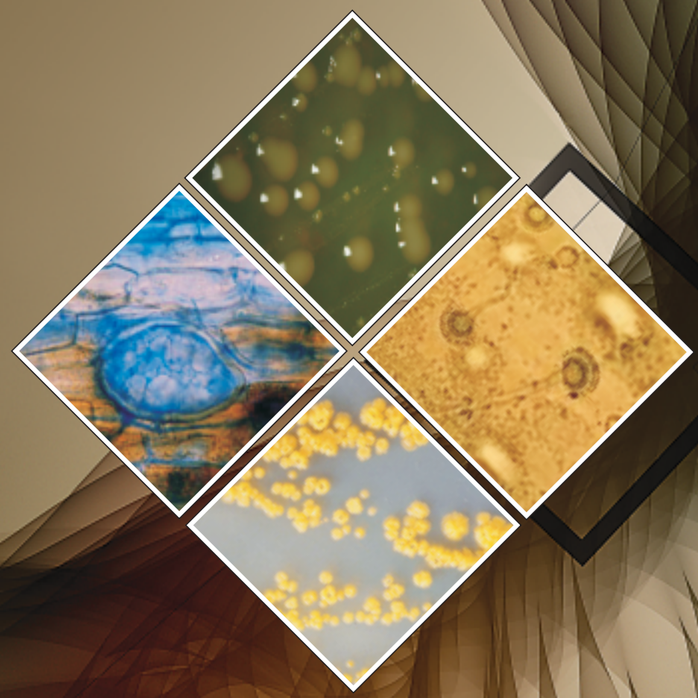

Corresponding Address

Prof. Dilip K. Arora

Director

National Bureau of Agriculturally Important Microorganisms

Kusmaur, Post Box No. 6, P. O. Kaithauli, Mau Nath Bhanjan - 275 101 (Uttar Pradesh) INDIA

Tel : 0547-2530080, Fax : 0547-2530358 E-Mail : nbaimmau@yahoo.com, nbaim2000@yahoo.com

visit us at : www.icar.org.in/nbaim/index.htm

भाकृअनुप
ICAR

Annual Report 2006-07

वार्षिक प्रतिवेदन 2006-07

NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT MICROORGANISMS

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो

NBAIM

Understanding and conserving our national heritage of agriculturally important microorganisms

Dr. Mangala Rai, Secretary DARE and D.G. ICAR, **Dr. H. P. Singh,** D.D.G. (Hort.), **Prof. Dilip K Arora**, Director NBAIM with research group of NBAIM

NBAIM Family

NBAIM

Annual Report 2006-07

वार्षिक प्रतिवेदन 2006-07

भाकृअनुप
ICAR

भारतीय कृषि अनुसंधान परिषद
Indian Council of Agricultural Research

NBAIM
रा.कृ.उ.सू.ब्यू.

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो
NATIONAL BUREAU OF AGRICULTURALLY
IMPORTANT MICROORGANISMS

Understanding and conserving our national heritage of agriculturally important microorganisms

Published by

Dilip K. Arora
Director, NBAIM

Editorial Board

A. K. Saxena
Rajeev Kaushik

Secretarial Assistance

Manish Kumar Jain

Hindi

Ashok Kumar

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Beautiful world of different species of Actinomycetes

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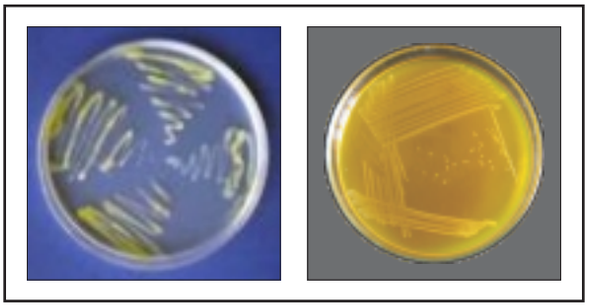


The NBAIM has the mandate "to act as nodal centre at national level for acquisition and management of indigenous and exotic microbial genetic resources for food and agriculture, and to carry out related research and human resource development, for sustainable growth of agriculture". Since the Bureau shifted to NISST building, Mau Nath Bhanjan in 2004, it has taken long strides in developing the infrastructure and carrying out the major research programmes. The major thrust of the Bureau is on the isolation, identification, characterization, preservation and conservation of Agriculturally important microorganisms (AIMs). Four externally funded projects are operative at the Bureau. With a view to strengthen research work in the area of microbiology ICAR gave a major responsibility to NBAIM to develop a network project on 'Application of Microorganisms in Agriculture and Allied Sectors' involving various Institutes of ICAR, SAU's and other Universities. The project is operative at 61 centres all over the country. The network project on AMAAS seek to initiate and strengthen the R&D efforts on various microbe based technologies that can be utilized to increase crop production, utilize agrowaste, manage abiotic stress, biocontrol of important insect pests, diagnostics of important groups of microbes and post harvest technology. It also seeks to strengthen research in the area of microbial diversity, identification and genomics.

NBAIM has developed strong linkages with several National Institutes, SAU's and International Microbial Resource Centres covered under the umbrella of WFCC and OCDE. NBAIM is now an affiliated member of World Federation of Culture Collection (WFCC). The bureau is growing as a nodal center for research on Agriculturally Important Microorganisms. It has developed facilities for the rapid identification of microorganisms and is on the threshold of offering this facility to the nation. The commissioning of additional lyophilization and cryopreservation facilities would further strengthened the state of the art facility for conservation and preservation of microorganisms.

In the last one year various facilities like PCR, Gel electrophoresis unit, Gel Documentation system, Lyophilizer, Microscopes, fermentor, DNA Sequencer, Microbial identification system, TGGE system, Gas chromatograph for FAME/ PLFA analysis, Automatic N- analyzer, Ultracentifuge, High speed centifuge, Sonicator, Freeze drier, Bead beater, ELISA reader with washer, water purification system, PCR four block, RT-PCR were added through different projects. The Bureau has HRD component in which training programs are organized in the field of molecular identification of AIMs and tools for development of microbial technology and its implementation. A Website (www.icar.org.in/nbaim/index.htm) of NBAIM was created and all the units of the NBAIM are linked with various ICAR research institutes and

research organization. The internet facility through VSAT was established at the Bureau and has enhanced the connectivity through wireless LAN.

There are six ongoing Institute research projects dealing with various aspects of diversity analysis of microorganisms in Indo Gangetic plains and extreme environments. Surveys were carried out and soil samples were collected from Northern Indo Gangetic plains, thermal springs mangroove ecosystem of Sunderbans, Western Ghats and Eastern Ghats. The DNA fingerprinting protocols using different molecular markers techniques for some important plant pathogenic fungi such as *Fusarium and Macrophomina phaseolina* have been developed. A combination of PCR and RFLP analysis of 28S r-DNA was used to differentiate *Fusarium* species and to assess their genetic relationships along with two housing keeping gene *Topoisomerase-II* and *Cellobiohydrolase-C* genes. NBAIM has taken lead in molecular analysis of some important group of bacteria like *Bacillus*, fluorescent *Pseudomonas* and *Serratia*. A simple procedure was developed for identification of genus *Bacillus* per se and to identify species based on sequencing of only a small fragment of 16S rRNA. More than 200 isolates of actinomycetes were obtained from different ecological regions including exotic zones. Six isolates belonging to *Streptomyces* species were found to be good producer of protease and could grow upto 50^{o}C.

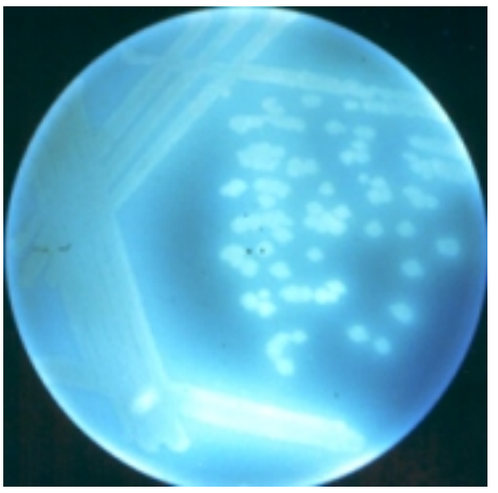
In an endeavor to develop human resource in the field of microbiology and biotechnology, the Bureau organized four training programmes and Kisan Goshthi. The major emphasis was to impart knowledge on various techniques and tools to study microbial diversity, identification, preservation and conservation of AIMs, DNA fingerprinting of microbes, bioinformatic tools and issues related to IPR. The Bureau is also continuing with its HRD activity to impart short and long term training to post graduate students from various National Universities/Institutes.

Dilip K. Arora
Director, NBAIM

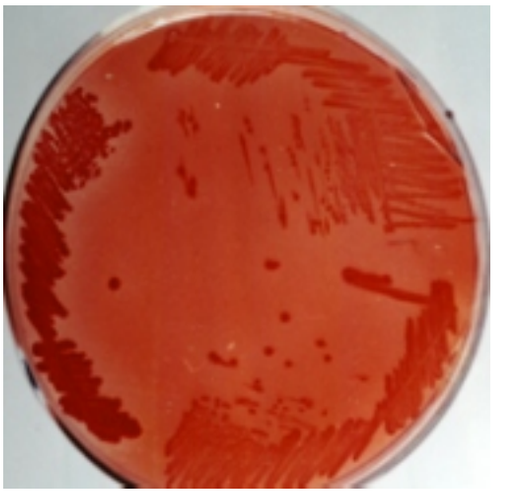
Preamble

The National Bureau of Agriculturally Important Microorganisms (NBAIM) is located at Mau Nath Bhanjan, Uttar Pradesh. The Bureau at present has four divisions viz. Microbial Conservation, Microbiology, Microbial Biotechnology and Microbial Isolation & Preservation. Microbial conservation division has the objectives to plan short-term and long-term conservation of AIMS, Microbiology Division has the objectives to identify, characterize and document AIMs. Identification of AIMs is also carried out for utilization as bio-fertilizers, bio-pesticides, growth promotion, bio-indicator, bio-degradation, bio-remediation, bio-composting, food processing etc. preparation of monographs of AIMs, synoptical keys for the identification of germplasm collection. Microbial Biotechnology division is vested with biochemical and molecular characterization, development of molecular markers, and diagnostic tools of AIMs. Microbial Isolation and Preservation Division is responsible for isolation and collection of AIMs from different agro-climatic zones of India.

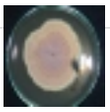
Besides these, the Bureau has HRD component under which training programs are organized in the field of morphological, biochemical and molecular identification of AIMs and tools for development of microbial technology and its implementation. In addition, the Bureau has five cells namely Agriculture Research Information System (ARIS), Technical Cell, Culture Collection Cell, Planning Monitoring and Coordination Cell and Hindi Cell.



Pseudomonas fluorescens



Azospirillum brasileme



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).

Streptomyces sp.

Detailed Research Activities

Exploration and collection of AIMs

- From soils, plants, freshwater etc.-covering different agro-climatic regions of India
- From existing culture collection centers, institutions and universities. The Bureau will function as a repository for all the AIMs available in the country.
- By repatriating 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 to 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

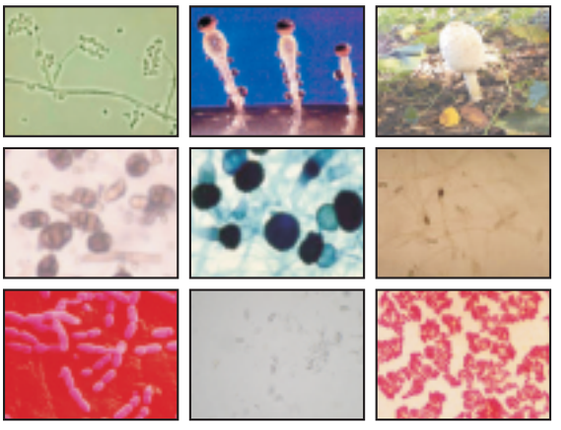
- Isolation and collection of exotic AIMs from different agro-climatic zones of India.
- Characterization of exotic AIMs on the basis of morphological, biochemical and molecular characters.
- Isolation and identification of bioactive compounds produced by exotic AIMs.
- Exploitation of AIMs for sustainable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

Specific targets and monitoring during the Xth 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

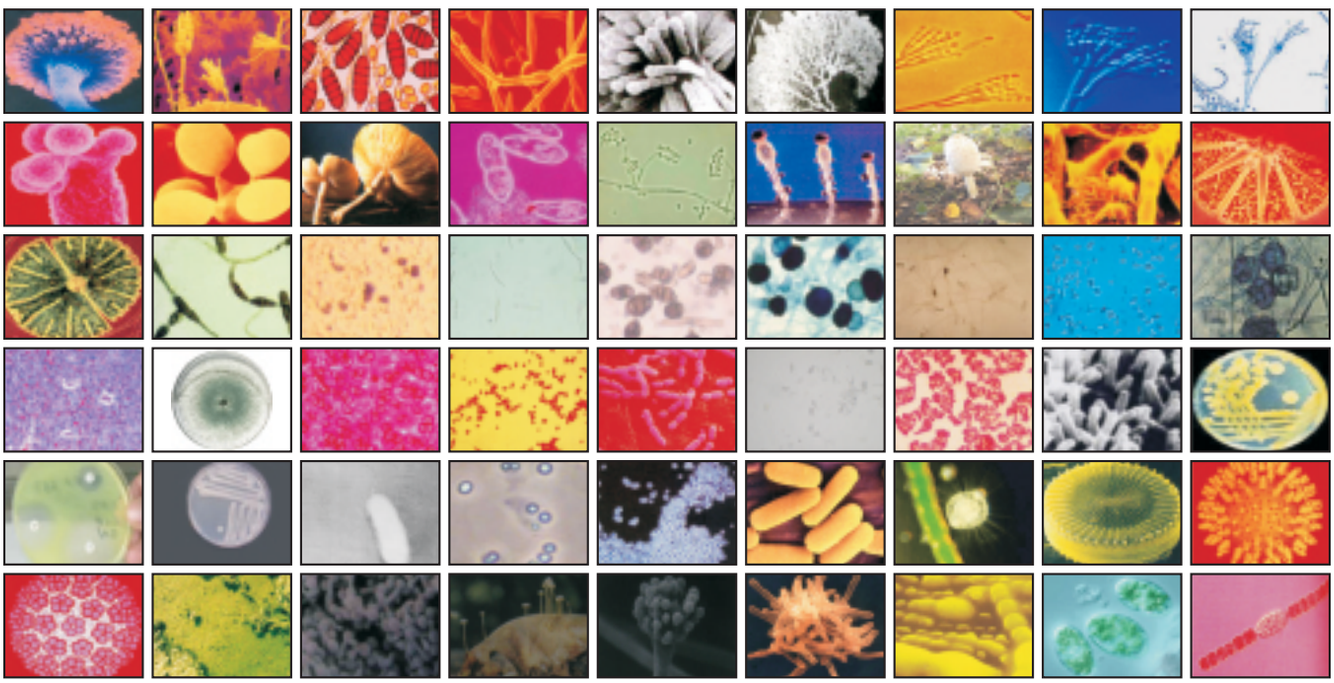
- Morphological, physiological, and biochemical.
- Molecular characterization based on prioritization with emphasis on IPR regimes.
- Development of molecular diagnostic tools.

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 etc.

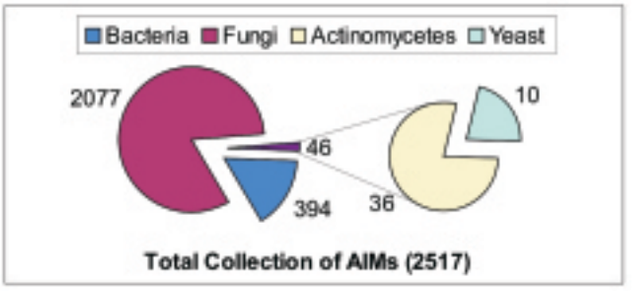


A unique world of microbes

Salient Achievements

Germplasm conservation

NBAIM follows strict quality and biosafety standards in the laboratories as well as culture collection. NBAIM repository has 2517 cultures, which includes filamentous fungi, bacteria, actinomycetes and yeasts isolated from various sources like soil, plant, insect, etc. Each culture is preserved by two methods according to the type of microorganism, either under mineral oil storage, or freeze-drying/ lyophilization, or storage in glycerol at -80° C.



Culture Collection of AIMs at NBAIM

- Exploration missions were undertaken in different states like Uttar Pradesh, Himachal Pradesh, Madhya Pradesh, Arunachal Pradesh, Assam, Rajasthan, Bihar, Sunderbans and Kerala for collection and isolation of AIMs. Over 725 fungi, 65 bacteria and 27 actinomycetes have been isolated during the exploration missions.
- Of the various isolates obtained during the surveys carried out by NBAIM, several strains belonging to *Pseudomonas fluorescens*, *Pseudomonas aeuriginosa*, *Bacillus subtilis*, *B. brevis*, *Fusarium oxysporum*, *Hypocrella discoidea*, *Metarhizium anisopliae*, *Pseudomonas sp. Trichoderma harzianum*, *T. koninghii* and *Verticillium lecanii* were found to suppress the growth of soil borne pathogenic fungi *in vitro*.
- About 100 strains of fusaria pathogenic to chickpeas, lentils and oilseeds were fingerprinted using a variety of experimental protocols and analytical procedures. Diagnostic probes for *Fusarium* were developed.
- A combination of PCR and RFLP analysis of 28S r-DNA was used to differentiate *Fusarium* species and to assess their genetic relationships along with two house keeping gene Topoisomerase-II and Cellobiohydrolase-C genes.
- Two species-specific primers and an oligonucleotide probe were designed from the conserved sequence of ITS region for identification of *Macrophomina phaseolina*. The gene sequences of partial ITS-1 region, complete 5.8S rDNA and partial ITS-2 region were submitted to GenBank.
- NBAIM has taken lead in molecular identification of some important group of bacteria like *Bacillus*, fluorescent *Pseudomonas* and *Serratia*.
- A simple procedure was developed for identification of genus *Bacillus* per se and to identify species based on sequencing of only a small fragment of 16S rRNA. Existence of twenty different groups of *Bacillus* were identified in Indo gangetic plains based on 16S rDNA-RFLP analysis.
- Fluoescent pseudomonads were characterized at molecular level and fingerprints were developed for identification of various genotypes.
- More than 200 isolates of actinomycetes were

isolated from different ecological regions including exotic zones. Six isolates belonging to *Streptomyces* species were found to be good producer of protease and could grow upto 50°C.

