist pathogens, the study should be a caution for both Government and local bodies.

Sub Project 3: Diversity of Actinomycetes in Indogangetic Plains

PI : D. K. Arora, Director
Co-PI : Mahesh Yandigeri, Scientist
SRFs : Nityanand Malviya

Rationale:

The actinomycetes are gram positive bacteria which have a characteristically high G+C content in their DNA (>55%). They are phenotypically diverse and are found in most natural environments. Many species produce a wide variety of secondary metabolites, including antihelminthic compounds, antitumour agents, and the majority of known antibiotics, which have been exploited by their use in medicine and agriculture. The actinomycetes were originally considered to be an intermediate group between bacteria and fungi but now recognized as prokaryotic. Majority of the actinomycetes are free living, saprophytic bacteria found widely distributed in soil, water, and colonizing plants. In soil, they are involved in the decomposition and mineralization cycles with the production of extracellular enzymes. Actinomycetes can be found almost in any substrate, although they prefer alkaline and neutral conditions in order to grow. The optimal pH range is between 7 to 8. Most of the actinomycetes grow at temperatures between 15 to 30°C. Actinomycetes show a compact, leathery appearance with dry surfaces and are reported as source of an earthy smell as they produce geosmin and 2-methyl isoborneol.

Indo-gangetic plains (IGP) is the major crop production belt of the country and is known to have rich biodiversity of flora and fauna. Hence, this project has been formulated to isolate, utilize and conserve actinomycetes diversity of IGP in order to exploit the actinomycetes for agricultural productivity and human welfare.

Objectives:

- Isolation, characterization and identification of actinomycetes from Indo-gangetic plains.

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

Significant Achievements:

- In earlier report, results of diversity from Dehradun, Nanital, Mau, Assam, Saharanpur, Meerut, Bagpat and Muzaffarnagar regions falling under Indo-gangetic plains were reported. In this report, surveys of Lucknow to Allahabad regions, Kanpur region, Central Punjab regions are reported. From these surveys 95 more isolates were isolated using starch casein agar, glucose yeast extract malt extract agar, actinomycetes isolation agar, Soil extract agar and various medium suggested by International Streptomycetes Project (ISP). The purified isolates were preserved in slants and glycerol stocks.
- The isolates were characterized morphologically by using parameters like aerial mycelium colour, substrate mycelium colour and colour of soluble pigments.
- The isolates were physiologically characterized by means of temperature and salt tolerance. A total 40 isolates were able to tolerate up to 55 °C, 28 isolates were able to tolerate 8% NaCl concentration. Functional characterization was done for plant growth promotion with regards to ammonia production, biodegradation activities like filter paper assay, carboxymethyl cellulose (CMC) assay, xylanase assay, cyanogenesis (HCN production), antifungal activity and production of enzymes.
- Thermotolerance study showed that 200 isolates showed growth at 35°C, 48 isolates at 45 °C and 39 isolates at 55 °C. Also, 5 isolates were able to grow at 8% NaCl stress. Out of 200 isolates tested 58 isolates were positive for production of hydrogen cyanide and 178 isolates capable to produce ammonia.
- Biodegradation study showed that a total of 18, 12, 12 and 13 isolates were able to degrade cellulose, hemicellulose (xylananse), carboxymethylcellulose (CMCase) and filter paper (FPase) respectively.
- Molecular diversity analysis of 23 isolates was carried out by phylogenetic analysis using universal primers of 16S rDNA and metabolic gene 'Nitrile hydratase' followed by restriction analysis with *Mbo*I by using Neighbour- Joining algorithm. An amplicon of ~1200 bp was obtained with 16S rDNA amplification and 1200 bp amplicon was obtained with nitrile hydratase gene amplification. The amplicons were digested with *Mbo*I for the restriction analysis and clustering.

Conclusion:

Actinomycetes isolates obtained from different regions under Indo-gangetic plains were found diverse in morphological and biochemical characteristics. Earlier we reported Dehradun region has more diversity, but in recent survey and studies, we found Lucknow region has more diverse actinomycetes in terms of morphology followed by Dehradun region. Also results showed cultivated lands of central Punjab region has lesser diversity than Lucknow cultivated lands. Sufficient functional diversity was also observed from the various biochemical assays. Molecular characterization of 23 isolates also revealed that 16S rDNA formed 6 clusters and nitrile hydratase gene showed 9 clusters at >70% similarity. This huge database of actinomycetes from Indogangetic region could be applied for identification of potent isolates.

Sub Project 4 : Diversity of Actinomycetes in Extreme Conditions

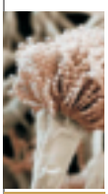
PI : D. K. Arora, Director
Co-PI : Mahesh Yandigeri, Scientist
SRFs : Arvind Yadav

Objectives :

- Microbial diversity analysis in extreme climates.
- To look for the production of enzymes (amylase, protease, cellulase, CMCase and xylanase).
- Identification of osmolyte production by actinomycetes
- Molecular characterization of actinomycetes from extreme environment.

Significant Achievements :

- Soil and water samples were also collected from extreme climates: Manikaran thermal springs, Rajgiri hot springs, Sundarbans Mangrove, cold climates of Leh and Laddakh regions, hot desert of Rajasthan, Sambhar Lake (Rajasthan) and Pulicat salt lake (Tamilnadu). A total of 217 isolates were collected from different extreme environments on different media like starch casein agar, actinomycetes isolation agar, soil extract agar, and various ISP media. Isolates were characterized by different morphological and functional features like mycelia colour, spore colour, pigment production and various biochemical tests *e.g.,* PGPR activities, physiological properties, salinity tolerance, metal tolerance, biodegradation activities, biocontrol activity and enzyme production.



- Thermotolerance study of all isolates showed that 217, 45 and 35 isolates showed growth at 35°C, 45°C and 55°C respectively. Also, salinity tolerance studies showed that 163, 132, 14 and 5 isolates showed growth at 2, 4, 6 and 8% NaCl respectively.
- Metal tolerance (for copper salt) of all isolates were tested and found that 85, 28 and 1 (ACY164) isolate showed resistance up to 16, 40 and 250 µg/L, The plant growth promotion activity of ACY164 isolate was tested by designing pot experiment for analysis of effect of co-inoculation of metal resistant actinomycete on defensive enzymes of chickpea.
- PGPR activities like ammonia production and siderophore production of all isolates was also tested and found 183 and 6 isolates showed ammonia and siderophore production respectively. Biocontrol attributes of actinomycetes isolates against known fungal pathogens and gram positive bacteria *viz., Macrophomina phaseolina, Fusarium solani, F. ciceri, Bacillus licheniformis, B. thuringiensis, B. israelensis, B. ciriculans* and *B. subtilis* was checked by using dual culture technique. Cyanogenesis process was also tested and found that 38 isolates were able to produce hydrogen cyanide.
- Ability of isolates to degrade cellulose, hemicellulose and xylan was assayed and found 14, 8, 8 and 9 number of isolates were capable to show xylanase, cellulase, CMCase and FPase activities respectively. A total 8 isolates were able to produce protease enzyme. The production medium and condition were optimized. Best protease activity with casein (5%) after 120h followed by malt extract (3%) after 144h and was 157 and 94.5 U/ml/min respectively. Maximum activity was recorded at pH 8.0 and temperature 70°C. Out of 217 actinomycetes isolates, 6 isolates were able to produce amylase. The best amylase production was shown by ACY161 isolate. The substrate, temperature and pH were also optimized and found that starch as a carbon source which produce maximum of 86U/ml/min at 60h; its optimal temperature and pH on which it shows maximum enzyme production was 42°C and 6.8 respectively.