- Genomic DNA isolation from actinomycetes was optimized and good quality DNA was isolated from 56 isolates of actinomycetes for 16S rDNA PCR amplification.
- A total of 107 bacteria, 133 actinomycetes and 35 fungi were isolated from Rajgiri thermal springs. Out of 107 bacterial isolates, 41 survived at 45°C and 9 isolates survived at 55°C. Out of 133 actinomycetes, 24 survived at 45°C and 19 isolates survived at 55°C.
- Temperature tolerant bacteria capable of producing cellulase and xylanase were identified. These isolates appear to be promising for biomass degradation.
- Bacterial inoculants were developed that can alleviate the harmful effect of salinity and improve the growth of wheat in salt affected soils. These isolates have the capability to produce IAA and solubilize phosphorus at a salt concentration of 8%.
- The microbial shift was observed in paper mill effluent irrigated soils (Lal Kuan, Pantnagar, Uttrakhand), the population of xylan and cellulose degrading bacteria was more in the effluent irrigated soils.
- A network project on "Application of Microorganisms in Agriculture and Allied Sector" (AMAAS) with NBAIM as the nodal centre was approved during the mid term appraisal of Xth five year plan, and was formally launched on Auguest 27, 2006. The total budget outlay for the project is Rs 1600.05 lakhs and is operational at 61 different Institutes/ SAUs/ Universities in the country.
- The AMAAS project has six thematic areas viz. Microbial diversity and identification; Nutrient management, PGPR & biocontrol; Agrowaste management, bioremediation & PHT; Microbial management of abiotic stress; Microbial genomics; and Human resource development.
- To strengthen the HRD on microbial diversity analysis, the Bureau organized training programme on "Microbial Diversity Analysis of Extrimophiles" and "Microbial Community Analysis through Metagenomics".
- The Bureau also organized a Kisan Mela for transfer of technology in association with Directorate of Seed Research, Mau (UP).

Major On Going Research Projects

On-Going Institute Projects :

- Molecular and functional diversity of microorganisms isolated from extreme environments.
- Assessment of genotypic diversity of *Bacillus, Bacillus*- derived genera and fluorescent Pseudomonads in Indo-Gangetic Plains.
- Exploration germplasm collection and characterization of antagonistic micro-organisms of soil borne fungal pathogens in Indo-Gangetic plains of India.
- Microbial diversity analysis of soils contaminated with industrial effluents in northern plains of Indo-Gangetic regions.
- Exploration, Collection, biochemical, molecular and genetic characterization of actinomycetes in Indo-Gangetic Plains of India.

On going AMAAS Projects:

- Diversity Analysis of Microbes in Extreme Conditions.
- Development of Diagnostic Kit for the Identification of *Bacillus.*
- Diversity of Actinomycetes from Indo-gangetic Plains.
- Development of Microbial Consortium to Alleviate Salinity Stress for Improved Growth and Yield of Wheat.
- Complete Genome Sequencing of *Mesorhizobium ciceri* Ca181.
- Microbial shifts in soils contaminated with pulp and paper mill effluent

Externally Funded Projects:

- 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.

Molecular and functional diversity of microorganisms isolated from extreme environments

PI : D. K. Arora
CO-PI : A. K. Saxena, Rajeev Kaushik, A. K. Singh, A. Chaurasia, R. C. Tripathi, A. B. Dash

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 agriultural residues, bioremediation and mining genes for abiotic stress tolerance.

Research Achievements

A total of 130 bacteria were isolated from the rhizosphere of wheat growing in the salt affected soils of Mau, Varanasi, Balia and Gorakhpur. Five different media were employed for the isolation of bacterial diversity. All the isolates were 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.

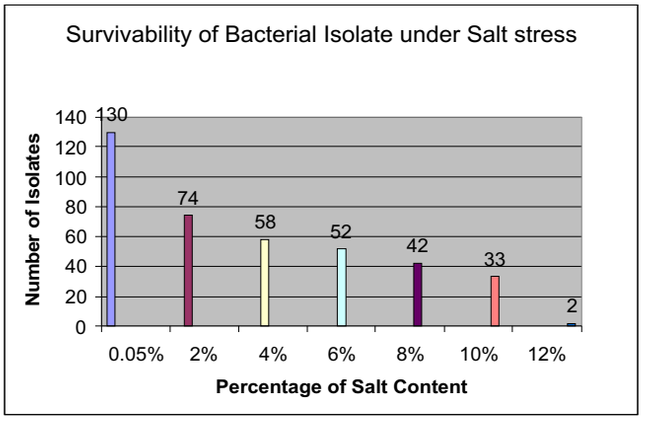
Media	Number of isolates obtained from different sites			
	NBAIM Campus	Varanasi	Balia	Ghazipur
Nutrient Agar	20	18	8	9
King's B	5	3	4	4
Jensen's N Free	9	8	3	6
Soil extract Agar	9	1	4	3
Trypticase Soya Agar	16	0	0	0

Screening of bacterial isolates for the production of osmolytes

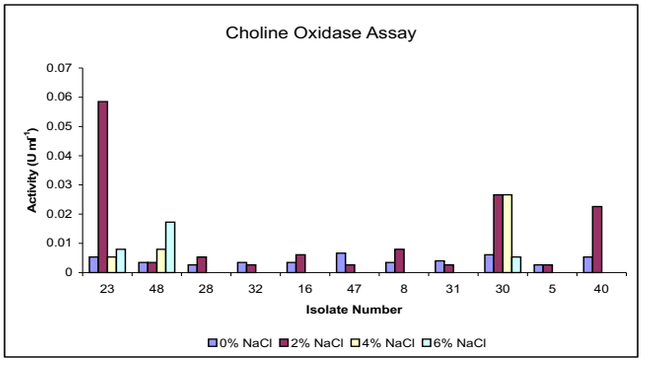
A total of 24 isolates that showed tolerance to 8% NaCl or above were screened for the activity of enzymes involved in salt stress tolerance.

Choline Oxidase Activity

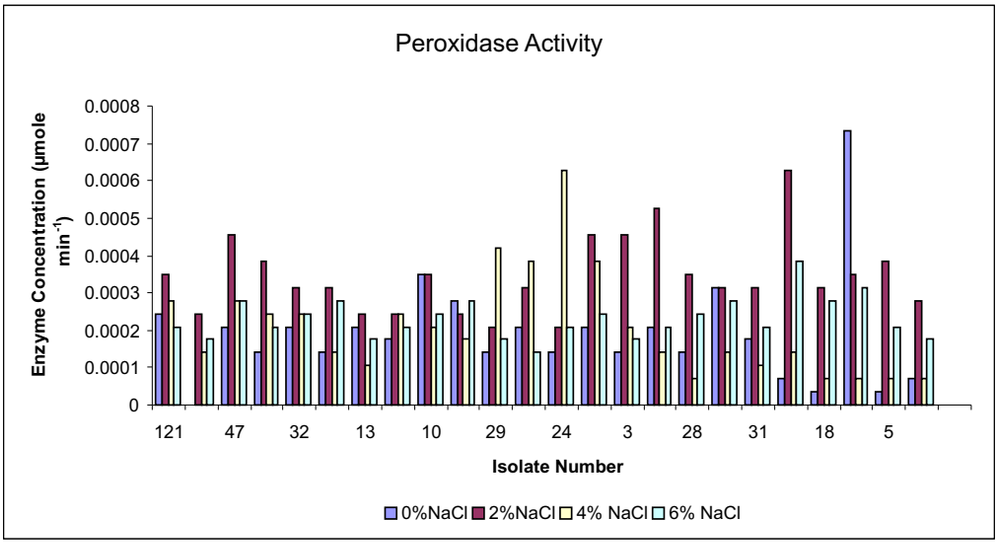
Enzyme choline oxidase is responsible for the conversion of choline to glycine betaine in a single step coupled with both H_2O_2 generation and oxygen consumption. It has been reported from isolates of *Arthrobacter globiformis* and *Arthrobacter pascens*. Of the 24 cultures screened for the activity



Intrinsic resistance of bacterial isolates to salt stress



Activity of enzyme choline oxidase in salt tolerant bacterial isolates at different NaCl concentrations



Peroxidase activity in salt tolerant bacterial isolates at different NaCl concentrations

of choline oxidase, only 11 were able to show activity in the absence of salt. Except for the isolates 23, 30 and 48 others did not show any activity at NaCl concentration greater than 4%. In general for all the isolates except isolate 48, maximum activity was found at 2% NaCl and with further increase in NaCl concentration, there was a decline in the activity of choline oxidase. One unit causes the formation of one micromole of hydrogen peroxide (half a micromole of quinoneimine dye) per minute under the experimental conditions used.

Activity of enzyme Peroxidase

The activity of enzyme peroxidase was measured for 24 isolates at different salt concentrations. Maximum activity was found for isolate 30 in the absence of salt stress. One unit of peroxidase was defined as the amount of enzyme that converts 1 µmol of H_2O_2 to quinoneimine dye (A_{480nm}=2.86) per min.

Assessment of genotypic diversity of *Bacillus*, *Bacillus*-derived genera and fluorescent Pseudomonads in Indo-Gangetic Plains

PI : A. K. Saxena
Co-PI : Rajeev Kaushik

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.

Major objectives

1. Survey and collection of soil samples from Indo-gangetic plains of India.
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

Isolation of *Bacillus* species was carried out from the soil samples collected from the northern Indo-Gangetic plains (near Dehradun) following enrichment technique. One gram of soil sample was added to 10 mL of nutrient broth and heated in a hot water bath at 80^{0}C for 15 minutes in triplicates. The broths were incubated at different temperatures- 30, 37 and 55^{0}C to isolate mesophilic and thermophilic species of *Bacillus*. The isolates obtained from different locations and at different temperatures are shown in table. A total of 74 isolates were obtained from Dehradun area and were purified on Nutrient agar medium amended with methyl red. From the samples collected from Rajgir, Assam and Sunderbans, isolations were

Rajgiri (Gaya, Bihar)	Temperature of Incubation		
Location	**30^{0}C**	**37^{0}C**	**45^{0}C**
Brahmakund with sediments, pH5.8	1	2	0
Brahmakund with sediments, pH6	1	2	0
Brahmakund with surface water, pH6	3	4	0
Saptdhara water, pH5.9	4	1	0
Suryakund surface water, pH5.5	1	1	0
Suryakund surface water with sediment, pH6.5	3	1	0
Brahmakund hard surface water with algae mats, pH5.8	1	1	0
Sandiemnts of Brahmakund, pH6.0	3	1	0
Indo-Gangetic Plains			
Location			
Meerut	3	3	0
Muzaffarnagar	11	15	5
Saharanpur	3	0	4
Baghpat	1	3	1
Dehradun (Sample Number)			
1A	2	4	5
5A	2	2	5
6A	2	1	3
7B	3	4	1
8A	1	5	2
9A	3	2	1
10B	2	2	4
11B	2	3	2
12A	1	4	0
13	1	2	3
Assam			
Location			
Golaghat	2	3	0
Bokaghat	0	1	0
KNP	8	11	1

made both for *Pseudomonas* and *Bacillus*. In total 199 *Bacillus* isolates were obtained of which initially 106 isolates were characterized at molecular level.

Fluorescent Pseudomonas was also isolated from the soil samples collected from Indo-Gangetic plains, Sunderbans and Mau Nath Bhanjan using King's B medium. The plates showing fluorescent colonies under UV light were isolated and purified. Interestingly only four soil samples showed the presence of fluorescent pseudomonas and total of five isolates were obtained from Indo-Gangetic plains.

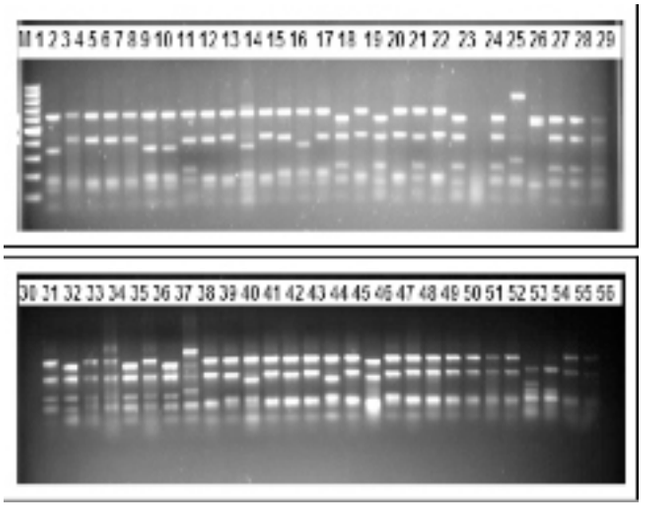
Molecular diversity analysis of *Bacillus* isolates

The molecular diversity analysis of *Bacillus* isolates was carried out using 16SrDNA- PCR-RFLP and 16-23S IGS-PCR-RFLP analysis. The study was carried out to generate database on the distribution of different species of *Bacillus* and *Bacillus* derived genera in Indian soils. Genomic DNA was isolated from all the isolates along with 34 reference strains of different species of *Bacillus* collected from NBAIM culture collection. PCR amplification of 16S rDNA was carried out and all the isolates and reference strains yielded a single amplicon of 1.6Kb.

The results obtained with the 16S rDNA-RFLP analysis with three restriction endonucleases *Alu*I, *Msp*I and *Mbo*I showed a high level of genetic diversity among the *Bacillus* isolates. A total of 32 different fragments were obtained and used for analysis of similarity coefficient. However, bands smaller than 90 bp were not taken into consideration. Restriction endonucleases *Alu*1, *Msp*1 and *Mbo*I produced 26, 24 and 22 RFLP patterns for the 106 isolates and 34 reference strains included in the present study. All the three enzymes used were equally discriminatory

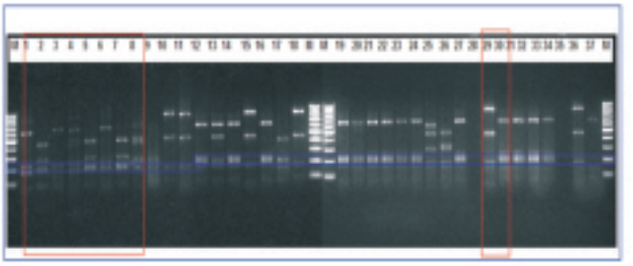
Isolationof fluorescent Pseudomonas from soil samples collected from different locations

Location	Fluorescent Pseudomonas
Indo-Gangetic Plains	05
Rajgiri	29
Sunderbans	09
Maunath Bhanjan	35
Total	**78**



Restriction patterns of PCR amplified fragement of 16S rDNA of *Bacillus* isolates digested with *Msp*1M: Molecular Marker 100 bp ladder, 1-56 *Bacillus* isolates

The combined *Alu*I, *Msp*I and *Mbo*I restriction patterns of the amplified 16S rDNA were used for cluster analysis by UPGMA. Dendrogram was constructed from the similarity matrix by the unweighted pair group method with airthmetic mean (UPGMA) (Sneath and Sokal, 1973). In order to test the goodness of fit of cluster analysis, cophenetic value matrices were calculated and compared with the original similarity matrices that were UPGMA clustered by using the NTSYS-PC analysis package (version 1.6; Exeter Software, Setauket, N.Y.). The similarity coefficient varied from 0 to 100% indicating high degree of genetic variability. The analysis revealed 21 main clusters and the Cluster I was the largest and included 31 isolates and nine reference strains belonging to *B. subtilis*, *B. marcescens*, *B. sphaericus* and *B. amyloliquefaciens*. Many closely related species could not be separated by RFLP analysis. It is also evident from the literature that the similarity among species of *Bacillus* is as high as 99%.

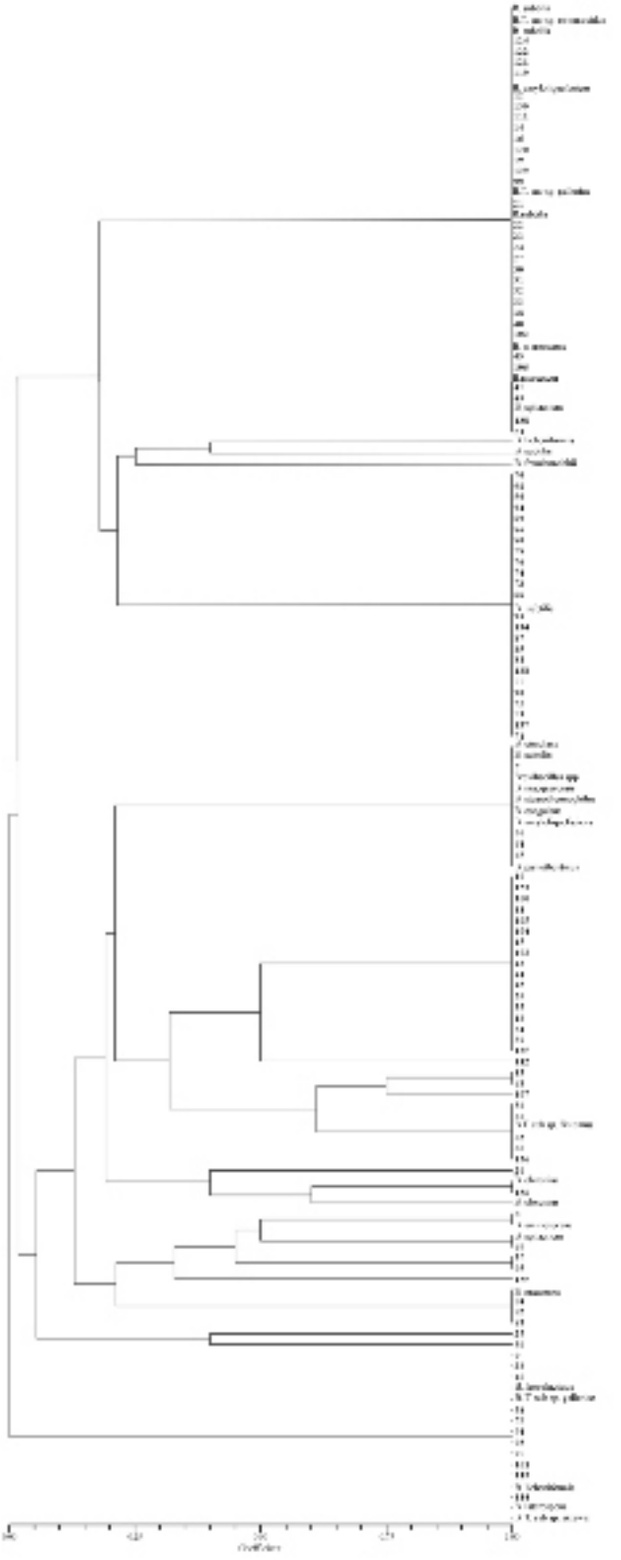


Restriction patterns of PCR amplified fragement of 16S rDNA of *Bacillus* isolates digested with *Alu*1 M: Molecular Marker 100 bp ladder, 1-36 *Bacillus* isolates

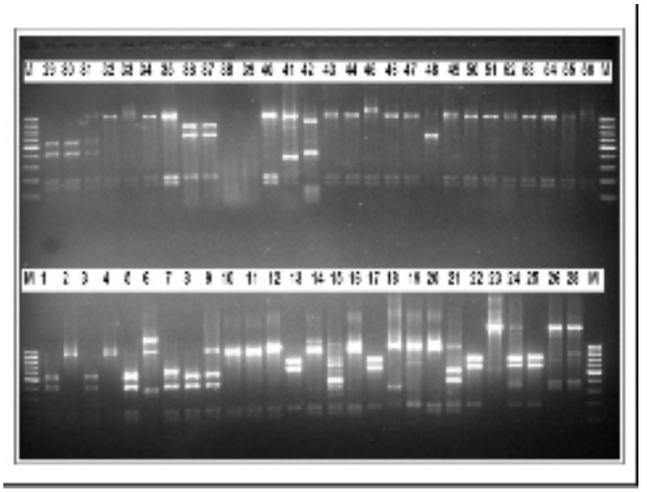
As evolutionary distance decreases, the diversity found in 16S rDNA is often insufficient and thus genetic relationship of closely related species cannot be accurately defined. Noncoding region like intergenic gene spacer (IGS) between 16S-23S rDNA evolves more rapidly and are consequently more variable than coding regions. Polymerase chain reaction has been used as a rapid means of amplifying the spacer region for the specific purpose of detecting heterogeneity between and within species. The amplification of 16S-23S IGS gene sequence revealed large variation in the product size also among different isolates of *Bacillus* and ranged between 1500 to 2000bp.