Conclusion

A total of 217 actinomyctes were isolated different extreme climates which have various potentials like PGPR activities, biocontrol attributes, degradation assay and various enzymes production. We have isolated metal resistant isolates which have potential attributes to enhance defensive enzymes of chickpea crop. We have isolated many isolates which is capable to show potential biocontrol activities against major crop disease and were also taken into consideration as future plan. One culture has been isolated which produces excellent protease and amylase enzymes whose production media optimal condition has also been optimized. In future we are looking for osmolyte under extreme conditions and identification of such isolates.

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

Project Title: 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 of Effluent Contaminated Soils

PI : Rajeev Kaushik, Senior Scientist
Co-PI : A. K. Saxena, Principal Scientist
SRFs : Binu Mani Tripathi and Anamika Srivastava

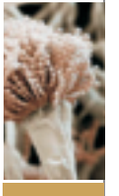
Objectives:

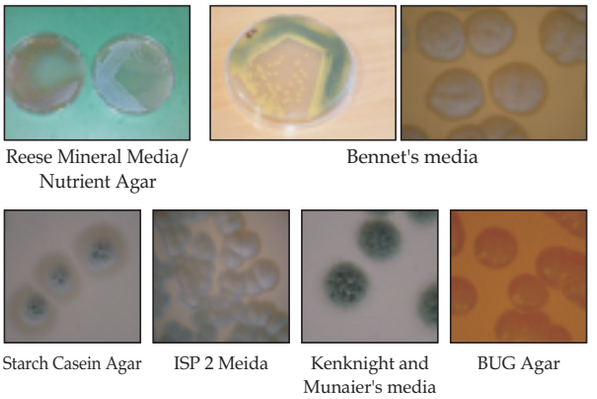
- Isolation of microorganisms from agricultural soils irrigated/contaminated with waste water of medium and large scale paper mills.
- To assess the functional shift in soil microbial population as a result of long term irrigation or dumping of paper mill effluent on the agricultural lands.
- Screening 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 in two cropping season i.e. *Rabi* and *Kharif*. For the sampling of soil and plants three fields were selected (i) Control field where effluent irrigation only with fresh water, (ii) Diluted effluent irrigated field (DEIF) and (iii) Concentrated effluent irrigated field (CEIF). Soil samples were collected from rhizosphere, and non-rhizospheric regions of the crops grown.
- 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. It might be due to the incorporation of lignocellulosic and hemicellulosic fractions of effluent into the soil on regular basis. Total microbial count of the pulp and paper mill effluent irrigated fields over control field reveled significant reduction in the population of gram negative bacteria, free living nitrogen fixers and fungi in effluent irrigated soils. However, significant increase was observed in case of Gram positive bacteria and actinomycetes in effluent irrigated soils over control. The total fluorescent Pseudomonad's did not show any significant change in the population count. Significant decrease was observed as a result of pulp and paper mill effluent in the population of Phosphate solubilizers and *Azospirillum* over the control soil, but there was significant increase in Xylan degrading, cellulose degrading and Mercury tolerant bacteria in effluent irrigated soil over the control soil.
- 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.

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





Colony characteristics and pigmentation of the *Sterptomyces* sp isolate on different media

- Using BIOLOG microbial identification system the bacterial isolate was identified as *Burkholderia glumae* and submitted to the NBAIM Culture Collection.
- The isolate of the actinomycetes was fast growing and could utilize xylan in 12-18 hrs and reach in the stationary phase. It is an uncommon property of actinomycetes to grow at this rapid rate and showed different colony pattern and pigmentation on different media and spiral sporulation pattern The DNA from different isolate was isolated and its 16SrDNA was amplified and sequenced. The isolate at the initial stage seems to be novel species of *Sterptomyces* as revealed by NCBI Blast search of the 16SrDNA sequence. The further characterization of the isolate and purification of its xylanases is in progress.

Conclusions

In order to decipher the functional changes in the soil microbial community as a result of long term application of pulp and paper mill effluent into the agricultural lands, the soil samples were collected from the selected control and effluent irrigated sites in Uttranchal state. Initial studies revealed certain changes in the native microbial population as 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 from the soil irrigated with the paper mill effluent several isolates were isolated and in preliminary stage encouraging results were 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.

Project: Microbial Management of Abiotic Stress

PI : A.K. Saxena, Principal Scientist
Co-PI : Rajeev Kaushik, Senior Scientist
SRFs : Shweta Tiwari, Rameshwar Singh, Brijendra K. Kashyap

Rationale

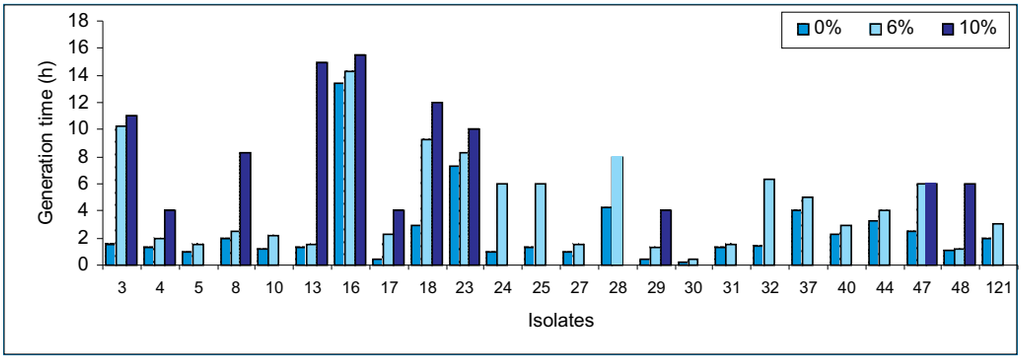
In India about 10 million ha of arable land is salt affected. The high concentrations of salt in the soil have a detrimental effect on crops and microorganisms. Microorganisms have been implicated in alleviating the effects of abiotic stress by different mechanisms. They can alter the availability of nutrients so as to maintain Na:K ratio in the plant. They are also involved in production of antioxidants to prevent injury to the plant because of salt stress. They can alleviate salt stress by production of growth promoting substances. Bacterial exo-polysaccharides have been implicated in providing protection from environmental stresses and host defenses. Yield losses of wheat in moderately saline areas average 65%. Thus an attempt was made to alleviate the effect of salt stress by inoculating wheat crop with rhizobacteria to improve its growth and yield in saline soils. The microorganisms and wheat cultivars were initially screened for salt tolerance. A total of 130 bacteria were isolated from the rhizosphere of wheat growing in the salt affected soils and screened for salt tolerance at graded concentrations of NaCl. Of the 130 isolates, about 42 isolates were able to tolerate NaCl stress of 8% while only two isolates showed tolerance to 12 % NaCl.

Objectives

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

Growth kinetics of bacteria at different salt concentrations.

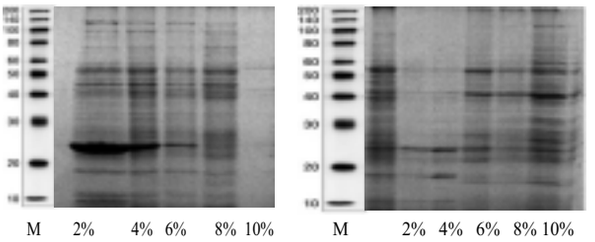
Twenty four isolates reported earlier to be salt tolerant were analysed for their growth kinetics using Nutrient broth. The generation time (gt) for each isolate at different salt concentration was calculated and is shown in fig. In general with the increase in salt concentration, the gt increased for all isolates. Some of the isolates (5, 10, 27, 28, 30, 31, 32, 37, 40, 44 and 121) that could grow on plates up to 10% NaCl failed to grow in broth with 10% NaCl.



Generation time (h) of different isolates at three salt (NaCl) concentrations.

Protein profile of salt tolerant isolates grown at different salt concentrations.

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.



Protein profile of bacterial isolates grown at different salt concentration

Field experiment for evaluation of salt tolerant bacteria for their ability to alleviate salt stress for growth of wheat:

Nine isolates that performed better in the pot experiment were selected for field trial. Inoculations with isolate 44 produced significantly higher root dry weight whereas isolate 8 gave maximum shoot biomass both after 60 and 90 DAS. Proline, reducing sugars (RS) and total soluble sugar (TSS) level was significantly higher for all treatments at 60 DAS as compared to 90 DAS. Inoculation with isolate 8 induced significantly higher proline and TSS accumulation in plants where as isolate 44 resulted in higher accumulation of RS.

The results on analysis of N, P K and Na in the shoot after 60 and 90 DAS are shown in Table 2. The percent accumulation of these nutrients decreased with the age of the plant and was more at 60 DAS as compared to 90 DAS. Inoculation of isolate 121 resulted in significantly higher accumulation of N both at 60 and 90 DAS which was at par with isolates 8 and 40. The %P in plants was significantly influenced due to inoculation of isolates 3, 8 and 24 as compared to uninoculated control. Plants inoculated with isolates 121, 24 and 47 showed significantly high % K in the shoots as compared to other treatments and uninoculated control. Inoculation with all the isolates showed significantly less %Na in the shoots as compared to uninoculated control. In treatments inoculated with isolates 40, 121, 3, 24 and 47 the % Na was statistically at par and significantly less than uninoculated control (Table 2). Grain yield and total biomass was significantly influenced by the inoculation and isolate 121 was found to be the best and gave a 23% increase in grain yield over control.

Influence of rhizobacteria on nutritional and biochemical parameters of wheat (cv. K7903) under saline conditions (field experiment, average of three replicates, 5 plants replicate^{-1})

Treatment	%N		%P		%K		%Na		Na/K		Proline (µg mg^{-1})		RS (µg mg^{-1})		TSS (µg mg^{-1})	
	60d	90d	60d	90d	60d	90d	60d	90d	60d	90d	60d	90d	60d	90d	60d	90d
Control	1.94	0.89	1.04	0.69	1.11	0.71	1.01	0.78	0.91	1.10	0.72	0.64	160.05	87.53	128.89	121.42
3	2.40	1.08	1.44	0.84	1.40	0.82	0.83	0.66	0.59	0.80	0.97	0.96	138.28	88.99	127.47	104.90
8	2.59	0.99	1.49	0.83	1.31	0.79	0.96	0.74	0.73	0.94	2.16	0.83	203.18	128.13	234.44	154.14
13	2.36	0.98	1.34	0.76	1.28	0.79	0.98	0.76	0.77	0.96	0.61	1.19	159.34	122.63	118.89	120.88
18	2.50	0.99	1.38	0.81	1.40	0.82	0.94	0.76	0.67	0.93	0.82	1.04	159.09	104.65	144.14	120.69
24	2.50	1.00	1.42	0.76	1.46	0.74	0.81	0.64	0.55	0.86	0.85	0.63	151.06	88.49	120.15	114.60
40	2.59	1.04	1.13	0.73	1.17	0.74	0.78	0.60	0.67	0.81	1.18	0.76	187.22	85.00	166.59	142.03
44	2.52	0.92	1.18	0.63	1.32	0.73	0.99	0.77	0.75	1.05	0.53	0.99	224.80	102.63	167.32	170.54
47	2.38	1.00	1.34	0.78	1.41	0.87	0.85	0.62	0.60	0.71	0.97	0.84	230.00	52.78	250.00	174.48
121	2.64	1.11	1.20	0.72	1.44	0.82	0.82	0.62	0.57	0.76	1.49	0.85	203.69	65.00	157.66	154.52

Conclusions

Earlier researchers have shown that selected PGPR ameliorated the deleterious effect of salinity on growth of tomato, pepper, canola, cotton and *Arabidopsis thaliana*. Studies have indicated that mobilization or solubilization of nutrients, increasing water use efficiency, stimulation of root growth, could cause these positive effects by production of phytohormones and enzymatic lowering of plant ethylene concentrations. In addition several physiological, enzymatic and biochemical changes in plant due to inoculation of PGPR have been suggested that helps in alleviating the salt or drought stress. PGPR are known to increase the uptake of N, P and K of wheat. It was reported that inoculation of wheat with *Pseudomonas* sp. stimulated plant growth by reduction of toxic ion uptake, increases in auxin contents and formation of stress-specific proteins in plants. Treatment with PGPR strains could alleviate the effect of potentially toxic ions and thus improve the plant growth. Recent studies have revealed that plants inoculated with PGPR containing ACC deaminase were better able to thrive through the salinity stress. Salt stress resulted in the synthesis of some new polypeptides and in most cases inoculation with *A. brasilense* inhibited the synthesis of some of these new polypeptides**.** It seems that with PGPR, the plants no longer need their innate defense mechanisms represented by the expression of stress proteins in the shoots and roots of barley plants to combat salinity stress. Nutrient imbalance in plants contributes significantly to the stunted growth under salt stress. It is the ionic balance rather than the absolute Na^+ content which determines the salt tolerance of a plant. Low Na^+ : K^+ ratio favours growth and yield of crop. Exopolysaccharide (EPS) production by PGPR strains also helps in binding cations, including Na^+ and thus could decrease the content of Na^+ available for plant uptake and thus helping to alleviate salt stress in plants. Among the nine isolates used in the field experiment, 5 were found to produce copious amount of EPS and could have contributed to decreased Na uptake in plant. In the present study two isolates 40 and 121, significantly enhanced the yield of wheat under salt stress. Isolate 40 that gave the maximum yield was found to be poor with regards to both IAA and P-solubilization. It is also not a nitrogen fixer but could produce EPS. The results suggest that PGPR inoculation does help to overcome salinity stress but the reasons are too complex to be clearly indicated. The reports on induction of certain genes, polypeptides or regulation of high-affinity K^+ transporter *HKT1* by soil microorganisms suggests that the mechanisms could be many, yet it did not underscore the utilization of PGPR for improving the productivity of crop plants under saline conditions.

Network project on 'Application of Microorganisms in Agriculture and Allied Sectors'

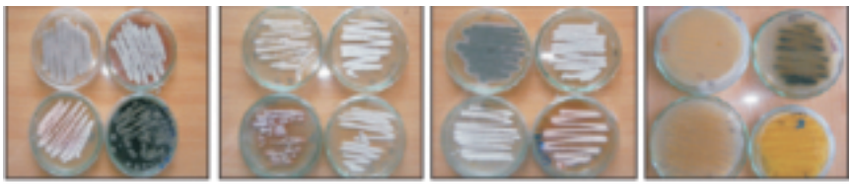
Significant Achievements (All Coordinating Centres)

Theme: Microbial Diversity and Identification

- Samples of soil, root, leaf, decaying wood, mushrooms, leaf litter and termite mound were collected from 48 grids in the study area of Western Ghats and used for isolation of AIMs. 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. Out of 84 fluorescent pseudomonads 52 were found to solubilize TCP in the range of 9.05 - 84.87%.
- 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 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.The huge database of microorganisms generated from extreme environments will help in identification of new species and genera. 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
- Developed simple approach for identification of *Bacillus 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.