The RFLP analysis of 16-23S rDNA was carried out with two restriction endonucleases -*Alu*1 and *Hae*III (Fig 7,8). A total of 46 different fragments were obtained and used for analysis of similarity coefficient. Restriction endonucleases *Alu*1 and *Hae*III produced 34 and 36 RFLP patterns for the 106 isolates and 34 reference strains included in the present study.

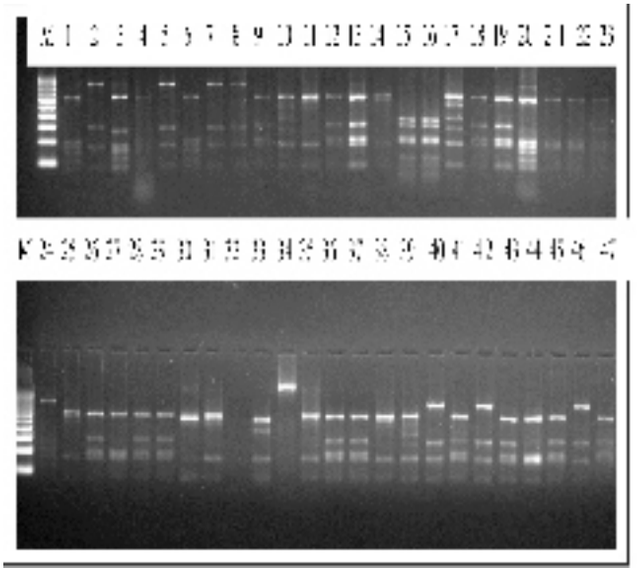
The combined *Hae*III and *Msp*I restriction patterns of the amplified 16S rDNA were used for cluster analysis by UPGMA and a dendrogram was constructed.A total of 34 clusters were obtained. The results revealed that RFLP analysis of 16-23S rDNA is more discriminatory as compared to 16S rDNA as some of the refernce species used that clustered together by 16S rDNA-RFLP could be distinguished by 16-23S rDNA-



Dendrogram depicting the phylogenetic relationship based on 16S rDNA-RFLP analysis with three restriction endonucleases- *Alu*I, *Msp*I and *Mbo*I among the isolates and the standard strains of *Bacillus* spp.

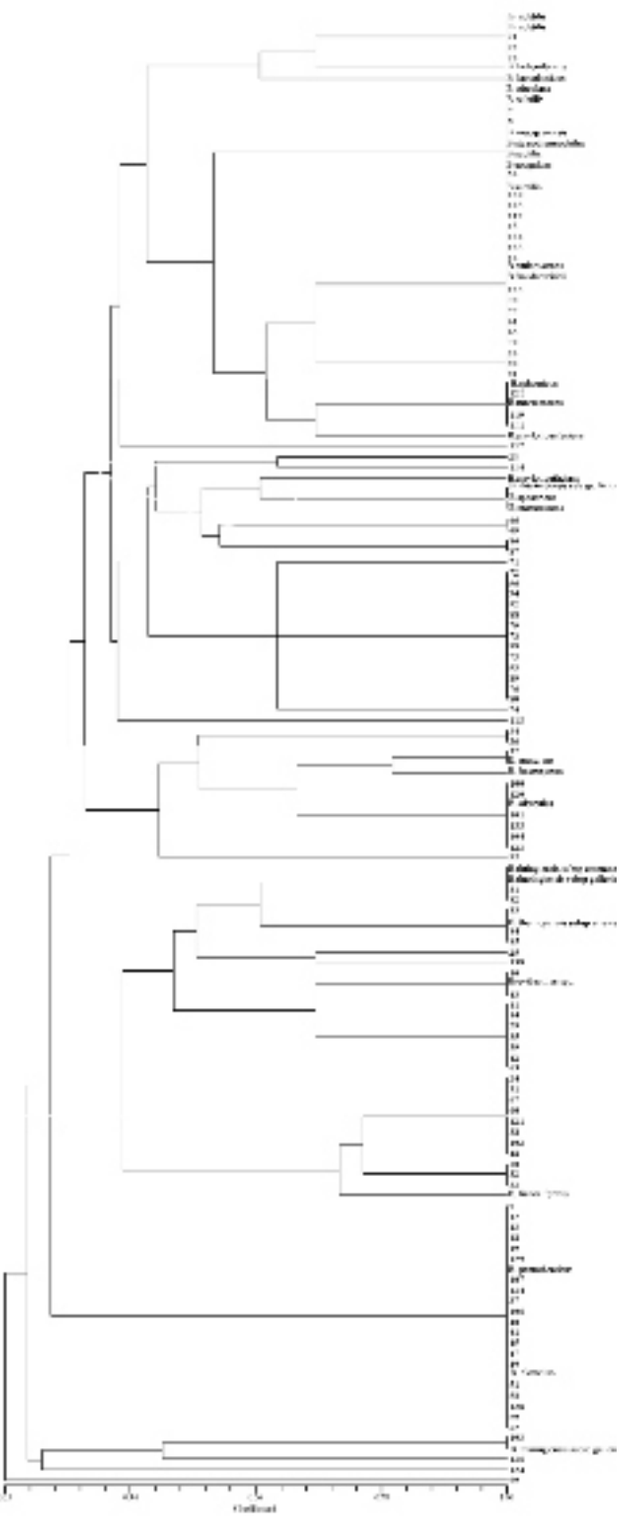


Restriction patterns of PCR amplified fragement of 16 -23S rDNA of *Bacillus* isolates digested with *Hae*1II, M: Molecular Marker 100 bp ladder, 1-56 *Bacillus* isolates



Restriction patterns of PCR amplified fragement of 16 -23S rDNA of *Bacillus* isolates digested with *Msp*1, M: Molecular Marker 100 bp ladder, 1-47 *Bacillus* isolates

RFLP analyses. The molecular diversity analyses revealed that large number of species of *Bacillus* and *Bacillus*-derived genera are distributed in Indian soils. The preponderance of *Bacillus* isolates in soils indicates good health of the soil as many species have been implicated as PGPR involved in nitrogen fixation, P-solubilization, production of plant growth hormones and also as biocontrol agents for pathogenic fungi and insect pests.



Dendrogram depicting the phylogenetic relationship based on 16S-23S rDNA-RFLP analysis with two restriction endonucleases- *Msp*I and *Hae*III among the isolates and the standard strains of *Bacillus* spp

Microbial diversity analysis of soils contaminated with industrial effluents in northern plains of Indo-gangetic region

PI : Rajeev Kaushik
Co-PI : A. K. Saxena

Rationale

The rice-wheat (RW) cropping system occupies 10 million ha of the productive area in the Indo-Gangetic plains (IGP). It is an important cropping system for the food security of our country. In recent past land degradation has reduced the productive capacity of this area. These soils suffer physical, chemical and biological degradation due to intensive and mechanized management practices. 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. Indiscriminate use of industrial effluents for irrigation purposes in many parts of IGP is the most common practice followed by the farmers, which may deteriorate the soil fertility in long run.

In India distillery and paper mill industry falls under 17 identified categories of the highly polluting industries defined by the Ministry of Environment and Forests, Government of India. Pulp mills require enormous quantity of water for debarking the trees, washing the wood chips, bleaching the pulp as well as the final papermaking step. Distilleries using molasses generate enormous quantity of organic rich acidic effluent. The heterogeneous natures of these effluents often having BOD and COD levels well above those of their recipient waters, pose a significant wastewater treatment challenge to mill operators. It has been observed that these industries find it difficult to achieve the standards and pollute the surface water bodies when the effluent from these industries is discharged. In India, among various pulp and paper mills, the larger ones have installed the chemical recovery plant but the colour problem still persists. Most of the smaller mills do not have such chemical recovery plants and are the cause of severe environmental pollution of soil and ground water

Indiscriminate use of these effluents for irrigating agricultural land by the farmers in northern plains of IGP might cause shift in the functions and structure of native microbial communities. 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. There is a need to isolate, identify and conserve microorganism which are able to detoxify the toxic or organic components present in the effluent that are contaminating the soils.

Objectives

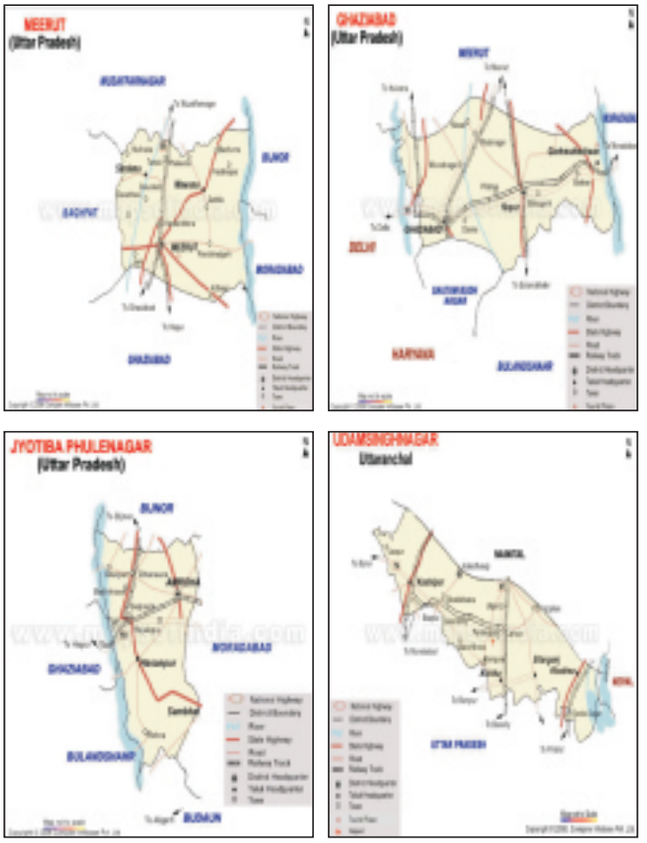
- Isolation, characterization and identification of bacteria from agricultural sectors contaminated with paper and alcohol industry effluent in Northern plains of Indo Gangetic Region.
- Deciphering shifts in bacterial structural and functional diversity in soils contaminated with such effluents.
- Development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

industries 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 collecting soil samples, the fields were selected in the identified regions, where effluent is



Geographical location of sampling area in Northern region of Indo Gangetic Plains

being used for irrigation since last 20 years. For the sampling of soil and plants three fields were selected (i) Control field where effluent irrigation was not done at all and is being irrigated only with fresh water, (ii) Diluted 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.

Analysis of soil physicochemical properties

Soil pH, moisture content and texture **:** 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. The higher OC content of the effluent irrigated field over the control soil might be due to the incorporation of lignocellulosic and hemicellulosic fractions of effluent into the soil on regular basis.

In contrast, the distillery effluent contaminated soil showed acidic pH as compared to the control soils from the same region of sampling. The irrigation of soils with distillery effluent for long term in these regions might have reduced the pH due to the presence of organic acids and high carbon content in these effluents.

As the samples were collected during the fallow period the moisture % was not more than 20%. The texture of soil in the paper mill and distillery effluent contaminated soils was clay loam and sandy loam respectively.

Soil moisture (%), pH and total organic carbon content of soil samples receiving irrigation of paper mill and distillery effluent.

Treatment	Paper mill effluent Contaminated soils			Distillery effluent contaminated soils		
	Moisture (%)	pH	OC (%)	Moisture (%)	pH	OC (%)
CF	7.93	7.33	0.68	23.6	7.68	0.21
DEIF	16.57	8.17	0.81	21.8	6.84	0.34
CEIF	20.16	7.54	1.21	20.9	5.98	0.38

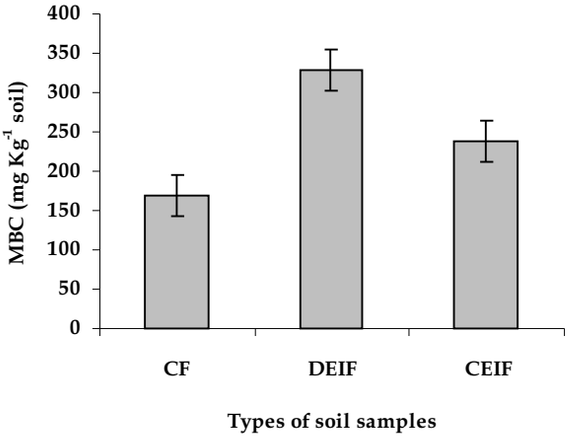
*CF: Control Field; DEIF: Diluted Effluent Irrigated Field; CEIF: Concentrated Effluent Irrigated Field

Texture of different soil samples.

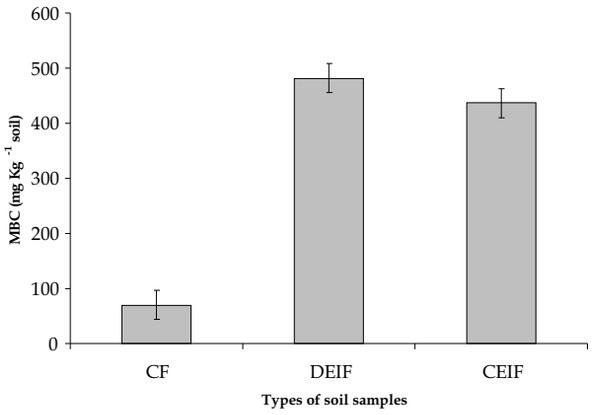
Treatment Site*	Paper mill effluent Contaminated soils	Distillery effluent contaminated soils
CF	Clay Loam	Sandy Loam
DEIF	Clay Loam	Sandy Loam
CEIF	Clay Loam	Sandy Loam

*CF: Control Field; DEIF: Diluted Effluent Irrigated Field; CEIF: Concentrated Effluent Irrigated Field

***Soil Microbial Biomass Carbon*:** The nutrient availability and productivity of an agro-ecosystem mainly depends on the size and activity of the microbial biomass, which is sensitive to any kind of ecological disturbance. Application of high



Microbial Biomass Carbon of the soil as influenced by paper mill effluent application



Microbial Biomass Carbon of the soil as influenced by distillery effluent application

organic matter containing wastewater to soil influences microbial biomass. Therefore, before adopting the use of paper or distillery mill effluent for irrigation on large scale by the industries on farmer's field, it is essential to understand the changes that may occur in soil microbial biomass due to application of such wastewaters. 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 chemical and physical properties resulting from soil management and environmental stresses in agricultural ecosystems.

The increase in soil microbial biomass carbon due to the application of paper mill effluent in CEIFand DEIF fields over control clearly indicated that due to the incorporation of effluent rich in lignocellulosic and hemicellulosic compounds enriches the soil with more of carbon and slowly with time these compounds get degraded and increase the soil microbial biomass carbon. However, increase in soil microbial biomass carbon over the period of time indicates good soil health but application of effluent may change the microbial community structure of the soil. The shift in microbial community over the period of time may not be beneficial for the sustainable crop productivity.

Isolation of bacteria from the samples:

In total 192 bacterial isolates were isolated from the rhizospheric, non-rhizospheric and endorhizospheric regions of the soil and plant samples. The media used were methyl red agar, crystal violet agar, King's B, Jensens N free media and Actinomycetes isolation agar. The isolates were tested for their ability to utilize cellulose and xylan as sole C source and it was observed that, off the 192 isolates, 95, 112 and 49 isolates showed cellulase, xylanase and CMCase activity, respectively.

Change in soil microbial community structure was observed due to excessive use of effluent for long term. In non-rhizospheric soil samples, significant reduction in microbial population was observed in samples from DEIF and CEIF over control field samples in all the media used, except Methyl Red (MR) Agar (specific for Gram positive bacteria) where significant increase was observed. Similar response was observed in case of rhizospheric soil, however, the MR media showed statistically at par total microbial count. Endorhizosphere colonizing of bacteria was only

observed in control field and in effluent treated field no colonization was observed except that of samples from CEIF showed only 2 x 10^5 bacteria population in Jensen's media.

Isolation of thermo-alkalophilic microorganisms:

From the soil samples of paper mill effluent irrigated soils, 32 bacterial isolates capable of growing at 45-65 °C and pH 9-11 were isolated showing xylanase activity. The isolates are preserved for further studies.

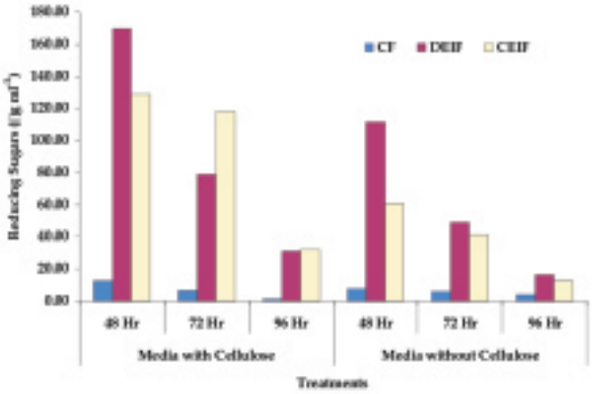
Variation observed in total microbial count (x10^5 gm^{-1} soil) of fields irrigated with paper mill effluent for 20 years in succession in different concentrations over control by using different types of semi-selective media

Media Types	Non-Rhizospheric Soil			Rhizospheric Soil			Endo-Rhizospheric Count		
	CF*	DEIF	CEIF	CF	DEIF	CEIF	CF	DEIF	CEIF
Jensens Media	5	6	2	15	8	18	0	0	2
CV Agar	70	15	4	37	19	4	0	0	0
MR Agar	23	65	12	56	15	56	0	0	0
PDA	0	0	2	0	0	0	0	0	0
KB Agar	108	0	5	150	26	67	24	0	0

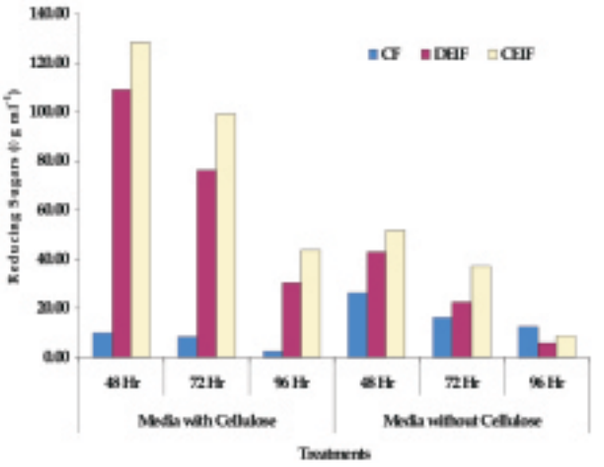
*CF: Control Field; DEIF: Diluted Effluent Irrigated Field; CEIF: Concentrated Effluent Irrigated Field

***Soil microbial cellulase and xylanse activity*:**

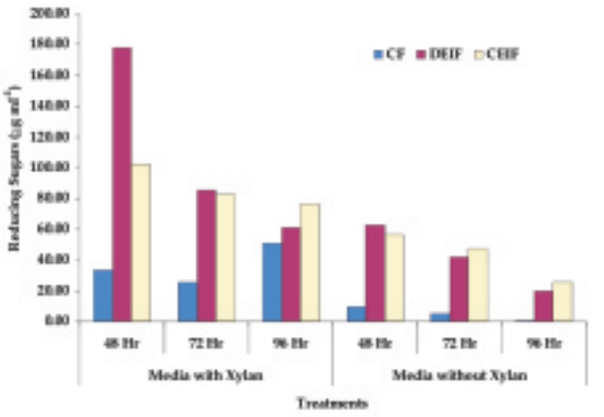
In order assess the changes that have occurred in cellulase and xylanse activity of total soil micro flora due to effluent irrigation, soil was incubated with cellulose and xylan and formation of reducing sugars were estimated. Variation in total soil microbial functional activity for cellulase and xylanse was observed in the soil samples irrigated



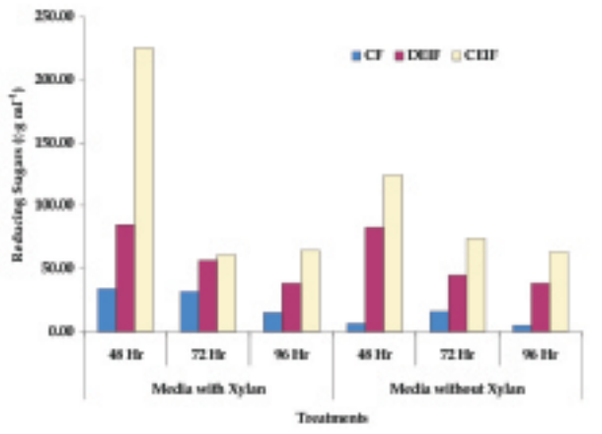
Soil microbial cellulase activity of soil irrigated with distillery effluent



Soil microbial cellulase activity of soil irrigated with paper mill effluent



Soil microbial xylanse activity of soil irrigated with distillery effluent



Soil microbial xylanse activity of soil irrigated with paper mill effluent

with diluted and concentrated effluent over control field samples. Soil irrigated with paper mill effluent showed significantly higher cellulase and xylanse activity over soil irrigated with distillery effluent. In both the cases significantly higher production of reducing sugars was observed in effluent irrigated soils over control.

Variation was also observed with time of incubation. Maximum production of reducing sugar was observed at 48 hrs of incubation and as the time increased, reduction in the amount of reducing sugar was observed in the medium. This could be due to the utilization of simpler sugars which have been formed during the course of incubation rather than utilizing cellulose and xylan.

Upon comparing the results with the control field, it can be concluded that, by the utilization of effluent in succession for over 20 years, changes in soil functional and structural properties have occurred. The change in soil microbes carbohydrate utilization pattern reveled that shift has been occurred from autochthonous plant growth promoting rhizobacteria to allochthonous complex structural polysaccharide utilizing microbes. Hence, in years to come the productivity of such lands may decrease if measures are not taken to improve the soil microbial C:N ratio, which is an indicator of soil fertility.

Exploration, germplasm collection and characterization of antagonistic microorganisms of soil borne fungal pathogens under Indo-Gangetic plains of India

PI : A. K. Singh
Co-PI : R. C. Tripathi

Rationale

Rice-wheat cropping system is most prevalent in the Indo-Gangetic plains and occupy almost to 10 million hectares. Over the years there has been a decline in the yield of wheat and rice. One of the reasons could be the losses due to fungal diseases and pests. Soil-borne fungal pathogens have been recognized in playing a major role in the root disease complex causing seed decay, damping off, seedling blight, collar-crown rots and wilt. For these soil-borne pathogens, chemical control methods are arduous, uneconomical and non advisable owing to risk of ground water pollution, death of non-targeted beneficial flora and evolution of fungicide resistant pathogens variants. Biological control by antagonistic microorganisms is a potential, non chemical and eco-friendly tool for crop protection against many phytopathogens including the soil borne pathogens. Bio-control agents like species of *Trichoderma*, *Gliocladium* and *Pseudomonas* have been used for managing soil borne diseases. Exploration and collection of antagonistic microorganisms for management of important soil-borne pathogens was undertaken from soil samples collected from different locations of north IGPs of India.

Objectives

- Germplasm collection of antagonistic microorganisms from soil, plants from different location of Indo-Gangetic plains of India.
- *In vitro* screening of fungal and bacterial isolates against soil-borne fungal pathogens

Significant achievements

Surveys were conducted at different locations in four districts namely Dehradun, Aligarh, Mathura and Mau (IGPs). Forty eight soil samples were collected from farmer's fields, uncultivated lands, forest areas and canal side plantations. A total of 110 fungi were isolated and purified by using standard protocols.

In vitro screening for antagonistic activity of 50 fungal isolates were carried out against *Macrophomina phaseolina* by using dual culture plate technique. Mycelial disc of 6 mm dia. was cut from the margin of three days old cultures of both test antagonist and target pathogens were placed

List of fungi isolated from rhizospheric soil samples

S.No	Fungus	No of isolations
1.	*Trichoderma* spp.	15
2.	*Fusarium* spp.	08
3.	*Macrophomina*	06
4.	*Aspergillus* spp.	10
5.	*Phoma* spp.	02
6.	Unidentified	69
	Total	**110**

Zone of inhibition of *Macrophomina phaseolina* by the isolated fungal antagonists on dual culture

S. No.	Fungal antagonist	Zone of inhibition (in mm)*		
		3 day	5 day	7 day
1	*Trichoderma harzianum*	0.8	7.0	15.0
2	*T. viride*	0.9	8.0	16.0
3	Unidentified Isolate 1	1.2	6.0	09.5
4	Unidentified Isolate 2	0.8	7.0	11.0

*Mean of 10 replicates

Lysis of mycelium of *Macrophomina phaseolina* by *Trichoderma harzianum*

opposite to each other on PDA in Petri dish. The pathogen were placed 24 h before placing of antagonist to make the two organisms meet near around the centre of plate. Following inoculation at 28°C the plates were observed for zone of inhibition.

Two isolates of *Trichoderma* spp. showed antagonism against *M. phaseolina in vitro.* The visual observation revealed that isolates started lysing mycelium *of M. phaseolina* on contact (5-6) days and completely lysed after 8-10 days of contact. The two unidentified fungal isolates showed >9.5 mm inhibition zone against *M. phaseolina*. These isolates seems to have potential for the control of *M. phaseolina* and could be exploited further.

Exploration, Collection, Biochemical, Molecular and Genetic Characterization of Actinomycetes in Indogangetic plains of India

PI : Anurag Chaurasia

Rationale

Actinomycetes are a group of morphologically diverse Gram positive bacteria with higher GC content. Morphological diversity ranges from micrococci which divide as cocci to members of the antinobacteria that forms pleomorphic rods or go through a coccus-rod-coccus life cycle. More complex still are the branched filaments forming actinomycetes that multiply by variation on a theme of fragmentation division. Spherical cells of the nocardioform actinomycetes grow into branched filamentous mycelia that multiply fragments at the onset of stationary phase to form the spherical cells. Actinomycetes can be found almost in any substrate, although they prefer alkaline and neutral conditions in order to grow. The optimal pH range in which they grow is between 7 and 8. Nevertheless, they can live under acidic conditions between pH 4.8-5; however this is a critical condition for these bacteria. Most of the Actinomycetes grow at temperatures between 15 and 30°C, however, some like the thermophiles Actinomycetes live in very high temperature, about 60°C. Once plated, Actinomycetes have a compact, leathery appearance with a dry surface. They are reported as the source of an earthy smell as they produce Geosmin and 2-methylisoborneol (MIB) an organic metabolites They are also a group of physiologically diverse bacteria. The diversity is seen in the production of secondary metabolites, enzymes and in the thousands of kinds of metabolic products that they synthesize and excrete. Many of these products are antibiotics with the ability to inhibit growth of other bacteria, fungi, viruses and protozoa. Indo-Gangetic plains 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 Indo-Gangetic plains which will be exploited for agricultural productivity and human welfare.

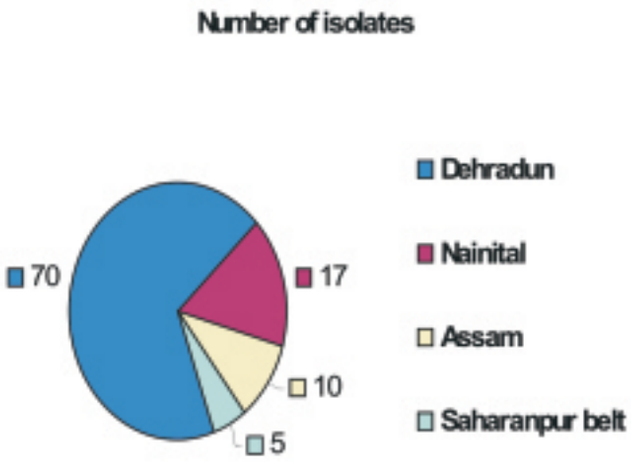
Objectives

- Survey and collection of soil samples from Indo-Gangetic plains of India.
- Isolation of actinomycetes diversity from soil samples.
- Biochemical, molecular and genetic characterization of identified isolates.
- Application of useful isolates for agricultural productivity and human welfare.

Achievements

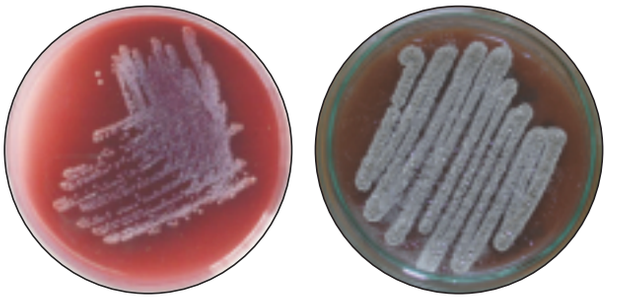
Using CaCO3 enrichment technique, 102 actinomycetes were isolated from soil samples collected from Dehradun, Nainital, Assam and Saharanpur belt of Indo-Gangetic plain. These strains were isolated using media like Actinomycete Isolation Agar Medium and Starch casein agar. Isolates have been morphologically characterized using soluble pigment they produce, color of substrate mycelium and texture of the colony as the criteria. They are able to grow at wide ranges of temperature and saline conditions. Isolates have been preserved for short term duration in agar slant culture and kept at 4^0 C. For long term preservation they have been kept in 10% glycerol and kept at -80^0 C.

Number of isolates from different soil samples



Actinomycetes Identification

Digital atlas comprising of 121 macroscopic and scanning electron microphotograph of various actinomycetes genera has been compiled with the help of Society of Actinomycetes, Japan (SAJ). Initial identification of some of our own isolates at the genus level was carried out by phenotypic comparison with the species available in the atlas.

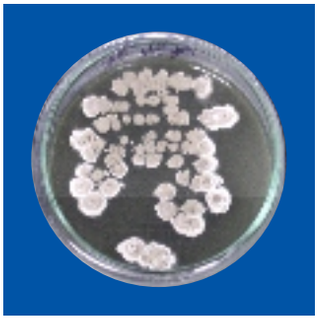


Dactylosporangium sp (SAJ) **NBAIM isolate**

Future Plan

Molecular identification of the isolates to be standardized by using amplified rDNA restriction analysis (ARDRA). This rapid method for identifying filamentous actinomycetes genera is based on 16S rRNA gene restriction fragment patterns. The patterns were generated by using specific restriction endonucleases to perform *in silico* digestions on the 16S rRNA gene sequences of all validly published filamentous actinomycete species. The method is being applied to identify actinomycetes isolates from soil.

Streptomyces sp. (SAJ)



NBAIM isolate

Network project on "*Application of Microorganisms in Agriculture and Allied Sectors*" (AMAAS)

With a view to strengthen research work in the area of microbiology ICAR gave a major responsibility to NBAIM to develop a network project on AMAAS involving various Institutes of ICAR, SAU's and other Universities. The project was prepared and submitted as a part of revised Xth plan EFC to ICAR and was finally approved for a period of one year with a total budget outlay of Rs.1600.09 lakhs. The project is operative at 61 centres all over the country. The project was formally launched on September 27, 2006 at NBAIM.

Rationale

Microorganisms present in the soil play an important role in nutrient solubilisation, mobilization and recycling. They have very wide potentialities to control soil borne pathogens, stimulating plant growth, increasing nutrients availability, accelerating decomposition of organic materials, and are anticipated to increase crop production.

Sustainable agriculture involves the successful management of agricultural resources to satisfy changing human needs while maintaining or enhancing the environmental quality and conserving natural resources. The continuous decline in soil organic matter levels due to continuous cropping without recycling enough crop or animal residues, and insufficient application of nutrients has led to serious nutrient imbalances, impaired soil health and declining factor productivity. Currently, there is a gap of nearly 10 million tones of nutrients between what crops take out and what is added through fertilizers and manures. Thus, there is an urgent need to recycle all available organics in a more efficient way and improve and expand biofertilizer usage. These are the only feasible and low cost and eco-friendly way of improving nutrient supply and improving soil health in the short and medium run. With the crude prices having crossed the 65 dollars per barrel level, the continuously escalating prices of nitrogen fertilizers poses a serious burden on marginal farmers. Also it has to be anticipated that the subsidies on fertilizers would be slowly but surely phased out. Fertilizer nutrient use efficiencies continue to be notoriously poor, 35-50% for N, 15-20% for P, 60-70% for K and 30% for S. Therefore continuous addition of nutrients under such poor efficiency scenarios is a waste of money and foreign exchange involved in importing some fertilizers. In many cases such nutrients are locked in unavailable forms in soils. Mobilizing such reserves through microbes is an urgent imperative. Hence it is now strongly realized that integrated plant nutrient supply systems involving a combination of chemical, organic and biofertilizers is the only alternative to improve nutrient use efficiency, sustain crop production and improve soil health. This requires us to strengthen microbiological alternatives as nutrient sources. Neglecting biofertilization will therefore be dangerous. It is essential to study the influence of microbes such as nitrogen fixers, P-solubilisers, VAM fungi and PGPR in the retention of absorption and release of nutrients to plants to augment crop yields.

Most of the work done in this area has focused on the use of individual beneficial soil microorganisms in crop production as microbial inoculants in the past. But now combined inoculations of mixed cultures of beneficial

organisms by farmers is the rule rather than the exception. In mixed cultures, there is better interaction of the introduced compatible organisms based on the principle that greater the diversity and number of inhabitants, the higher the order of interaction and more stable the ecosystem. Based on these principles, attempts are being made to develop consortia of predominant compatible organisms isolated from the rhizosphere/endorhizosphere and diverse ecological niche.

Although research has been focused on most areas and soil types in the country, lesser attention has been paid to extreme environments like arid, saline and acid soils. Presently over 73% area under crop production is dependent on rainfall and more than one half of this area is located in a low rainfall zone. Dryland soils are deficient in N and P and farmers seldom apply fertilizers. Biological nitrogen fixation (BNF) and biofertilizers thus have a promise in dryland agriculture which needs to be fully exploited. These areas generally experience adverse weather conditions such as high temperature, low moisture etc. In India, nearly 8.4 million ha of arable land is salt (salinity and sodicity) affected. The high concentrations of salts in the soil have a detrimental effect on plants and microorganisms. Microorganisms present in the rhizosphere are reported to alleviate the salinity stress by different mechanisms. Thus it is proposed to develop a consortium of microorganisms that can help the plant to survive and yield more even under saline conditions. Thus emphasis should be given to management of abiotic stress through microbial inoculation.

The PGPR related research programme in India is unorganized in several centers on several crops. There is tremendous scope for working in a network mode in India as the crops are varied and with diverse climate and soil factors. There is a need to consolidate the research efforts on PGPR for reduced use of chemical fertilizers and plant protection chemicals.

Microorganisms have the ability to rapidly adapt to varied environmental conditions and utilize new substances they encounter as their sole source of carbon and energy. India is basically an agricultural country where a variety of crops are grown throughout the year that generates large quantities of agricultural wastes. Agriculture residue can be put to use in different ways by using microorganism that use it as substrate: development of enriched compost, vermicompost; production of bioethanol and certain enzymes like phytases, proteases, lipases and amylases. Therefore there is a need to develop cost-effective, eco-friendly and appropriate technology to maximize economic value of nutrients contained in agrowastes for sustainable agriculture.