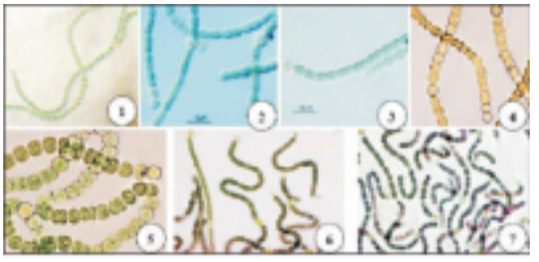
Sampling sites for regions having extreme climatic conditions

- Employing the sequence data of rDNA genes alongwith other functional genes and housekeeping genes could unravel the phylogenetic position of different species and *forma specialis* 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.
- Soil samples were collected from regions around Dehradun, Nainital, Mau, Assam, Saharanpur, Meerut, Baghpat and Muzaffarnagar falling under Indogangetic plains. A total of 230 isolates of Actinomyctes were isolated from soil samples collected from northern IGP. The isolates were characterized morphologically and 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 $CuSO_4$.



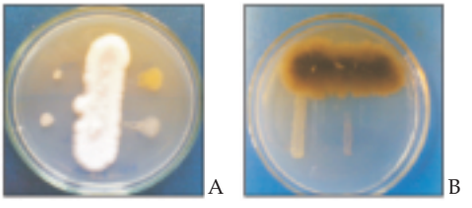
Some of Actinomycete isolates obtained from Indo Gangetic Plains

- Soil samples were collected from different districts of Kerala and 76 N-fixers, 81 P-solubilizers, 25 pseudomonads, 22 *Trichoderma*, 16 lignin degraders and 3 cellulose degraders were collected and characterized. Four isolates of P-solubilizing fungi were characterized and identified as *Penicillium glabrum.* Two pigmented bacteria *viz*., deep purple and pink were identified by morphological and biochemical characters and 16S rRNA sequence. Purple bacteria was identified as *Chromobacterium violaceum* and pink as *Serratia marcescens*.
- Different Cyanobacterial species viz. *Anabaena anomala, Anabaena doliolum, Anabaena oryzae, Aulosira fertilissima, Cylindrospermum muscicola, Hapalosiphon intricatus, Nostoc muscorum, Tolypothrix tenuis, Anabaena fertilissima, Nostoc rivulare, Anabaena torulosa, Nostoc paludosum, Nostoc piscinale* and *Nostoc* species were isolated from rice fields of different districts of Eastern UP and Western Bihar IGP region.



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.

- 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, of which 141 isolates showed positive reactions for IAA production. Twenty most promising isolates of fluorescent *Pseudomonas* spp. isolated from alluvial soil of CRRI farm and coastal saline soils of Kendrapara and Jagatsingpur districts of Orissa. Three of the isolated *Pseudomonas* spp. showed growth inhibition against plant pathogenic fungi *Rhizoctonia solani* and *Xanthomonas oryzae*. Partial sequencing of the 16s rDNA of a new isolate of *B. thuringiensis* 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*.



A.Inhibition of *Xanthomonas oryzae* against *Pseudomonas* sp.; B. Inhibition of *Rhizoctonia solani* against *Pseudomonas* sp.

- From 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. The results 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.

- 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 biochemical characterization, especially sugar fermentation pattern was used as a phenotypic method for species determination of the *Lactobacillus* isolates. 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 the 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 accession numbers were obtained from NCBI, USA (GenBank accession EU637371-EU637403).

- Wild mushroom specimens were collected from Himachal Pradesh, Uttarakhand and Rajasthan and identified 434 collections upto the genus level. Based on 5.8S rDNA sequencing and RFLP two new lignicolous *Volvariella* spp., a new species of *Flammulina*, were identified. Two wild *Hericium* spp. (edible and medicinal mushrooms) namely as *H. coralloides, H. racemosum, Cantharellus applachenesis, Cantharellus cibarius* var. *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. Artificial domestication for *Flammulina* spp. and *Macrocybe giganteum* on wheat straw were successful which could help in diversification of new mushroom species from India.
- 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, including rhizobia and plant growth-promoting fluorescent pseudomonads, 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. A water soluble bioactive compound has been obtained from *Pseudomonas fluorescens* biovar V active against three soil borne fungal pathogens of groundnut.
- 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.
- From different districts of Madhya Pradesh *viz.* Tikamgarh, Nimad, Neemuch, Raisen, Bhopal, Ujjain and Indore 350 bacterial strains from agricultural fields were isolated. Among these, 5 isolates of fluorescent *Pseudomonas*, 10 isolates of *Bacillus* sp., 80 free living as well as symbiotic nitrogen fixing bacteria were obtained. 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*. For *A.beijerinckii* species – L-tartrate, o-hydroxybenzoate, D-glucuronate or D-galactouronate and pH of 6 favours the growth of bacteria. For *A. armeniacus* species – caprylate is used as carbon source.

- Two hundreds new isolation of bacteria including free living, symbiotic and actinobacteria were made from different regions of Haryana. 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 three isolates were identified as *Bacillus* sp. 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. *Rhizobium* isolated from rabi legumes (chickpea and berseem) were positive for infection on their respective hosts and showed the presence of *nod* C gene.
- In Bihar, 59 villages of 32 blocks of 12 districts were surveyed. From the soil samples, 480 agriculturally important microbes were isolated. Out of 480 organisms, 112, 52, 44, 27 and 8 were identified as *Azotobacter, Rhizobium*, PSB's, *Azosprillium* and *Glucanocatobacter*.
- 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. 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 Gene Bank (Acc. No. EU159585).
- Four hundred and forty two fungal isolates were 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. 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.
- 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.
- 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. 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.
- 61 villages in South Andaman and 20 villages in Middle Andaman were surveyed for collection of soil and plant samples. Off 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., one is *Sclerotium rolfsii* and one is *Fusarium* sp. Three *Colletotrichum* spp were identified at species level, *i.e., C. gloeosporioides*. Among the 399

bacterial antagonistic microorganisms 54 were identified as *Pseudomonas* spp. and 47 as *Bacillus* spp. by morphological and biochemical characterization.

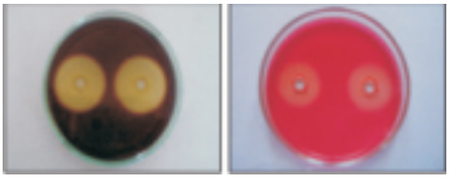
- From freshwater ecosystems of Bhubaneshwar and Khurda, water and sediment samples were collected. Using enrichment culture technique 41 bacterial isolates were obtained showing CMCase activity. Using conventional morphological tools and sequencing of 16S rDNA region the isolates were identified as *Bacillus*. Effect of salinity and temperature was also studied.
- Soil samples representing six different agro climatic regions of Punjab were collected. In total 122 diazotrophic isolates were obtained. 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.

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

- 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 against *Fusarium oxysporum* f. sp. *ciceri*. Most of these antagonists also showed PGP 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 Distt. 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.
- Soil and plant samples were collected from Assam (Kaziranga national biodiversity park and Jorhat district), Uttaranchal, West Bengal (Sunderban mangrove forest areas) and 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. After primary screening through dual culture 14 *Pseudomonas fluorescens*, 14 *Bacillus* and 9 fungal isolates were selected for evaluation of their secondary metabolite against *Fusarium oxysporum* f. sp. *ciceri* and *M. phaseolina*. The selected fungal isolates were subjected for the qualitative and quantitative assay of hydrolytic enzymes like chitinase, protease, cellobiase and cellulase. Out of these three, produced high quantity of chitinase, the main enzyme that hydrolyze the chitin and is involved in mycoparasitism.
- 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 of *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%.