Despite the many achievements of modern agriculture certain cultural practices have actually enhanced the destructive potential of disease. Almost 30% of the yield in agriculture is lost because of combined effects of biotic and abiotic stresses with pathogenic fungi alone responsible for a reduction of about 12% plant disease control, therefore, now has become heavily dependent on pesticides to combat the wide variety of fungal diseases that threaten agricultural crops. A wide spread application of chemical pesticides/ fungicides inundates the agro-ecosystem with toxic compounds that affect the balance of natural food chain. Biological control of pathogens by application of specific antagonistic microorganisms to seeds or planting material has been studied intensively, however, only few of these biocontrol agents have been effective some year to year, and over a broad range of conditions very few are successful. Few commercial preparations based on *Trichoderma*, *Pseudomonas*, *Bacillus* are available in the market for biocontrol of phytopathogens. The last decade has witnessed a tremendous break-through in the research efforts on biological control of plant diseases in India especially by using species of *Trichoderma* and *Gliocladium*. The future holds tremendous possibilities for efficient use and manipulation of

Trichoderma and other biological agents for management of crop diseases under varied agro-climatic conditions.

Since the first complete microbial genome was published in 1995, more than 100 microbial genomes have been completely sequenced and published, and another 300 microbial genome-sequencing projects are estimated to be in progress worldwide. The significance of the information that has been derived from these complete individual genomes cannot be underestimated. Every genome that has been sequenced to date has provided new insight into biological processes, activities, and potential of these species that was not evident before the availability of the genome sequence. Sequence databases and comparative tools are now more easily accessible and allow for successful comparisons of different genomes, the identification of metabolic pathways and the analysis of transporter profiles across various species. Most significantly, the tremendous success of genome sequencing has allowed us to pursue other avenues where we can now derive genomic information from the multitudes of uncultivable prokaryotic species and complex microbial populations that exist in nature.

The role of microorganisms in sustainable agriculture is enormous. Worldwide micro-organisms are potentially exploited to enhance the food grain production under sustainability. Considerable progresses have been made in the microbial exploration and utilization in India also and it has been clearly demonstrated that these technologies are powerful tools for enhancing the application of microorganisms in crop improvement. Unawareness among farmers is one of the important limitations in the spreading of these low cost technologies. For successful launching of a exploration and exploitation of microorganisms, it is necessary to equip the extension machinery at the grass root level with technical backup of exploration of micro-organisms. There is a need to train scientists/ researchers/ technicians/ farmers for the exploration and application of Microorganism in agriculture.

The proposed ICAR network project on 'Application of microorganisms in Agriculture and allied sectors' seek to initiate and strengthen the R&D efforts on various microbe based technologies that can be utilized to increase crop production, utilize agrowaste, manage abiotic stress, biocontrol of important insect pests, diagnostics of important groups of microbes and post harvest technology. It also seeks to strengthen research in the area of microbial diversity, identification and genomics. It seeks to strengthen infrastructure, research capacity and human resources of ICAR institutions with respect to various microbe-based applications.

The network project has six components:

1 Microbial diversity and identification

2 Nutrient management, PGPR and Biocontrol

3 Agrowaste management , Bioremediation and Post harvest processing

4 Microbial management of abiotic stress

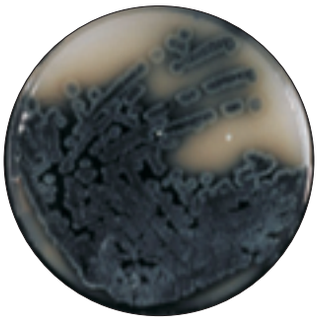
5 Microbial genomics

6 Human resource development

The overall objectives

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

- Development of microbe-based technologies for agrowaste management and bio-degradation for sustainable crop production.
- Harnessing microbial activities for bio-remediation of organic and inorganic environmental pollutants.
- Management of abiotic stresses using microorganisms.
- Development of microbe mediated processes for product development and value addition in agriculture.
- Unraveling microbial genomics for its utilization in agriculture and industry.
- Development of technologies for rapid microbial diagnostics.
- Human resource development in microbe conservation and utilization.



Significant Achievements of AMAAS

Theme 1:

Microbial Diversity and Identification

Objectives

a) Microbial diversity analysis from various eco-regions/exotic environments.

b) Identification of microorganisms using conventional and molecular techniques

c) Application of some important microbes in agriculture and allied sectors for enhancing food productivity

Achievements

- Soil, plant, water and sediments samples were collected from all over India from different ecological regions, e.g. Northern plains of IGP, Bihar, Eastern UP, North Eastern Region, West Bengal, South Andaman, Middle Andaman, North Andaman, Neil & Havelock, Hut Bay, Car Nicobar, Nancowrie Islands and Great Nicobar, Hot water Springs, Nilgiris, western and eastern cost, etc.
- A huge database of nitrogen fixer, plant growth promoting rhizobacteria, phosphate solubilizers, lignin and chitin degraders was developed.
- Actinomycetes capable of producing hydrolytic enzymes have been characterized.
- Sulphur oxidizing and reducing bacteria isolated from marine environment can help in reducing the pollution.

Theme 2

Nutrient Management, PGPR & Biocontrol

Objectives

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

Achievements

- Large number of microbes were screened for the development of microbial inoculants for enhancing the productivity of crops like soyabean, rice, coconut, black pepper and ginger.
- Low temperature tolerant rhizobacteria that would improve crop productivity at high

altitude areas like Almora, Nainital and Pithoragarh were selected.

- It was demonstrated that colonization of AM fungi increased due to application of Zn.
- Many potential bioagents were identified against *castor botrytis*. Two *Trichoderma* species (Tv-32 and Th-10) could effectively enhance sunflower growth and reduce *Alternaria* blight severity.

Theme 3

Agrowaste Management, Bioremediation and PHT

Objectives

- Isolation, identification and characterization of microorganisms from various selected agro, industrial and urban wastes.
- Development of microbial consortia for rapid degradation and effective utilization of selected waste.
- Production of value added products like bio-fuels, enzymes and mushroom using selected agro, urban and industrial wastes.
- To assess the impact of organic waste application in agriculture on shifts in soil microbial community structure and functions in relation to soil physiochemical properties.
- Expand understanding of microbial genetics as a basis for enhancing the capabilities of microorganisms to degrade pollutants.
- Conduct microcosm/mesocosm studies of new bioremediation techniques to determine in a cost-effective manner whether they are likely to work in the field, and establish dedicated sites where long-term field research on bioremediation technologies can be conducted.
- Development of fermented products from fruits, vegetables and cereals.
- Value addition of pulses, millets and Horticultural produces through microbial fermentation.
- Biopreservation of vegetables for extension of shelf life and control of spoilage in processed products.

Achievements

- Bacterial isolates obtained from PAH Contaminated Soils were found to utilize mixture of PAH at concentration as high as 100 ppm. These bacteria are able to use 2, 3 & 4 ring PAH compounds as sole C-source and can be used for bioremediation.
- Sphingomonads, able to degrade HCH, showed the presence of HCH degradative genes in PCR analysis. " Significant changes in soil structural and functional diversity was observed due to long term use of pulp and paper mill effluent in agriculture..
- Industrially important fungi and bacteria capable of producing pectinase, cellulase, amylase, natural colours were isolated from fruit wastes and lignocellulosic rich Agrowaste.

Theme 4

Microbial Management of Abiotic Stress

Objectives

- Isolation of microorganisms from rhizotic zones of cereal crops (wheat and millets) grown under stress conditions of salt, drought and extreme temperatures.
- Selection of bacteria capable of growing under stress conditions of salt, drought and extreme temperatures.
- Evaluation of the selected organisms in the rhizosphere of wheat and millets (phytotron studies).
- Biochemical characterization of selected microorganisms.
- Development of consortium of micro-organisms that can alleviate the effect of

drought, salinity and extreme temperature.

- Field evaluation of consortium of microorganisms for improvement of wheat, rice and millets under stress conditions.

Achievements

- Microbes capable of tolerating high salt concentration upto 10% NaCl and carrying out plant growth promoting activities were found to alleviate the effect of salinity and improve the growth of wheat under salt stress.
- A strain of *Pseudomonas* was found to provide protection to pearlmillet at 50°C and overcome high temperature stress.

Theme 5

Microbial Genomics

Objectives of Sructural Genomics

- Complete genome sequencing of *Mesorhizobium ciceri* strain Ca181 with genome size of 8Mb.

Objectives of Functional Genomics

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

Achievements

- General characteristics of the culture *Mesorhizobium ciceri* Ca 181 were studied and the culture was found to be able to tolerate NaCl concentration of 7% and was resistant to antibiotics Ampicillin (50 ug/ml) and Nalidixic acid (30 u g/ml).
- Protocol for total DNA isolation was standardized and DNA isolated from M. ciceri Ca 181.
- Construction of genomic library of M. ciceri Ca181for complete genome sequencing.
- Selection and collection of soil DNA from higher altitudes and longitudes of Uttaranchal including Ranichauri, Pithorgarh, Chamoli and Pantnagar
- Soil DNA isolation and, amplification and and cloning using Eubacterial universal primers has been accomplished
- Pilot experiments have been done for preparation for small insert DNA library in pUC 19 plasmid vector

Theme 6

Human Resource Development and Training

Two trainings were organized:

1. Microbial diversity analysis of Extremophiles
2. Microbial community analysis through Metagenomics

Theme: Microbial Diversity and Identification

Sub Project 1: Diversity analysis of microbes in extreme conditions

Rationale

Life in extreme environment has been studied intensively focusing attention on the diversity of the organisms and molecular and regulatory mechanisms involved. The 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

- Microbial diversity analysis in extreme climates.
- Identification of osmolytes production by extremophilic bacteria.
- To look for production of enzymes (amylase, cellulase, CMCase, FPase, xylanase & protease) in thermophilic bacteria
- Molecular characterization of extremophilic bacteria

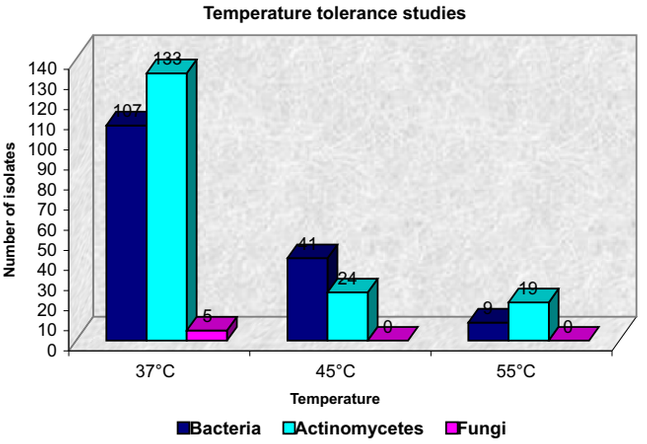
Research Achievements

Isolation and enumeration of bacteria, fungi and actinomycetes population from water and soil samples

Water and sediment samples collected from Rajgiri thermal springs were analysed for the isolation of bacteria, fungi and actinomycetes. Large variations were observed in the colony morphology of different isolates. Population of Gram positive, Gram- negative bacteria, fluorescent *Pseudomonas*, *Bacillus* spp., free living nitrogen fixers and *Azospirillum*, fungi and actinomycetes were determined using both specific and non-specific media. The results revealed that among the eight samples analysed the population of Gram positive bacteria was maximum and ranged between 5-20 x 10^3 cfu g^{-1} or mL^{-1}. The population of actinomycetes (filamentous Gram positive bacteria) ranged between 8-28 X 10^2 cfu g^{-1} or mL^{-1} on Actinomycetes Isolation agar medium. Fungal population was as low as 100 cfu g^{-1} or mL^{-1}. Some of the fungal isolates were identified as *Penicillium*, *Trichoderma spp, Fusarium* spp and *Aspergillus niger*. The proportion of Gram +ve bacteria and Gram -ve bacteria was calculated and it revealed that hot springs differentially enrich Gram positive bacteria. The cell wall structure of Gram+ve bacteria and also other modifications like the presence of endospore in few genera must have contributed to the differential enrichment of Gram +ve bacteria.

Screening of isolates for temperature tolerance

Isolations were made from each sample based on the morphotypes of the isolates. A total of 107 bacteria, 133 actinomycetes and 35 fungi were



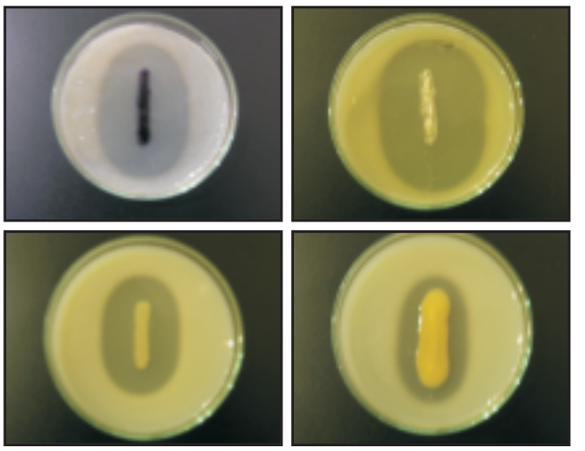
Location	Bacteria cfu 10^3		Fungi cfu x cfu 10^2	Actinomycetes cfu x 10^2	Proportion of Gram +ve Bacteria (in%)
	Gram +ve	Gram -ve			
Brahmakund water with sediment (Pradhankund), pH 5.8	15	6	7	21	71.8
Brahmakund water with sediment, pH6.0	17	3	4	28	86.4
Brahmakund surface water, pH6.0	7	1	4	16	86.0
Saptdhara water, pH5.9	8	0	1	8	98.9
Suryakund surface water, pH5.5	20	0	2	20	99.1
Suryakund water with sedimen,t pH6.5	5	3	6	18	99.1
Brahmakund hard surface water with algae mats, pH5.8	10	1	6	9	65.3
Sand sediment of Brahmakund, pH6.0	7	4	4	13	91.2

isolated, purified and screened for temperature tolerance. Of the 107 bacterial isolates screened, 8 isolates were able to grow at a temperature of 55^{0}C or more. Out of 133 isolates of actinomycetes only 19 were able to grow at 55^{0}C whereas none of the fungal isolates could grow beyond 37^{o}C.

Screening for enzyme production

Thermophilic bacteria are a source of enzymes that have wide application in research, industry and biomass degradation. These isolates were further screened for the production of enzymes important in industry (Proteases and amylases) and in biomass degradation (cellulases, carboxymethyl cellulases and xylanases). Of the 41 isolates screened, 20 were found to produce amylases in a qualitative assay. Quantitative estimation of enzyme activity in these bacteria revealed that isolate no. 21 could produce maximum amount of amylase followed by isolate no. 61. Screening for enzyme proteases with casein as the substrate revealed that 9 isolates could produce the enzyme and gave clearing zones around the colonies.

Thermophilic organisms play an important role in the decomposition of agricultural wastes. During the thermophilic phase of decomposition, the temperature goes as high as 65^{0}C. The temperature



Qualitative screening for Protease production

tolerant bacteria identified were also screened for the production of enzymes involved in decomposition chiefly xylanases and cellulases. Of the 41 isolates screened, cellulase and carboxy methyl cellulase was found in only four isolates. Isolate no. 73 showed the maximum activity of cellulase whereas isolate no. 55 showed maximum CMCase activity. Xylanase activity was present in 22 isolates and ranged between 0.48 to 1.7 ug xylose/ug protein. Of all the isolates only isolate no. 55 exhibited both xylanase and cellulase activity and can be exploited for the bioconversion of agricultural residue.

Sub-Project 2: Development of Diagnostic kit for the identification of *Bacillus*

Rationale

The genus *Bacillus* is a large, heterogeneous group of Gram positive, 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

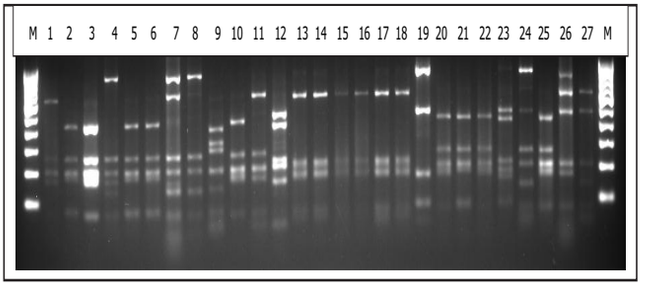
- To isolate and characterize the soil microbes (*Bacillus* and *Pseudomonas*)
- To develop rapid diagnostic kits for identification of soil microbes.

Research achievements

16S rDNA was amplified from twenty seven strains belonging to different *Bacillus* species. A strong amplification band of about 1540 bp from each *Bacillus* strain was obtained. Amplified restriction digestion of rDNA analysis (ARDRA) from the twenty seven strains showed limited variation among the strains.

Interestingly it was observed that a 265 bp band is common to all the strains of *Bacillus* used in the present study. In order to confirm this observation, the possible cleavage sites of *Alu*I in the complete rRNA gene sequences available in the GenBank for many different species of *Bacillus*, *Bacillus*- related genera and *Bacillus*- derived genera were compared. Many species of *Bacillus* yielded 265 bp fragment on digestion with *Alu*I. This fragment was present almost at the same location in different *Bacillus* species and the exact location. However, none of the *Bacillus*- derived genera - *Alicyclobacillus, Brevibacillus, Geobacillus, Filobacillus, Paenibacillus* or *Bacillus*- related genera like *Lactobacillus, Sporosarcina, Arthrobacter* showed the presence of 265 bp fragment when digested with *Alu*I. *Bacillus cereus* and *Bacillus thuringeinsis*, two closely related species were separated from all other species by the presence of two additional *Alu*I sites within the 265 bp fragment thereby yielding fragments of 186, 48 and 21 bp (totaling to 265 bp).

The presence of 265 bp fragment is linked to identification of *Bacillus* as a genus was further confirmed on analysis of strains that have been derived from *Bacillus* or those that showed



Restriction patterns of PCR amplified fragement of 16S rDNA of *Bacillus* isolates digested with *Alu*I M: 100 bp DNA ladder, 1: *B. marscescens*, 2: *B. coagulans*, 3: *B. subtilis*, 4: *B. licheniformis*, 5 & 6: *B. circulans*, 7: *B. freudenreichii*, 8: *B. globigii*, 9: *B. saccharolyticus*, 10: *B. subtilis*, 11 & 12: *B. sphaericus*, 13: *B. thuringiensis* sup sp. *finitimus*, 14: *B. thuringiensis* sub sp. *entomocidus*, 15:*B.thuringiensis* sup sp. *aizawai*, 16: *B. thuringiensis* sub sp. *galleriae*, 17. *B. cereus*, 18: *B. thuringiensis* sup sp *israelensis*, 19: *Brevibacillus* sp., 20: *Bacillus macquariensis*, 21: *B. stearothermophilus*, 22: *B. pasteurii*, 23: *Aneurinibacillus aneurinolyticus*, 24: *B. laterosporus*, 25: *B. pantothenticus*, 26 & 27: *B. oleronius*.