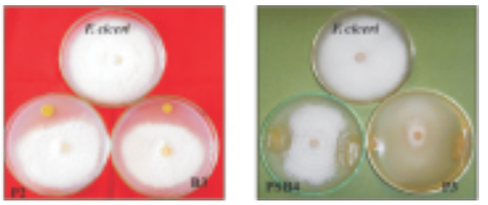
- Based on TCP solubilizing ability at 4 °C fourteen isolates were selected and designated as elite isolates. The P solubilizing ability of *Pseudomonas fragi* and *P. lurida* under cold incubation temperatures have been reported for the first time. The isolate *Pseudomonas poae* strain RT5RP2 registered the highest level of soluble P (26.64 ppm) from Tricalcium 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. Nine elite isolates were identified based on their 16S rRNA sequences *viz., Pseudomonas fragi* strain CS11RH1, *P. poae* strain NS12RH (1), *P. poae* strain RT5RP2, *P. 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.
- 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.



Production Pectinase and Cellulase enzyme by rhizobacterial Ginger isolate GRB-35

- 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 and 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.
- 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. 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.
- More than hundred cyanobacterial, and bacterial plant growth promoting microorganisms were isolated from wheat and rice rhizosphere. 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. Isolates also showed PGPR traits such as IAA production, P-solubilization, ammonia production.

- A total of 76 heterotrophs, 78 heterotrophic nitrifiers, 8 denitrifiers, 14 oligotrophs and 6 *Azospirrilum* isolates were obtained in pure culture. Methane oxidation potential of 15 soils from rice-based cropping system of Orissa varied from 6.16 to 81.53 indicating high variability. Twelve pure cultures of methanotrophs with particulate methane monooxygenase (pMMO) activity were isolated. Two heterotrophic nitrifying bacteria were identified through 16S rDNA sequencing as *Bacillus* sp. and *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.
- Mycorrhizal inoculation in Maize showed increased acid phosphatase activity by 20-40% besides improving the availability of Zn. These changes facilitate lowering of pH which resulted in the release of Zn from the fixed pool. 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. 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.
- 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). 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.
- 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.
- 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.



Biocontrol action of PGPR against *Fusarium oxysporum pv.*

- Plant growth promoting microorganisms were isolated from endorhizosphere of different varieties of pigeonpea. Five *Azotobacter* strains improved chickpea growth with maximum increase of 52% produced within 4 weeks of plant growth. Out of 15 PSB strains tested, PSB 24 and 27 accumulated significantly, greater biomass. 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 *in vitro* 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 pigeonpea & chickpea using enrichment technique.
- Different explorative surveys were carried out for fungal pathogens of a range of grassy weeds across India. The fungi isolated from the diseased weed parts were confirmed to be pathogens by satisfying Koch's postulates. 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

- 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, 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 as 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.
- From the soil samples contaminated with PAH from four different geoclimaic locations of India 15 bacterial strains were found to have ability to degrade Anthracene, Phenanthrene, Fluorene, Naphthalene and Pyrene at concentration as high as 100 ppm. Five novel partial gene sequences of 16S rRNA gene of PAH utilizing bacterial cultures have been submitted online to the GenBank portal 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, as per the literature available. Conditions were standardized for recovery and

quantification of PAH degradation by HPLC method and best elution was found with 60:40:: Acetonitrile: water mobile phase.

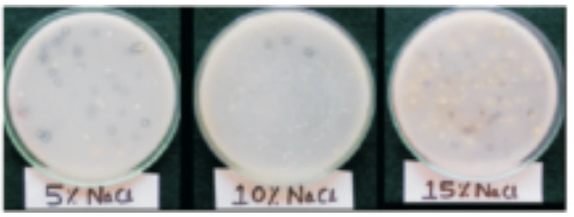
- Agricultural land situated around hexachlorocyclohexane (HCH) dumpsites was surveyed for HCH residues. Highly contaminated agricultural soil was selected for isolation of HCH-degrading bacteria. A total of 25 strains were isolated by enrichment method. Out of 25 isolated strains only 7 strains were 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.
- From different thermophilic fungi available at NRC on Mushrooms, Solan, strains of *Scytalidium, thermophilum* and *Humicola insolens* have been identified till date. 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 *Chaetomium 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. Optimum temperature for the growth of *S. thermophilum* was found to be 45 °C. Wheat straw substrate is the best and cheap source for the growth and multiplication of *S. thermophilum*. Strain of *S. thermophilum* can better be utilized for compost production, as this is a fast cellulose decomposer.
- 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. 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.
- 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. Based on 16S rDNA sequence analysis, the bacteria were identified as three novel species of *Bacillus* sp. which were grouped in a common clad with type strains of *B. mycoides* and *B. thuringiensis*. ARDRA analysis of the genomic DNA of the two *p*-nitrophenol degrading *Bacillus* sp. with *AluI* and *MspI* showed that the two isolates had almost similar restriction sites indicating species closeness. Both the *p*-nitrophenol degrading bacteria degraded *p*-nitrophenol as the sole source of carbon and energy releasing stoichiometric amount of nitrite. A novel *Bacillus* sp. having 92% homology with *Bacillus djibelorensis* degraded the organochlorine fungicide vinclozolin in mineral salts medium using it as a source of C and energy has been isolated and identified through complete 16s rDNA sequencing and FAME analysis. 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 soils enriched with various isomers of HCH. 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 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.
- Off the several isolates isolated from sewage contaminated sites 12 bacterial and 5 fungal strains were found to tolerate Cr, Cd, Pb and Ni at

different concentrations ranging from 50 - 100 ppm. Heavy metal Cd was found more toxic to growth of fungi as compared to Pb at 50 ppm conc.

- 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. 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 and thiosulfate oxidation has been isolated.
- 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.
- 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 % of alcohol could be recovered
- Several pectinolytic, amylolytic and cellulolytic microorganisms were isolated from different fruit wastes. 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.

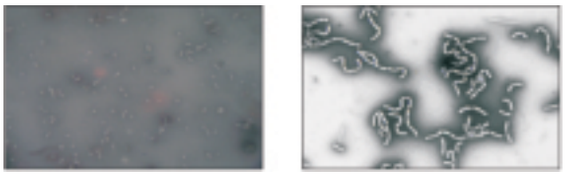
Theme 4: Microbial Management of Abiotic Stress

- 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.



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

- In total 175 samples of soil, rhizosphere soil, roots, leaves and decaying residue were 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). Four *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 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.
- 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.



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

- 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. 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. 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

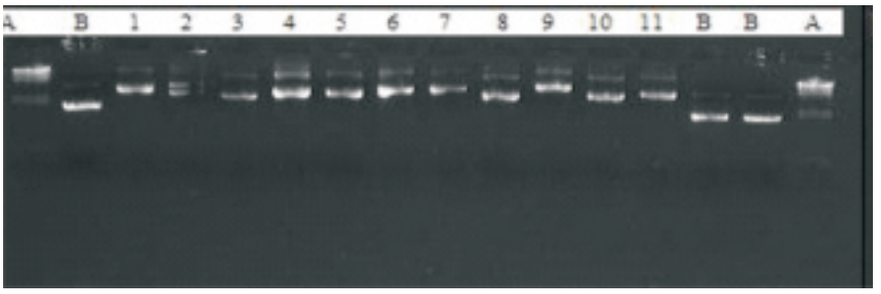
was observed in wheat plants inoculated with the cold tolerant isolates, which is critical to the plant's ability to tolerate stress conditions. Elite cold tolerant bacterial cultures having ice nucleation activity in the range of -6.12 to -9.83.

Theme 5 : Microbial Genomics

- 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.
- 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. Tn5 transposon insertion site in HQ tolerant mutant was mapped by genome walking technique. The site of insertion of Tn5 in HQ tolerant mutant was found to be in the PqqF gene involved in the biosynthesis of coenzyme PQQ.
- 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* sp. 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.

Complete Genome sequencing of *Mesorizobium ciceri* strain Ca181 with genome size of 8Mb:

- 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.
- 16S rDNA of *M. ciceri* ca 181 was amplified fromits genomic DNA 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 ciceri* strains and *Bradyrhizobium* sp.
- 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



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

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. The digested fragment was amplified using T7 and M13 universal primers and were sequenced.

Theme: Human Resource Development

Following trainings were organized under the theme HRD of ICAR Networking project on AMAAS

1. **"DNA Sequencing and Microbial Identification Module of Agriculturally Important Microorganisms"** from September 17-21, 2007.

Objectives:

- Isolation of genomic DNA from different groups of microbes.
- PCR amplification of 16S rDNA from select bacteria and actinomycetes; and ITS or 28S rDNA from fungi.
- Microbial identification based on gene sequencing.
- 16S rDNA-based microbial identification for bacteria and actinomycetes .
- 28S rDNA-based microbial identification for fungi and yeasts.
- Compilation of forward and reverse DNA sequencing into a consensus sequence.
- Sample comparisons by means of a phylogenetic tree and sample comparison to GenBank, an international DNA sequence database.
- Bioinformatics tools used in microbial sequencing analysis.

Participants of "DNA Sequencing" Training

2. **"Molecular and serological detection of plant viruses from February 24-March 1, 2008**

The training program has the following theme areas to address:

- Molecular detection of some important plant viruses.
- Serological detection of some important plant viruses.
- Bioinformatics in characterization of important plant viruses.

Participants of "Molecular and Serological Detection of Plant Viruses" Training

Publications

Research Papers

1. Babu, B.K., Saxena, A.K., Srivastava, A.K. and Arora, D.K. 2007. Identification and detection of *Macrophomina phaseolina* by using species-specific oligonucleotide primers and probe. Mycologia 99: 797-803.
2. Tilak, K.V.B.R. and Saxena, A.K. 2008. Response of onion (*Allium cepa* L.) to inoculation with *Azospirillum brasilense*. J. Eco friendly Agric. 3(1): 16-18.
3. Jyotsana, Srivastava, A., Singh R.P., Srivastava, A.K., Saxena, A.K. and Arora, D.K. 2008. Growth promotion and charcoal rot management in chickpea by *Trichoderma harzianum*. J Plant Prot. Res. 48: 81-92
4. Singh, A., Sahay, H., Kaushik, R., Saxena, A.K. and Arora, D.K. 2008 Optimization of α-Amylase production from a moderately thermophilic *Burkholderia glumae*. Bioresource Technology.
5. Singh D., Vardhan S., Singh R., Singh R. N., Singh, B.P., Kaushik R., Saxena A.K., Arora D. K. 2008 Predominance of Opportunist Human pathogens in Rajgiri thermal springs, India. Current Science.
6. Upadhaya, S.K., Tiwari, S., Kashyap, B. K., Mishra, B.K., Tiwari, R., Kaushik, R. Singh, D.P., Saxena, A.K., Arora, D.K. 2008 Utilization of salt tolerant PGPR for improving the productivity of wheat crop under saline conditions. Biol. Fertil. Soils (Comm.).
7. Singh, A., Sahay, H., Tripathi, B.M., Yadav, S., Singh, R.N., Kaushik, R., Saxena, A.K., Arora, D.K. 2008 Biochemical and Molecular characterization of thermo- alkali tolerant xylanase producing bacteria from Hot water springs of Manikaran. Microbiological Res (comm.).
8. Yadav, M., Singh, K., Saxena, A.K. and Arora, D.K. 2008 Phylogenetic analysis and identification of *Fusarium* spp. using genes encoding Cellobiohydrolase-C and Topoisomerase-II. Mycological Res. (Comm.).

Presentation in Conferences/symposia/ Seminars/ others:

1. National Conference on Climate Change and Indian Agriculture', Oct. 12-13, 2007
2. Brainstorming workshop for establishment of a National Genomics Resources Repository, Dec. 27, 2007.
3. "Rabi Kisan Mela" organized by NEFORD at Mau from 9-10th October 2007
4. International Training Course on Molecular Biology and Biotechnology Techniques at IVRI, Izatnagar, Bareilly on 15.10.2007.
5. National Symposium on "Recent Advances in Phycology: From Molecule to Ecosystem at Department of Botany, Punjab University, Chandigarh
6. 2nd Asian Congress of Mycology and Plant Pathology at Osmania University, Hyderabad on 19-22 December 2007.
7. International Conference on Agricultural Biotechnology AgriBio 2007" at FICCI, New Delhi
8. International Conference on Mushroom Biology at NRCM, Solan on 10-11 February 2007.
9. "Centenary Celebration of Babu Jagjivan Ram Ji" organized by Shri Montek Singh Ahluwalia at Shashtri Bhavan, New Delhi.
10. Nineteenth Executive Council Meeting on March 19, 2008, Allahabad Agricultural Institute-Deemed University, Allahabad.

Manuals Published

1. Saxena, A. K. 2007 DNA Sequencing and Microbial Identification Module of Agriculturally Important Microorganisms.
2. Srivastava, A. K. 2007 Microbial Identification Module of Some Agriculturally Important Microorganisms.
3. Kaushik, R. 2008 Molecular and Serological Detection of Plant Viruses.

Library Information and Documentation

Books:

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Reviews:

- Annual Review of Microbiology (Vol. 46 to 56, 59 to 610
- Annual Review of Phytopathology (Vol. 30 to 41, 44 & 45)4
- Clinical Microbiology Reviews
- Microbiology and Molecular Biology Reviews

Journals:

- Journal of Eco-Friendly Agriculture
- Indian Journal of Microbiology
- Journal of the Indian Institute of Science
- Journal of Plant Biochemistry and Biotechnology
- The Asian Journal of Experimental Chemistry
- Microbiological Research
- Eukaryotic Cell
- The Plant Pathology Journal
- The Indian Journal of Genetics and Plant Breeding
- Journal of Bacteriology
- Mycological Research
- Journal of Clinical Microbiology
- Indian Journal of Sugarcane Technology
- Current Science
- Pestology
- Journal of Biosciences
- Fungal Genetics and Biology
- Current Contents
- Applied and Environmental Microbiology
- Nature
- Plant Disease
- Journal of Mycology and Plant Pathology
- Indian Phytopathology
- Mycobiology
- Journal of Indian Academy of Sciences
- The Journal of the Indian Botanical Society
- Asian Journal of Microbiology, Biotechnology and Environmental Sciences
- Microbiology

Miscellaneous Literature:

- ICAR Annual Reports
- ICAR Bulletins
- ICAR News
- Annual Reports of ICAR Institutes
- News Letters of ICAR Institutes
- Vision of ICAR Institutes
- Advanced Biotech
- Hindi Books
- Current Contents of Life Sciences
- Catalogues
- Dictionaries