Presence of 265 bp fragment after Alu1 restriction digestion in various *Bacillus* and *Bacillus*-derived genera and its position in the 16S rDNA.

Name	New Name	Accession Number	Total Sequence available	Presence of 265bp fragment	Position of 265bp fragment
Bacillus sp. SD-B1		AB189316 1443		+	1042-1306
Bacillus amyloliquefacines CMB01		AF489591 1526		+	1063-1327
Bacillus sonorensis NRRL B-23154		AF302118 1410		+	1049-1313
Bacillus licheniformis GXN 151		AY2915821	1440	+	1038-1302
Bacillus licheniformis S2		AY0527671547		+	1073-1337
Bacillus megaterium		AJ880767 1519		+	1076-1340
Bacillus vallismortis		AB0211981530		+	1065-1329
Bacillus circulans		AY0430841496		+	1045-1309
Bacillus cereus		AJ853737 1552		_	1102-1366
Bacillus malacitensis		AY6036561420		+	1044-1308
Bacillus cereus		BCE288157	1340	_	1045-1309
Bacillus halodurans		AY9601181500		_	_
Bacillus coagulans - IDSp		AF466695 1512		-	-
Bacillus globisporous	*Sporosarcina Globispora*	X68415	1532	-	-
Bacillus stearothermophilus	*Geobacillus stearothermophilus*	AB0211961523		-	-
Bacillus halodenitrificans DSM 10037	*Virgibacillus halodenitrificans*	AY5431691521		-	-
Bacillus clausii		AB20179 1510		-	-
Arthrobacter globiformis		X80736	1464	-	-
Bacillus Sp. P1 4-7	*Paenibacillus*	AJ297712 1451		-	-
Bacillus Sp. R11400	*Paenibacillus*	AJ438302 1446		-	-
Gamma Proteobacteria N 4-7		U89956	1574	-	-
Lactobacillus Sp. 459		AY6811351512		-	-
Bacillus Sp. M4 (KU)		AB1934371223		-	-
Bacillus acidocaldarius	*Alicyclobacillus acidocaldarius*	X60741	1547	-	-
Bacillus acidoterrestris	*Alicyclobacillus acidocaldarius*	X60743	1542	-	-
Bacillus psychrosacchrolyticum		AB0211951507		-	-
Bacillus naganoensis		AB0211931540		-	-
Bacillus mojavensis		AB0211911526		+	1061-1325
Bacillus lentus		AB0211891535		-	-
Bacillus flexus		AB0211851529		+	1066-1330
Bacillus atropheus		AB0211811515		+	1050-1314
Bacillus thuringiensis		AF160221 1496		-	1053-1317
Bacillus thermocloacae		Z26939	1527	-	-
Bacillus pallidus	*Geobacillus pallidus*	Z26930	1516	-	-
Bacillus alcalophilus		X76436	1505	-	-
Bacillus psychrophilus	*Sporosarcina psychrophilus*	X54968	1552	-	-
Bacillus firmus		AY8335711579		-	-
Bacillus firmus		AJ717383 1533		-	-
Bacillus pumillus		AB2112281503		+	1065-1329
Bacillus myxolacticus		D16274	1484	-	-
Bacillus laevolacticus		D16269	1484	-	-
Bacillus fusiformis		AY548956	1547	-	-
Bacillus thermoglucosidasius	*Geobacillus thermoglucosidasius*	AY608991	1562	-	-
Bacillus subtilis		AB016721	1502	+	1049-1313
Brevibacillus sp. SSCT72	*Brevibacillus* sp. SSCT72	AB210966	1491	-	-
Bacillus sp. RF2-5	*Filobacillus* sp. RF2-5	AB191344	1491	-	-
Bacillus terranovensis	*Aneurinibacillus terranovensis*	Aj715390	1552	-	-

'+' indicate the presence of 265 bp fragment;
'-' indicate the absence of 265 bp fragment;
'*' indicate the presence of two additional AluI sites within the 265 bp seqence

similarity to derived genera but still are placed in *Bacillus*. For example *Bacillus coagulans* did not showed 265 bp fragment on digestion with *Alu*I. In earlier studies it has been shown to cluster with two *Geobacillus* species. Likewise *Bacillus laevolacticus* (or *Bacillus myxolacticus*) did not yielded 265 bp fragment and has been shown to cluster with *Virgibacillus panthothenticus* (Group III). Earlier reported *Bacillus psychrophilus* and *Bacillus globisporus* were later nomenclatured as *Sporosarcina psychrophilus* and *Bacillus globisporus* respectively, and as expected did not showed the presence of 265 bp fragment. Certain other species belonging to distant groups like *Bacillus clausii, Bacillus halodurans, Bacillus lentus, Bacillus thermocloacae* and *Bacillus firmus* did not gave 265 bp fragment on digestion with *Alu*I and needs to be scrutinized for their inclusion in the genus *Bacillus*. Based on our results we predict that all those species that could not yield a 265 bp long nucleotide fragment on digestion with *Alu*I could be assigned to new genera within the family Bacilliaceae.

Nucleotide sequence of 265 bp fragment for each representative species was selected for alignment studies. The BLAST algorithm was used to search for homologous sequences in GenBank, EMBL, DDBJ and PDB. The data obtained was analysed to draw inference. The Multi Align program was used for pair wise and multiple alignments of 265 bp nucleotide sequences of all strains used in the study. The percent homology among the sequences was analyzed to look for development of species-specific probes. The nucleotide sequence of 265 bp fragment from *B. subtilis*, *B. licheniformis, B. amyloliquefacieus, B. megaterium, B. cereus* and *B. circulans* was BLASTn searched for homologous sequences in GenBank. The results were encouraging as the sequence from a particular species, like *B. megaterium* showed 100% alignment with sequences of *B. megaterium* and significant alignment with closely related species *B. flexus.* This extent of homology only with small nucleotide sequence prompted us to carry out multiple alignment analysis of 265 bp fragment. The alignment results indicated that this is a hypervariable region of 16S rRNA and most of the species selected for analysis could be distinguished based on the sequencing of this hypervariable region. The alignment data further revealed that most of the variation existed only in the last 100 bp region of 265 bp fragment. This study indicates that ARDRA with *Alu*I gives identity of *Bacillus* species and its further confirmation can be carried out by partial sequencing of 100 bp fragment (165 nb to 265 nb within 265 bp fragment). To illustrate the point, three species of *Bacillus,* that is, *Bacillus* sp. SD-B1, *B. sonorensis* abd *B. licheniformis* that showed 100% similarity with ARDRA could be further distinguished on the basis of sequencing.

Based on the results of the present investigation we suggest a simple approach for identification of *Bacillus* sp. per se and to classify them into different species: PCR amplification of 16S rDNA; development of ARDRA with *Alu*I, look for the presence of 265 bp band (or three bands of 186, 48 and 21 bp for *B. cereus* and *B. thuringeinsis*) to identify genus *Bacillus* and also can predict species to certain extent and sequencing of 265 bp fragment to identify the species of *Bacillus*.

Theme: Microbial Management of Abiotic Stress
Project: Development of microbial consortium of alleviate salinity stress for improved growth and yield of wheat

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.

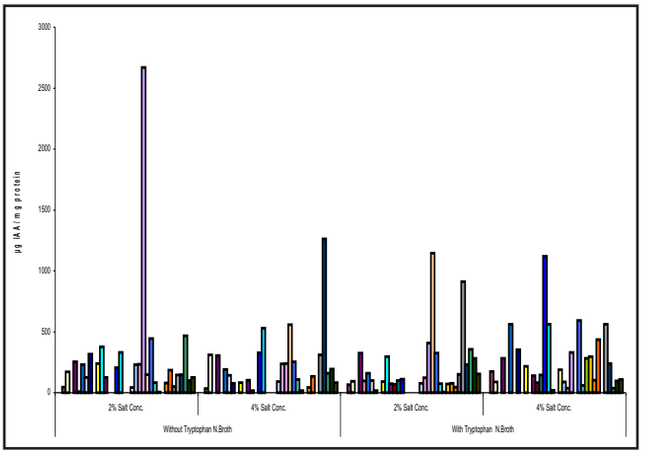
Research Achievements

Screening of wheat varieties for salt tolerance: Five varieties of wheat (RAJ-3077, PBW-373, PBW-343, UP-2382, K-7903) were screened for tolerance to salt on plain agar augmented with different concentrations of NaCl (0.5%,1.0%,1.5%). Percent germination of seeds was recorded every 24 h upto 72 h. The increase in salt concentration not only

Screening of wheat cultivars for salt tolerance

adversely affected the percentage germination of the seeds of wheat but also delayed their germination. Only one variety of wheat (K-7903) showed tolerance to NaCl stress upto 1.5%. This variety was selected for further plant-microbe interaction experiment.

Plant growth promoting attributes of salt tolerant bacteria



Production of IAA by different bacterial strains with or without tryptophan and in the presence of 2% and 4% NaCl

IAA Production:

Bacterial strains tolerant to 8% or more NaCl were analysed for their ability to produce IAA both in presence and absence of salt stress. Of the 42 isolates screened, 24 bacteria were found to produce IAA in the absence of salt stress. Isolate N12 showed the maximum production of IAA (11.87µg/ml). However in the presence of 2 and 4% NaCl, in general there was a decline in the production of IAA by different strains.

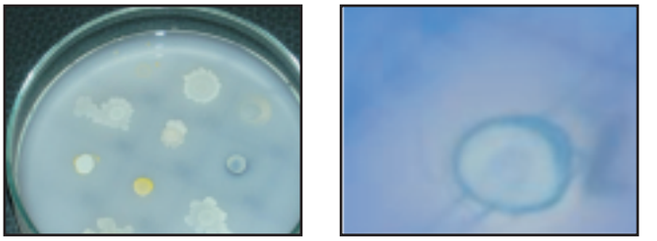
Phosphate solubilization

Salt tolerant isolates were also screened for their ability to solubilize tricalcium phosphate both in presence and absence of salt stress. Of the 42 isolates screened, 11 were able to solubilize phosphorus with varying efficiency. However with the increase in the salt concentration from 2 to 8% there was a drastic decline in the P-solubilization activity and only three isolates could solubilize P upto NaCl concentration of 8%. Quantitative assay of soluble P was carried with TCP as the insoluble source of P. The results were different from the qualitative assay. Isolates 5, 44 and 48 were found to solubilize P upto 8% NaCl and interestingly for most of the strains solubilization was more at higher salt concentration.

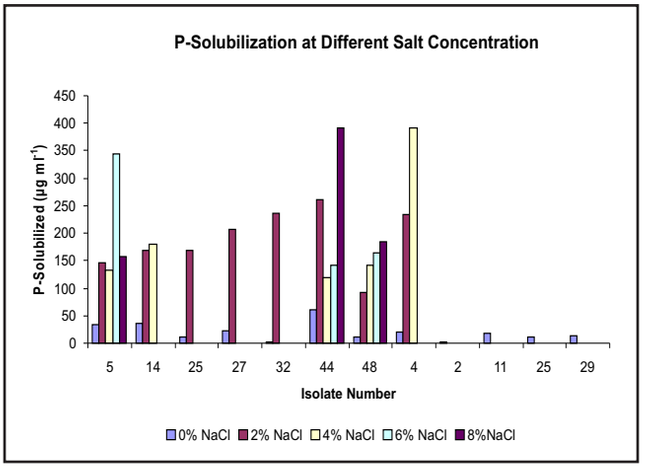
Evaluation of salt tolerant bacteria for their ability to alleviate salt stress for growth of wheat (Green house experiment)

Screening of bacterial cultures for P-solubilization (Qualitative assay)

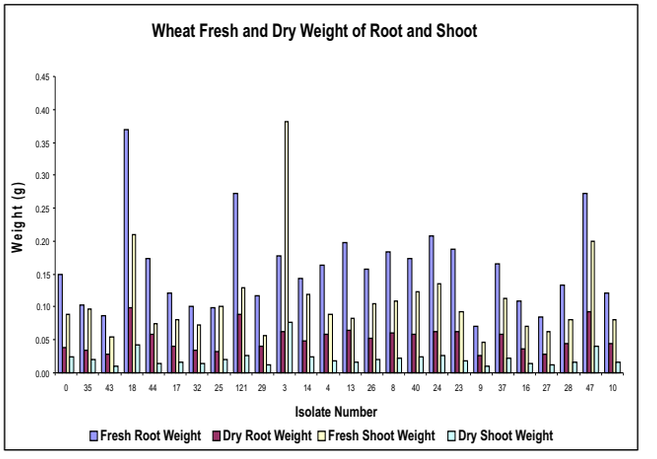
No of Isolates used	% NaCl				
	0	2	4	6	8
42	12	9	5	3	3



Qualitative screening of bacterial cultures for P-solubilization on Pikovskaya medium with TCP as insoluble source ofphosphorus.

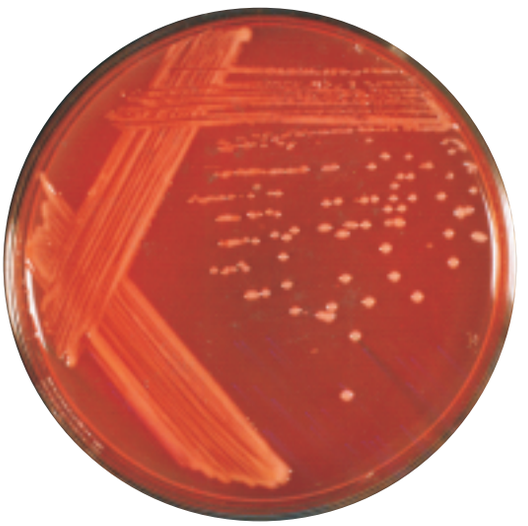


Quantitative estimation of P-solubilization by salt tolerant bacteria at different salt concentration.



Growth parameters of wheat as influenced by bacterial inoculation under salt stress.

A green house experiment was conducted using plastic cups and sand as the rooting medium. Artificial salinity was created using NaCl to achieve the ECe of 4.5 dS m^{-1}. Wheat cultivar K-7903 was selected for the study, as it was tolerant to salinity. The seeds were surface sterilized and two seeds were sown in each cup. After germination the cups were inoculated with the bacterial inoculants. A total of 25 isolates that showed good IAA production and P-solubilization and were tolerant to salt were selected for the inoculation studies. Uninoculated treatments were kept as control. Each treatment was replicated thrice and one plant per cup was maintained. The plants were irrigated with Jensen's N free medium. A basal dose of 60 kg N ha^{-1} as KNO_3 was given for all treatments. The plants were harvested after 40 days and the fresh and dry weight of root and shoot was recorded. Inoculation of isolates like 3, 18, 47 and 121 had a beneficial effect on growth of wheat and can help in alleviating the effect of salinity. These isolates were further selected for field trial. The field experiment is in progress and the analysed results will be reported next year.



Bradyrhizobium japonicum

Diversity and Conservation of Agriculturally Important Microorganisms and their Potential as Biocontrol Agents (APCESS Project, ICAR, New Delhi)

PI : Dilip K. Arora
RA : Sudhanshu Kashyap, Rakesh Kumar
SRF : Bhim Pratap Singh

Objectives

- To improve the use of, and develop new, biodiversity based selection of antagonistic microorganisms by surveying of different geographical/ecological sites of India eg. agricultural land, forest, sandy soil, hilly terrains of Uttar Pradesh, Himachal Pradesh, Madhya Pradesh, Arunachal Pradesh, Assam, Rajasthan, and isolation and characterization of microorganisms representing diversified groups eg. Bacteria, fungi, and actinomycetes. Development of methods for long-term preservation of isolated microorganisms.
- Identification and characterization of isolated antagonistic microorganisms using biochemical/molecular tools.
- Study of plant growth promoting and biocontrol activity of a few selected groups of microorganisms against some common, but economically important root diseases. Characterization of some of the active biomolecules from selected antagonistic microorganisms, and role of these biomolecules in disease control.

Research Achievements

The project was of three-year duration and completed on 23.11.06. The salient achievements of the project are:

- Extensive field surveys and samples from different states viz. Uttar pradesh, Madhya Pradesh, Assam, Jammu and Kashmir and Rajasthan were collected.
- A total of 304 isolates showed biocontrol activity against different plant pathogenic fungi and were identified to as: *Bacillus subtilis*, *B. brevis*, *Fusarium oxysporum*, *Hypocrella disciodea*, *Metarhizium anisopliae*, *Pseudomonas* sp., *Trichoderma harzianum*, *T. koningii* and *Verticillium lecanii*.
- Cultures were preserved in mineral oil (fungi), glycerol at –80^{o}C (bacteria), and lyophilized for long term preservation.
- Plant growth hormones like GA3, IAA , BAP and fungicide Bioforan were shown to influence the biocontrol potential of *Pseudomonas fluorescens* and *Trichoderma harzianum* against *Alternaria alternata* and *Phytopthora infestans*
- Mini–Prep" isolation of genomic DNA directly from the culture plates of fungi was developed. This technique saves time and genomic DNA for PCR reaction can be obtained within 2 hours (without the use of liquid N).
- PCR-ITS-RFLP revealed limited genetic heterogeneity among the Fusarium isolates.
- More than 80 RAPD primers were used to distinguish between different Fusarium isolates, but only 5 RAPD primers viz., OPA04, OPA13, OPB11, OPE07 and OPE11 produced variable polymorphic pattern.
- Of the 32 isolates of fluorescent *Pseudomonas* screened for phosphate solubilization, 23 were able to solubilize tricalcium phosphate with varying efficiency.
- The relationship between the genotype of fluorescent pseudomonad and its ability to solubilize inorganic phosphorus was determined. Genetic profiles were generated using RAPD primers and 16S rDNA-(RFLP). The combined pattern obtained with the four endonucleases defined 9 distinct genomic groups among 32 isolates and 10 reference strains at a similarity coefficient of 75%.
- Fluorescent *Pseudomonas* isolates were shown to have ability to produce HCN, ammonia and siderophore.