Distinguished Visitors

- Dr. P. L. Gautam, DDG (CS), ICAR, Krishi Bhavan, New Delhi
- Dr. H. P. Singh, DDG (H), ICAR, Krishi Bhavan, New Delhi
- Dr. S. Ayyappan, Deputy Director General (Fisheries), Krishi Anusandhan Bhawan-II, Pusa, New Delhi
- Dr. T. P. Rajendran ADG (PP), ICAR, Krishi Bhavan, New Delhi
- Justice Shishir Kumar, Administrative Judge, High Court, Allahabad
- Dr. Kirti Singh, Ex-Chairman, ASRB, New Delhi
- Sh. B. N. P. Pathak, Legal Advisor, ICAR, New Delih
- Dr. K. V. B. R. Tilak, Emeritus Scientist, Osmania Univesity, Hyderabad
- Dr. C. Manoharachary, Emeritus Scientist, Osmania University, Hyderabad
- Dr. C. P. S. Yadav, Director, General, U.P. Council of Agricultural Research, Lucknow.
- Dr. T. P. Trivedi, ADG (ARIS), ICAR, Krishi Bhavan, New Delih.
- Dr. S. K. Dwivedi, NRC Equine, Hissar
- Dr. R. C. Srivastava, CARI, Portblair
- Dr. R. P. Tewari, Director, NRC Mushroom, Solan
- Dr. S. A. Patil, Director, IARI, New Delhi
- Dr. D.J. Bhagyaraj, 41-RBI Colony, Anand Nagar , Bangalore
- Dr. A. N. Mukhopadhyay, Former Vice Chancellor Assam Agricultural University, Assam.
- Dr. A. N. Rai, Vice Chancellor Mizoram University, Mizoram.
- Dr. S. M. Paul Khurana, Vice Chancellor, Rani Rukmani Devi University, Jabalpur
- Dr. R. P. Tewari, Director, NRC for Mushroom, Chambaghat, Solan
- Dr. S. K. Sharma, Director, National Bureau of Plant Genetic Resources, New Delhi.
- Dr. Ramesh Sonti, Centre for Cellular and Molecular Biology, Hyderabad

कार्यकारी सारांश

- दसवें पंचवर्षीय योजना में एक नेटवर्क परियोजना ''कृषि एवं तत्सम्बन्धी क्षेत्रों में सूक्ष्म जीवों का अनुप्रयोग'' अनुमोदित की गयी थी, जिसका नोडल केन्द्र राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो को बनाया गया। पहले से अनुमोदित 6 विषय क्षेत्रों के अतिरिक्त एक नये विषय क्षेत्र ''सूक्ष्म जिनोमिक संसाधन रिपाजिटरी'' को ग्यारहवीं पंचवर्षीय योजना में समायोजित किया गया। भविष्य में निम्नलिखित नए दृष्टिकोण को सम्मिलित किया जाएगा।
  - ✦ भौगोलिक सूचना प्रणाली के माध्यम से सूक्ष्म जीव विविधता का मान-चित्रण।
  - ✦ बायोइन्फार्मेटिक्स के सहयोग एवं साफ्टवेयर के विकास द्वारा सूक्ष्मजीवों का प्रभावी प्रबंधन।
  - ✦ ओ.सी.डी.ई. एवं डब्ल्यू.एफ.सी.सी. सूक्ष्म संसाधन केन्द्रों के दिशा निर्देश पर आधारित गुणता प्रबंधन प्रणाली का विकास।
  - ✦ पेटेंट हो सकने वाले राष्ट्रीय महत्व एवं बौद्धिक संपदा अधिकार के अन्तर्गत आने वाले कृषि-उपयोगी सूक्ष्मजीव एवं आनुवांशिक फेरबदल वाले सूक्ष्मजीवों के संग्रहण की नीति एवं उनका दीर्घकालिक संरक्षण।
  - ✦ सूक्ष्मजीव जीन बैंक का विकास
  - ✦ शिक्षा, किसान एवं उद्योगों से संपर्क।
- वर्तमान में ब्यूरो मे कुल 9 वैज्ञानिक एवं 2 तकनीकी कर्मचारी हैं।
- ब्यूरो ने डब्ल्यू.एफ.सी.सी. एवं ओ.सी.डी.ई. के अन्तर्गत आने वाले अन्तर्राष्ट्रीय सूक्ष्मजीव संसाधन केन्द्रों एवं भारतीय कृषि अनुसंधान परिषद, सी.एस.आई.आर., डी.बी.टी. संस्थानों तथा राज्य कृषि विश्वविद्यालयों से अपने संपर्क मजबूत किये हैं। राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो एक जीवंत एवं गतिशील संस्थान की तरह विकसित हो रहा है एवं भविष्य में यह संस्थान कृषि उपयोगी सूक्ष्मजीवों के पहचान, गुण, चिन्हांकन एवं संरक्षण के अत्याधुनिक सुविधाओं के साथ सूक्ष्मजैविक आनुवांशिक संसाधन के सभी अनुसंधान एवं विकास कार्य कलापों हेतु एक राष्ट्रीय संस्थान के रूप में आगे आएगा।
- राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो में सूक्ष्मजीवों एवं पेटेंट हो सकने वाले सूक्ष्मजीवों की राष्ट्रीय रिपाजिटरी एवं क्रायो संरक्षण की सुविधा है। राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो सूक्ष्मजीव डाटाबेस एवं ''सूक्ष्मजीव सूचना प्रबंधन प्रणाली'' हेतू आधारभूत सुविधाएँ विकसित कर रहा है।
- परिप्रेक्ष्य योजना के मध्यावधि मूल्यांकन ने अब तक की उपलब्धियों के विश्लेषण एवं पहले की योजना में आवश्यक संसोधनों के निर्धारण में बहुत योगदान दिया है। सूक्ष्मजीवों के उपयोग एवं इसके बदलते वैश्विक परिदृश्य के कारण यह जरूरी हो गया था। इस प्रकार कुछ नए विषय क्षेत्र चिह्नित किये गये हैं जो अब इस ''सूक्ष्मजीव संसाधन'' कार्यक्रम के अभिन्न अंग होंगे। हमारे अनुसंधान सलाहकार समिति ने भी परिप्रेक्ष्य योजना को विधिवत संशोधित करने की सिफारिश की है।
- मऊ में स्थापना के बाद 4 वर्ष के अल्प कार्यकाल की उपलब्धियों में 2800 से अधिक एक्सेशन्स जिनमें बहुत से उपयोगी सूक्ष्मजीव हैं, प्रमुख रूप से उल्लेखनीय है।
- विभिन्न राज्यों जैसे उत्तर प्रदेश, हिमाचल प्रदेश, मध्यप्रदेश, अरूणांचल प्रदेश, असम, राजस्थान एवं केरल में कृषि उपयोगी सूक्ष्मजीवों के पृथक्करण के लिए 16 विशेष अन्वेषण मिशन चलाए गये हैं।
- राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो में जैविक खाद की तरह उपयोग में आने वाले सूक्ष्मवर्द्धन बड़ी संख्या में हैं, जिनमें *राइजोबियम, एजोस्पाइरिलम, एजेटोबैक्टर, बैसीलस, स्यूडोमोनास, ग्लूकोनोबैक्टर* एवं *पी.एस.एम.* हैं।
- रोग नियंत्रण क्षमता वाले अनेकों सूक्ष्मजीव जैसे *बैसीलस, स्यूडोमोनास, सेरेशिया, ट्राइकोडर्मा, वर्टीसीलियम* की प्रजातियाँ भी संरक्षित हैं।