Development of sustainable management strategies for the control of parthenium weed using biotechnological approaches (DBT, New Delhi)

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

Parthenium hysterophorus is an exotic invasive annual weed now causing severe infestation in north India. It is poisonous, problematic, allergic and aggressive weed posing a serious threat to live stock and human being causing Dermatitis, Asthma, naso-dermal and naso-bronchial type of diseases. If grown in fields, it reduces 30-40% of crop yield. It is an herbaceous erect and annual plant, is native to the Gulf of Mexico and central South America, and has become widespread in North America, South America, the Caribbean and many parts of Africa, Asia and Australia. *Parthenium* weed seedlings emerge from the vast reservoir of viable weed seeds in soils. The resulting weed infestations require repeated weed control treatments with chemical herbicides over a period of years for successful weed management. In recognition of the inadequacies of chemical herbicides, efforts have been developed for exploiting microorganisms for biological weed control. One such approach is to select microorganisms that specifically inhibit weed seedling development, thereby hindering the establishment of a weed population competing with crop plants for light, water, and nutrients. In previous studies with crop plants, the colonization of roots by certain bacteria was found to be detrimental to plant development and was implicated as a significant factor in limiting crop growth. This group of microorganism likely induces damage through the production of phytotoxins that are absorbed by the plant roots. Selection for microorganisms that are specifically detrimental to weed seedling growth could benefit agriculture by contributing to increased crop yields, by minimizing weed competition, and by reducing the use of chemical herbicides.

When plants are attacked by pathogens or detrimental microorganisms, they respond by activating a variety of defense mechanisms, including the rapid production and accumulation of reactive oxygen species (ROS). Generation of ROS is thought to be an early event that can fundamentally influence the interaction between the plant and the pathogen. ROS are predominantly represented by superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$) and singlet oxygen ($^1O_2$). The early oxidative burst of plant tissue releases O_2^- and H_2O_2, which are recognized events in an incompatible response by the indirect mediators and the hypersensitive response (HR) which is characterized by the formation of necrotic lesions at the infection site, it has been involved in a number of morphological, physiological and molecular changes that appear to coincide with a rapid cell death. Thus, when produced in a rapid and controlled manner, ROS may aid in early signalling of pathways that respond to both biotic and abiotic stress. Reactive oxygen species (ROS) formation in plants has also been described after several abiotic stresss such as herbicides. Among them, triazines, ureas, diphenyl ethers, bipyridyls and phenoxycarboxylic acids have been related to oxidative stress. However, there is no report to evaluate the stress caused by microbes on generation of ROS in weed plants.

Objectives:

- Isolation, characterization and formulation of the effective microherbicides, its metabolites and active compounds present in the

metabolite. The purified compounds will be used against the control of *Parthenium* under different environmental conditions. The method of delivery of active compound will be studied in detail.

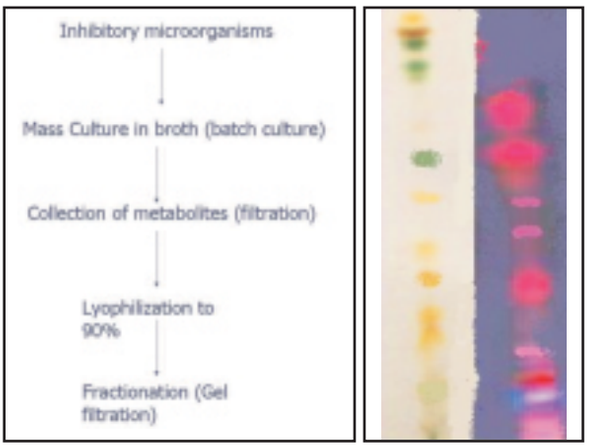
- Development of fermentation strategies and its optimization for mass production of the active compounds.
- Field level trial of the mycoherbicides and its active compounds either alone or in combination with herbicides to control *Parthenium* will be carried out in different fields of eastern UP infested with *Parthenium.*
- The inoculum-dose-response of selected microherbicides *viz: Fusariun pallidoroseum, Sclerotium rolfsii, Myrothecium roridum, Pseudomonas* strains etc.) as well as metabolites of these pathogens against the *Parthenium* at different environmental conditions.
- The effect of metabolites and active compounds on other plants and weeds will be studied.
- The metabolites and active compounds obtained after purification will be compared with chemical herbicides and will be used in integration with the herbicide to reduce the dose for effective control of *Parthenium*.
- Field level trial of the mycoherbicides and its active compounds either alone or in combination with herbicides to control *Parthenium* will be carried out in different fields of eastern UP infested with *Parthenium.*

Research Achievements:

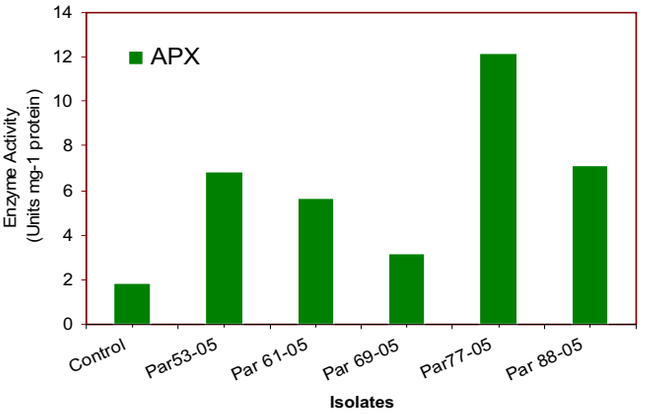
- The average densities of rhizobacteria on seedling roots were, ranging from 14×10^6 to 62×10^6 CFU g of root^{-1}.
- Rhizobacteria isolated from all weed seedling roots were comprised primarily of fluorescent and nonfluorescent pseudomonads, *E. herbicola, Flavobacterium spp.*, and *Alcaligenes spp.* The fluorescent isolates were predominantly *Pseudomonas fluorescens, P. putida,* and *P. syringae*. Non-fluorescent pseudomonads were mainly *P. cepacia, P. maltophilia, and P. stutzeri*. Additional gram-negative bacteria found infrequently included representatives of the genera *Acinetobacter, Citrobacter, Serratia,* and *Xanthomonas*. Gram-positive bacteria comprised less than 1% of all isolates. All weed samples showed high proportions (>25%) of fluorescent pseudomonads. The common fungi isolated comprises *Aspergillus, Cladosporium, Epicoccum, Fusarium, Humicola, Curvularia, Colletotrichum, Penicillium, Trichoderma, Rhizoctonia, Myrothecium* spp. *Sclerotium* spp. *Phoma* spp. *Helminthosporium, Chaetomium, Macrophomina phaseolina, Alternaria* spp. *Ascochyta* spp. Un-identified mycelium etc.
- The germination of *Parthenium* seeds was markedly influenced by the microorganisms colonizing the seed. In general 20-50% reduction in germination was recorded with potential microorganisms.
- The proportion of rhizobacterial and fungal isolates that inhibited seedling growth of *Parthenium* in pot assays ranged from 35% to 65%. The greatest proportions of inhibitory rhizobacteria were associated with the fluorescent cynogenic pseudomonads.
- Symptoms observed on foliar growth induced by inhibitory isolates ranged from general growth retardation to various types of leaf chlorosis, mottling, and distortions. Occasionally, root growth inhibition was exemplified as stunting and discoloration and poor lateral root development.
- The metabolite of isolate Par 49/05 exhibited significant reduction in plant length. A significant (P=0.05) negative correlation was observed in the concentration of metabolites and germination of *Parthenium* seeds.
- The HCN activity in the metabolite of

fluorescent pseudomonads seems to be the main growth inhibitory substance for *Parthenium.*

- The isolates showing inhibitory effect on growth of *Parthenium* were cultivated at 30 °C in a fully automated 3 L bioreactor and differents fractions of the metabolites showing reduction in germination of *Parthenium* were evaluated for presence of active compounds. The fractions with toxins and alkaloids showing potentiality to retard the growth of *Parthenium* were concentrated by lyophilization or through rotary vaccuum evaporator and characterized for functional group analysis.
- Reactive oxygen species (ROS) formation has been described after several abiotic stresss such as herbicides and microbial toxins. Therefore, the level of antioxidant enzymes in the *Parthenium* plant was evaluated after treatment with the fractionated metabolites.
- Fresh leaf and root samples were used for ascorbate peroxidase (and frozen for catalase glutathione reductase and superoxide dismutase. APX activity was measured by the decrease in absorbance at 290 nm of a reaction containing 50 mM Hepes-NaOH (pH 7.6), 0.25 mM ascorbate and 0.1 mM H_2O_2. Maximum increase in the level of APX was recorded with Par 77-05 followed by Par 53-05.

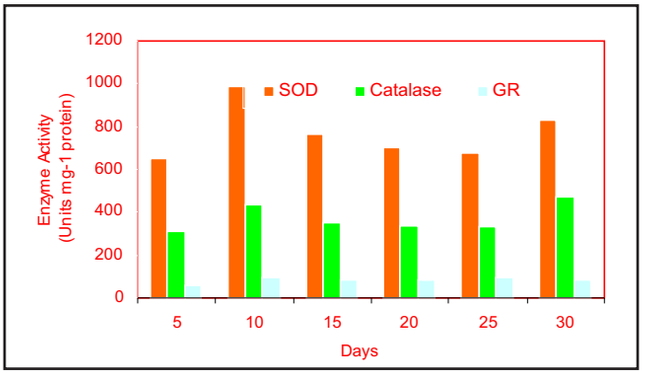


Schematic presentation for evaluation of the active compounds against *Parthenium*

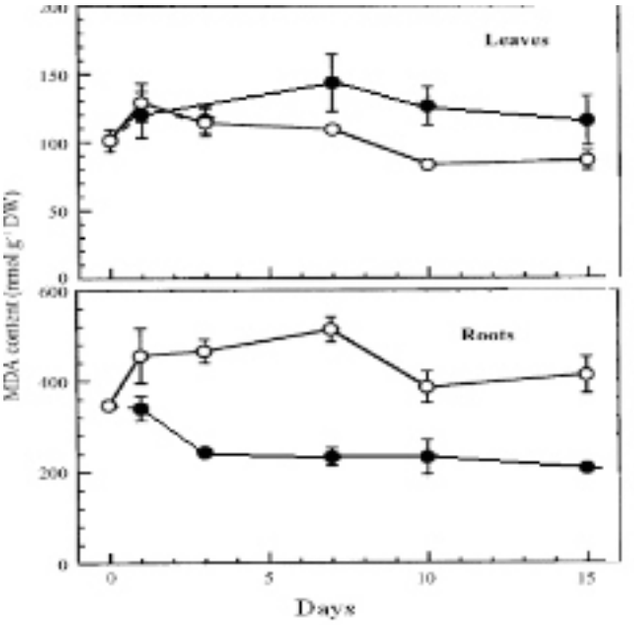


Level of Ascorbate peroxidase (APX) in *Parthenium* plants after treatment with the metabolites.

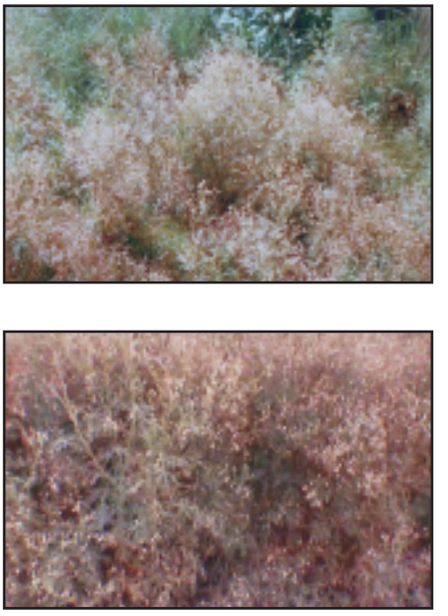
- Superoxide dismutase activity was measured spectrophotometrically at 505 nm and calculated as inhibition percent of formazan formation. The increase in SOD level was observed up to 10 days after metabolite treatment thereafter it was maintained at a steady rate till up to 30 days. The level of catalase ranged between 625-800 units.
- The levels of malondialdehyde (MDA), an indicator of lipid peroxidation, was higher after 7 days in leaves of metabolite treated plants, being significant only after 10 days of treatment. No increase in electrolyte leakage was detected in leaves Carbonyl content, considered an indicator of protein oxidation, was evaluated only 7, 10 and 15 days after treatment and showed a no significant increase after 15 days of treatment.



Level of Super Oxide Dismutase (SOD), Catalase and Glutathione Reductase (GR) in *Parthenium* plants after treatment with the metabolites.



Level of malondialdehyde(MDA) in *Parthenium* plants after treatment with the metabolites.



Oxidative burst and hypersensitive response in *Parthenium* treated with metabolite of *Par 77-05*

- In conclusion, these results suggest that oxidative stress is related to the abiotic stress caused by the metabolites of deleterious microorganisms.
- Although this study shows slight changes in lipid peroxidation and antioxidant systems after treatment, the slight potential oxidative stress generated seems very secondary in time and intensity.

Project on Collection Digitization of Agriculturally Important Microorganisms and their DNA fingerprinting (APCESS, ICAR, New Delhi)

PI : Dilip K. Arora
SRF : Mukesh Yadav
: B. Kishore Babu

In India, effort has been made to describe the microflora, taxonomically, morphologically as well as genetically for which the literatures on these aspects are abundantly available. However, till to date no work on digitization of agriculturally important micro-flora has been done which is available at different unorganized sectors, that is, universities/institutes/colleges. An attempt to digitize the microbial data available in records with different laboratories and scientific personnel/ teachers/students working in different research institution/ universities/ colleges was made in this project. In addition molecular techniques employed to decipher the unique fingerprints of some of the important AIMs.

Objectives

- Survey of different laboratories of Universities and research Institutions of Northern India for the purpose of obtaining information for microbial stock cultures collection holdings of AIMs .
- To contact scientific staff of different Universities and research Institution of India for providing of micro flora.
- Development of database and information about different available in different university/ research centre will be done.
- After developing a data base on manual format electronic software will be developed to provide information on morphological, biochemical, taxonomical, pathological and genetics aspects of AIMs.
- Efforts will be made to obtain at least 2000 AIMs from different Universities/labs in order to preserve them by modern techniques.
- DNA fingerprinting using set protocols of molecular techniques to ascertain the taxonomical status of some of very important agriculturally important microorganisms. The appropriate DNA probes for the detection of some AIMs will also be evaluated.

List of Universities/Institutes from where cultures were received during 2006-07.

Name of the Institute/ University	Fungi	Bacteria
Aligarh Muslim University	0	16
VPKAS, Almora	0	8
Assam Agriculture University	0	4
Allahabad Agricultural Institute		
University of Madras, Chennai	3	2
IIPR, Kanpur	40	0
DOR, Hyderabad	11	0
CIBA, Chennai	0	6
GBPUAT, Pantnagar	0	2

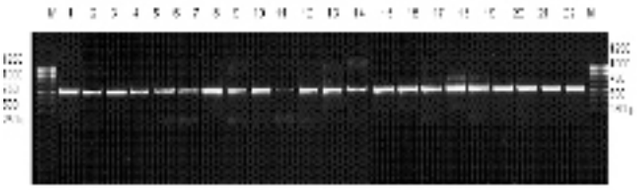
Research Achievements

Collection of cultures and digitization of data

Total 544 accessions were added to the culture collection.

- The passport data was created. The initial information e.g.date and place of isolation, mode of isolation, degree of pathogencity, mode of nutrition, type of enrichment technique used in the isolation, phase topology of the field, climatology of the place of isolation, nutrients requirements of the microbes, period of subculture, special and selective media etc.
- Database was developed containing various fields such as Name of microorganism, Accession number, Depositor, cultural medium, Source of isolation types of culture, form of preservation. After developing a data base on manual format, electronic software

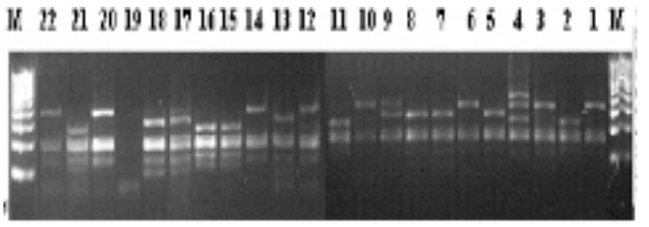
was developed to provide information on morphological, biochemical, taxonomical, pathological and genetics aspects of microorganisms. A total of 354 fungi and 190 bacteria have been digitized using digitization software "Micro NBAIM" developed at NBAIM.



PCR amplification of 28S rDNA gene cluster. Lanes 1-22 *Fusarium* isolates, M 1 kb ladder

The 28S r-DNA of 22 isolates of *Fusarium* representing 8 species were amplified using the primers ITS-1 and P3. All the strains produced a single amplified product of ranging from 1150 to 1200 bp.

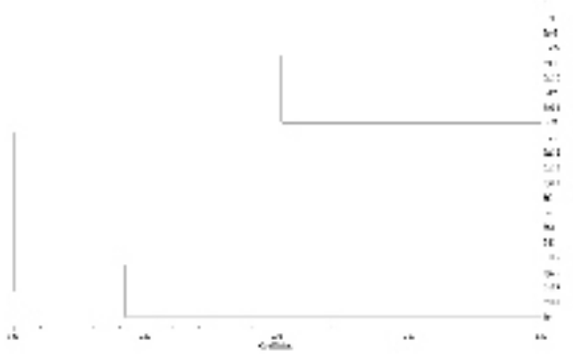
The amplified product was digested with tetra-cutter restriction endonuclease *HaeIII*. The enzyme could not distinguish among the closely related species. Both the isolates of *F. udum* showed identical RFLP pattern as isolate 2 of *Fusarium oxysporum*. Likewise both isolates of *Fusarium oxysporum* f.sp. *lycopersici* showed



PCR -RFLP of 28 S r-DNA gene cluster with restriction endonuclease *Hae*III. M: Marker 100 bp, , Lane 1-4: *Fusarium oxysporum,* Lane 5&6: *Fusarium oxysporum* f. sp. *ciceri,* Lane 7&8 *Fusarium udum,* Lane 9&10: *Fusarium colmorum* ,Lane 11&12*: Fusarium solani,* Lane 13 & 14: *Fusarim oxysporum* f. sp. *lentis,* Lane 15&16: *Fusarium oxysporum* f.sp. *lycopersici*, Lane 17&18: *Fusarium* oxysporum f. sp. *ricini*, Lane 19: *Fusarium* sambucium, Lane 20 *Fusarium* chlamydosporium, Lane 21 *Fusarium graminearium*, Lane 22: *Fusarium* cerealis

identical pattern to that obtained for *F. oxysporum* f. sp. *ricini*. On the other hand, among the four isolates of *Fusarium oxysporum*, three different patterns were obtained. The results indicated that developing fingerprints with RFLP analysis of 28S r DNA has limited potential to distinguish closely related species.