- कीट रोग जनक सूक्ष्मजीवों जैसे *मेटाराइजियम, ब्यूवेरिया, बैसीलस* प्रजाति जिनका उपयोग कीटप्रबंधन में किया जाता है, भी संरक्षित हैं।
- *बैसीलस, स्यूडोमोनास, फ्युजेरियम, मैक्रोफोमिना* एवं *सेरेशिया* की बड़ी संख्या के आइसोलेट्स के आण्विक अनुक्रम विकसित किए गये।
- महत्वपूर्ण नॉवेल जीन अनुक्रम एन.सी.बी.आई. जीन बैंक में जमा किए गए एवं उनका पहुँच क्रमांक प्राप्त किया गया।
- ब्यूरो डब्लू.एफ.सी.सी., ओ.सी.डी.ई. एवं जैव विविधता प्राधिकरण भारत (बी.डी.ए.) द्वारा मान्यता प्राप्त है।
- सूक्ष्मजीवों के पंजीकरण हेतु नोडल ऐजेंसी के रूप में, यह ब्यूरो भारतीय कृषि अनुसंधान परिषद द्वारा मान्यता प्राप्त है।
- *बैसीलस* प्रजाति की पहचान के लिए आर.एफ.एल.पी. नमूने एवं 16-एस आर डी.एन.ए. सूक्ष्म क्षेत्र के अनुक्रम के आधार पर एक तेज आण्विक जाँच तकनीक विकसित की गयी।
- *फ्यूजेरियम* समूह के प्रजाति स्तर पर पहचान के लिए हाउसकीपींग जीन अनुक्रम के उपयोग द्वारा जाँच विकसित की गयी।
- *मैक्रोफोमिना फेसिओलीना* की पहचान के लिए प्रजाति विशेष प्राइमरों एवं प्रोब जांच विकसित किये गये।
- प्रत्येक जमा के लिए एक ''पासपोर्ट डाटा'' विकसित किया गया। भविष्य में ब्यूरो के विकास के साथ कुछ कृषि उपयोगी सूक्ष्मजीवों का आण्विक मूल्यांकन किया जाएगा।
- कृषि उपयोगी सूक्ष्मजीवों का विस्तार राष्ट्रीय एवं अर्न्तराष्ट्रीय एजेंसियों द्वारा प्रत्यावर्तन से आरम्भ किया गया एवं यह चरण बद्ध ढंग से किया जाएगा।
- विभिन्न कृषि उपयोगी सूक्ष्मजीवों के प्रयोगशाला परिस्थितियों में ''अतिशीत संरक्षण'' के लिए अनुकरणीय एवं विश्वसनीय प्रोटोकॉल विकसित किया जाएगा, जो सूक्ष्मजीवों के दीर्घकालिक संरक्षण में उपयोगी होगा। फसल उत्पादन की वृद्धि में सहायक कुछ अतिविशिष्ट समूहों के अतिशीत संरक्षण प्रोटोकॉल विकसित करने का प्रयोग परिकल्पित है।
- दीर्घकालिक संरक्षण के लिए विकसित प्रोटोकॉल का उपयोग करते हुए मौजूदा रिपाजिटरी के सूक्ष्म आनुवांशिक संसाधनों के अतिशीत संरक्षण के प्रयास किए जायेंगे। एक्टीनोमाइसीट एवं धीमी वृद्धि वाले सूक्ष्मजीवों के अतिशीत संरक्षण प्रारम्भ किया जाना है। ऐसे सूक्ष्मजीव जिनका अतिशीत संरक्षण संभव नहीं है, के संरक्षण के लिए लाइफोलाइजेशन या खनिज तेल संरक्षण तकनीकी पर बल दिया जाएगा। अतिशीत संरक्षित सूक्ष्मजीवों की व्यवहार्यता को मूल्यांकित किया जाएगा।
- ऐरिस सेल के द्वारा सूक्ष्मजैविक रिपाजिटरी पर सूचना राष्ट्रीय डाटाबेस में एकीकृत की जायेगी जिससे इच्छित एवं विश्वसनीय परिणाम मिल सकें। देश के अन्य राष्ट्रीय रिपाजिटरी को एकीकृत करने के लिए इलेक्ट्रानिक माध्यमों से संपर्क विकसित किया जायेगा
- मानव संसाधन विकास क्रियाकलापों को ज्ञान विसरण के निम्न विभिन्न पहलुओं पर भविष्य में जारी रखा जाएगा।
  - ✦ कृषि उपयोगी सूक्ष्मजीवों की जैविक विविधता पर अध्ययन।
  - ✦ कृषि उपयोगी सूक्ष्मजीवों की पहचान एवं उनका संरक्षण।
  - ✦ नई तकनीकी एवं प्रोटोकॉल।
  - ✦ बायोसिस्टेमिक्स पर विशेष बल के साथ सूक्ष्मजैविक गुणवत्ता प्रबन्धन।
  - ✦ डी.एन.ए. फिंगरप्रिटिंग।
  - ✦ सूक्ष्मजीवों की आण्विक खोज एवं बौद्धिक संपदा अधिकार से सम्बन्धित नीतिगत मुद्दे।

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ICAR, New Delhi

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Principal Scientist, NBAIM

Important Recommendations:

- Development of separate set up for commencing the Registration of agriculturally important microbes at NBAIM.
- Scientific collaboration with ATCC and Japan Culture Collection Centres may be taken up.
- Profiles of various Sections of NBAIM as per the need may be developed.
- Commercialization of potential microbials through public-private partnership may be initiated.
- IPR issues related to microbial repository may be taken up in the light of ICAR guidelines.
- Guidelines of submission of microbial cultures may be kept in the website of NBAIM with a link to ICAR website.
- A pool of taxonomists in microbial identification may be created for being initialized as consultants under AMAAS Network Project in NBAIM.
- Publications of catalogues of various groups of microbials may be taken up.
- Repatriation of all national microbial cultures available in research projects under various APCess fund schemes, ICAR Institutes, State Agricultural Universities may be taken up immediately.
- While conceiving new research areas for the XIth five year plan period, care may be given to work on molecular approaches of taxonomy for Actinomycetes, bacteria and fungi.
- In order to facilitate supportive infrastructure for implementing future research programmes lyophilizer, mobile laboratory, cryo-preservation unit with liquid nitrogen plant and laboratory facility for diagnostics of fungal pathogens and soil microbes should be developed.

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Senior Scientist, NBAIM

Important Recommendations:

- Bureau should have the provision for flexi budget for need based consultancy for identification of microbes.
- IMC proposed the Mobile lab for the Bureau.
- Organization of seminar on Biosafety, IPR.
- Development of Culture Collection database and inventerization with provision to upgrade in future.
- Training of scientists/ office staff of NBAIM at appropriate institutions, so as to be helpful for the strengthening the mandated activities of the Bureau.
- To have a security system at NBAIM for the security of key laboratories and to safeguard unauthorized entry to the culture collections as well as the molecular laboratories.
- Filling up of the posts of Senior Scientists with specialization in areas of Microbiology and Biotechnology.
- Maintenance of campus, lighting, proper drainage system, cleaning of the channel nalas, improvement of water supply pipelines and sump well and also the boring of a new tubewell for safe drinking water supply.
- Electrification of unfinished quarters from plan budget.
- To organize Brain Storming Session inviting all Bureaus at Delhi for Registration of Microbes.

NOTES