The dendrogram obtained from 22 isolates of *Fusarium* species with UPGMA on Jaccards coefficient showed that at 70% similarity coefficient 22 isolates grouped into 5 clusters whereas at 85% similarity coefficient the isolates were further divided into 11 clusters indicating high level of genetic similarity among the species and forma specialis of *Fusarium*. This is evident

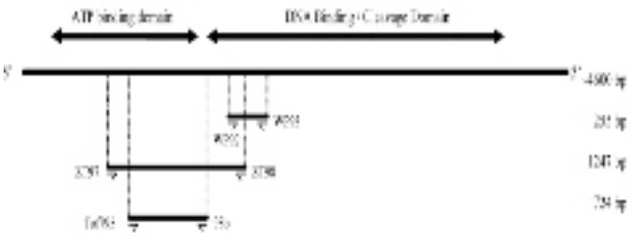


Dendrogram showing relationship among *Fusarium* species based on RFLP analysis of 28S rDNA with restriction endonuclease *Hae* III.

from the fact that one of the clusters contained isolates belonging to *F. oxysporum*, *F. colmorum*, *F. solani*, *Fusarium oxysporum f. sp. lentis*, *F. oxysporum* f. sp. *ricini*, *F. chamydosporium* and *F. cerealis*. The inability to distinguish isolates based on 28S rDNA–RFLP prompted to utilize other house keeping genes for molecular characterization.

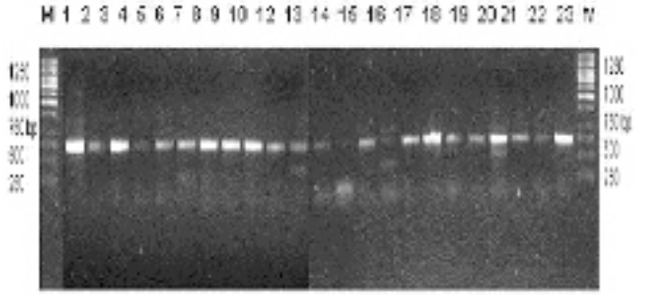
PCR-RFLP analysis of *Topoisomerase* –II gene

Topoisomerase II is part of the housekeeping genes. *Topoisomerase* II (EC 5.99.1.3) is essential for replication, transcription, condensation and



Sketch diagram of *Topoisomerase-II* gene.

separation of chromosomes and global genome stability. Type II topoisomerases cut both strands of the DNA helix simultaneously. Once cut, the ends of the DNA are separated, and a second DNA duplex is passed through the break. Following passage, the cut DNA is resealed. This reaction

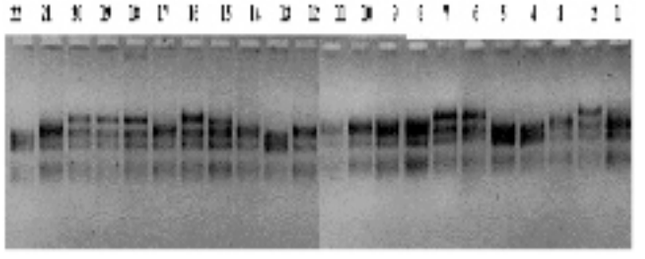


PCR amplification of *topoisomerase*-II gene using specific primers, Lane 1- 23: Different species of *Fusarium* M: Marker lane (1kb ladder, Fermentas Inc.)

allows type II topoisomerases to increase or decrease the linking number of a DNA loop by 2 units, and promotes

A part of *topoisomerase* II gene was amplified by using specific primers. A single amplicon size of 724 bp was obtained for all the isolates except isolate 11, 14 and 19 (Fig 19). These isolates even when further amplified using higher annealing temperature did not yielded single amplicon product.

This 724 bp PCR product when subjected to restriction digestion using tetra cutter restriction endnuclease *TaqI*, produced eight different

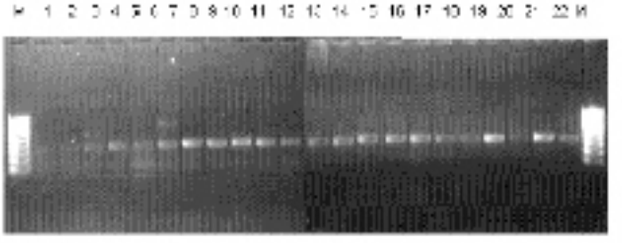


Restriction profile of *Topoisomerase-II* generated by *Taq*I Marker 100 bp, Lane 1-4: *Fusarium oxysporum,* Lane 5&6: *Fusarium oxysporum* f. sp. *ciceri,* Lane 7&8 *Fusarium udum,* Lane 9&10: *Fusarium colmorum* ,Lane 11&12*: Fusarium solani,* Lane 13 & 14: *Fusarim oxysporum* f. sp. *lentis,* Lane 15&16: *Fusarium oxysporum* f.sp. *lycopersici*, Lane 17&18: *Fusarium* oxysporum f. sp. *ricini*, Lane 19: *Fusarium* sambucium, Lane 20 *Fusarium* chlamydosporium, Lane 21 *Fusarium graminearium,* Lane 22: *Fusarium* cerealis.

restriction patterns as shown in Fig . However, again species-specific patterns were not obtained. Strains belonging to *Fusarium oxysporum*, *F. oxysporum* f. sp. *ciceri*, *F. udum*, *Fusarium oxysporum* f.sp. *lycopersici*, *F. oxysporum* f. sp. *ricini*, *F. sambucium* and *F. chlamydosporium* showed identical restriction patterns and could not be distinguished by endonuclease *Taq*1. The dendrogram (Fig-8) obtained from 22 isolates of *Fusarium* species with UPGMA on Jaccards Coefficient showed that at 85% similarity all 22 isolates grouped into 5 clusters and species were not clearly differentiated.

PCR Amplification of *Cellobiohydrolase-C* gene

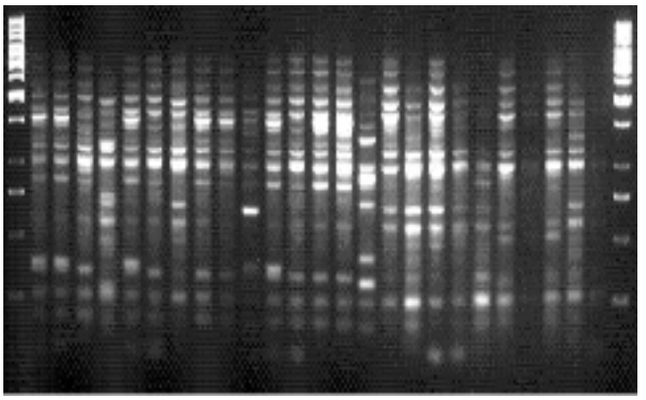
Cellobiohydrolase-C (Fig-2.)(EC 3.2.1.91) is part of a metabolic pathway and its secretion helps the fungi to degrade cellulose through the release of cellobiose from the nonreducing ends of the



PCR Amplification of *Cellobiohydrolase-C* gene in different strains of *Fusarium* spp. M: Marker 100 bp, , Lane 1-4: *Fusarium oxysporum,* Lane 5&6: *Fusarium oxysporum* f. sp. *ciceri,* Lane 7&8 *Fusarium udum,* Lane 9&10: *Fusarium colmorum* ,Lane 11&12*: Fusarium solani,* Lane 13 & 14: *Fusarim oxysporum* f. sp. *lentis,* Lane 15&16: ***Fusarium*** *oxysporum* f.sp. *lycopersici*, Lane 17&18: *Fusarium* oxysporum f. sp. *ricini*, Lane 19: *Fusarium* sambucium, Lane 20 *Fusarium* chlamydosporium, Lane 21 *Fusarium graminearium,* Lane 22: *Fusarium* cerealis.

chains.

A part of *Cellobiohydrolase-C* was amplified using specific primers and gave an amplicon of size 350bp. On restriction digestion the 350 bp fragment was cleaved into small fragments, but could not be detected by horizontal gel electrophoresis. For small size fragments RFLP analysis does not reveal heterogeneity and sequencing is the only alternative to look for species-specific variations. The products of representative species are in the process of sequencing and the differences in the sequence



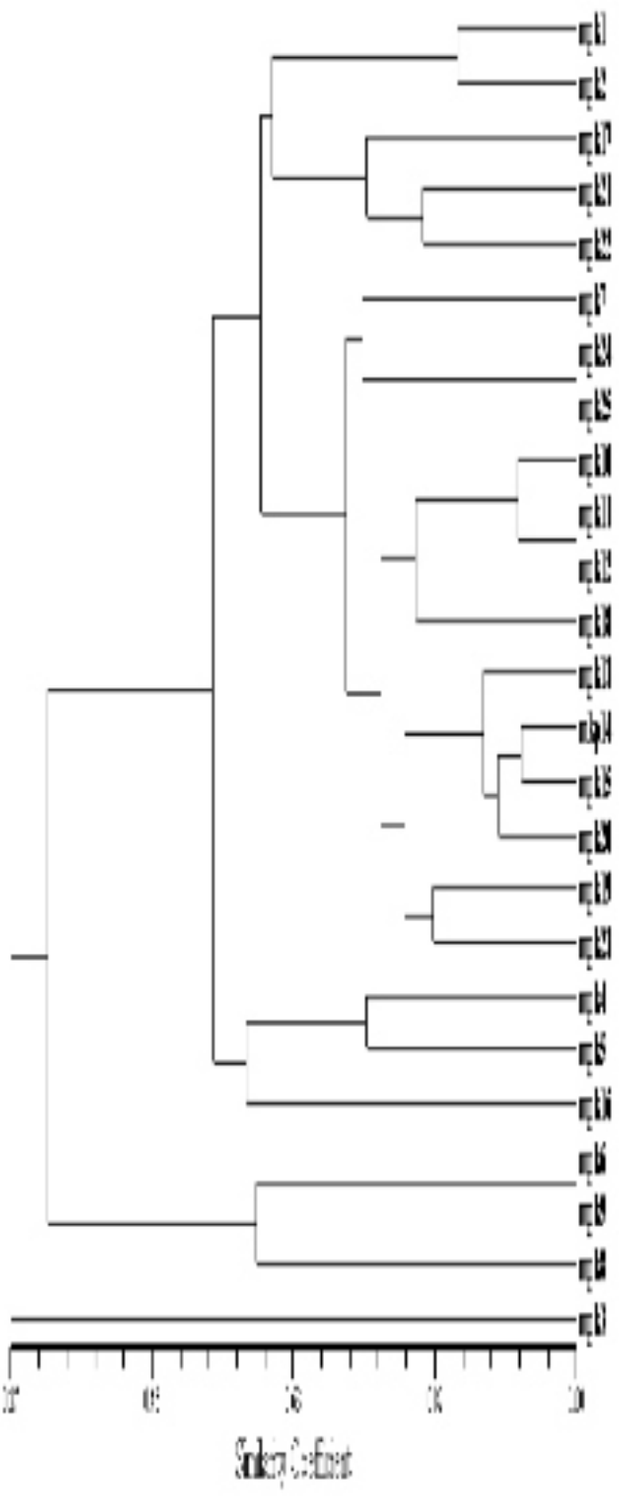
RAPD profiles of different isolates of *Macrophomina phaseolina* obtained with primer OPA 13. M= Marker (1 Kb marker, Fermentas). Lanes 1 to 25 are different isolates of *M. phaseolina*.

may help in the development of molecular probe.

The molecular analysis using 28S rDNA-RFLP, *topoisomerase* II- RFLP analysis and *cellbiohydrolase*-C-RFLP in combination could help in distinguishing few species and forma specialis. For example *F oxysporum* f. sp. *ciceri* strains that could not be differentiated by *topoismerase* –II-RFLP analysis could be distinguished by 28S-RFLP analysis. Likewise *F. chlamydosporium* and *F. cerealis* that gave identical patterns with 28S-RFLP analysis could be differentiated by *topoisomerase*-II –RFLP analysis. However further research is required using more restriction endonucleases for RFLP analysis and also sequencing of the amplified gene products to look for hypervariable regio that could be exploited for development of species-specific probe.

Molecular fingerprinting of *Macrophomina phaseolina*

Isolates of *Macrophomina phaseolina* collected from different geographical locations showed region and further analysis helped in the development of species-specific pimers and probe for *Macrophomina phaseolina*. The study was further carried out to look for genetic diversity of soil-borne populations of *Macrophomina phaseolina* using the RAPD primers. A set of 20 primers was used to identify the RAPD primer that could yield polymorhic patterns for different isolates. Primer OPA-13 was selected as it produced reproducible



Dendrogram obtained from 25 isolates of *Macrophomina phaseolina* with UPGMA on Jaccard's coefficient. Branches are labelled by isolate numbers. The line below the dendrogram represents the similarity index

RAPD profile with good polymorphism. Each isolate gave 8-15 bands. Very distinct pattern was obtained for each isolate and one band of 250 bp was common to all isolates.

Very limited diversity with regards to ITS – RFLP analysis (Annual report 2005-06). However based on sequencing of ITS region and further analysis helped in the development of species-specific pimers and probe for *Macrophomina phaseolina*. The study was further carried out to look for genetic diversity of soil-borne populations of *Macrophomina phaseolina* using the RAPD primers. A set of 20 primers was used to identify the RAPD primer that could yield polymorhic patterns for different isolates. Primer OPA-13 was selected as it produced reproducible RAPD profile with good polymorphism. Each isolate gave 8-15 bands. Very distinct pattern was obtained for each isolate and one band of 250 bp was common to all isolates.

UPGMA analysis of RAPD data showed that the isolates collected from geographically distinct regions could be broadly classified into 9 groups. Intra-regional variation between isolates was less apparent. However the variation based on geo-diversity was higher in *Mp* isolates. A total of 18 polymorphic bands were scored, and polymorphism was found among 25 isolates examined. Hence primer OPA-13 can be used as a marker for differentiation of *Mp* isolates based on geo-diversity.

Library, Information and Documentation

Books:

Administration	45
Bacteriology	26
Biochemistry	08
Bioinformatics	06
Bioinstrumnetation	01
Biotechnology	89
Botany	05
Environmental Sciences	14
IPM	12
Microbiology	222
Molecular Biology	72
Phycology	12
Plant Pathology	96
Plant Virus	03

Miscellaneous literature:

- Annual Reports of ICAR Institutes
- Journal of biosciences
- Indian Journal of Microbiology
- Journal of Mycology and Plant Pathology
- Complete Solution for Biotech Research
- Current Science
- Indian Phytopathology
- Mycobiology
- Journal of the Indian Academy of Sciences
- Nature
- Plant Diseases
- Fungal Genetics and Biology
- The nournal of the Indian Botanical Society
- Asian Journal of Microbiology, Biotechnology and Environmental Sciences
- Advanced Biotech
- Hindi books
- Current Contents of Life Sciences
- Catalogues
- Dictionaries
- ICAR News/ bulletins

Annual Reviews:

Annual Review of Microbiology
(Vol. 47 to 56, 59 & 60)
Annual Review of Phytopathology
(Vol. 30 to 41 & 44)
Applied and Environmental Microbiology
(Vol. 1 to 12), 2006

Journal List

1. Journal of Eco-friendly Agriculture
2. Indian Journal of Microbiology
3. Journal of the Indian institute of Science
4. Indian Journal of Sugarcane Technology
5. Current Science
6. Pestology
7. Advanced Biotech
8. Journal of Biosciences
9. Fungal Genetics and Biology
10. Genuine Chemical Corporation
11. Current Contents
12. Applied and Environmental Microbiology
13. Nature – Twenty year on
14. Plant Disease

Research Papers / Reviews / Books

Books

Vol. 5: Genes & Genomics (2005). Elsevier Science, U.K. (Edited by Dilip K. Arora)

Vol 6: Bioinformatics (2006). Elsevier Science, U.K. (Edited by Dilip K. Arora)

Research Papers

- Bhim Pratap Singh, Ratul Saikia, Mukesh Yadav, Rakesh Singh, V. S .Chauhan and Dilip K. Arora. Molecular characterizations of *F. oxysporum* f. sp. *ciceri* causing wilt disease of chickpea. African Journal of Biotechnology, 2006, 5(6). Pp. 497-502.
- Ratul Saikia, Mukesh Yadav, Bhim Pratap Singh, Dip K Gogoi and Dilip K Arora. Induction of resistance in chickpea by treatment with cell wall protein of *Fusarium oxysporum* f. sp. *ciceri* and *Macrophomina phaseolina.* Current Science, 2006, 91(11), 1543-1546.
- Ratul Saikia, Bhim Pratap Singh, Saju Varghese and Dilip K Arora (2006)- Influence of minerals amendment on diseases suppressive activity of *Pseudomonas fluorencens* to *Fusarium* wilt of chickpea. (Microbiological Research).
- Ratul Saikia and Dilip K. Arora (2007). Small Bugs, Big Business. In: ICAR-Industry Meet, Agricultural Transformation through Public-Private Partnership – An Interface, Edited by S. Ayyappan, Pitam Chandra & S. K. Tandon, Directorate of Information and Publications of Agriculture, ICAR, New Delhi. Pp 106-119
- Ratul Saikia, Mukesh Yadav, Saju Varghese, Bhim Pratap Singh, Dip K Gogoi, Rakesh Kumar and Dilip K Arora. Role of Riboflavin in Induced Resistance against *Fusarium* Wilt and Charcoal Rot Diseases of Chickpea, *Plant Pathol. J. 22(4) : 339-347 (2006)*
- Kishore Babu B., Saxena A. K. And Arora D.K. 2007. Identification and detection of Macrophomina phaseolina by using species-specific oligonucleatide primers and probes. Mycologia (Accepted)

Review

- Ratul Saikia, Bhim Pratap Singh, Tanuja Singh and Dilip K Arora. Use of Molecular Tools to Study the Diversity of Phytopathogenic Fungi Causing Diseases in Horticultural Crops. Indian J. Microbiology, 2006, 46 (4), 293-306.

Sequence

- Deposited sequence of 16S rDNA gene of *Pseudomonas aerunginosa* RsB29 to NCBI GenBank. Accession No- DQ 666628.

Conference, Training, Awards

Organized

- Organized the Launching meeting of the project "Application of Microorganisms in Agriculture and Allied Sectors" on August 27, 2006 at NBAIM.
- Training programme on "Mushroom Cultivation' jointly organized by NBAIM, Mau and NRC Mushroom, Solan, July 22-24, 2006.
- 'Kisan Mela' jointly organized by NBAIM, Mau and DSR, Mau on November, 18, 2006.
- Training Programme on 'Microbial Diversity Analysis of Extremophiles' organized by NBAIM from November 26-30, 2006.
- Training Programme in the area of "Microbial Community Analysis through Metagenomics" from 3-7 February 2007
- First Review Meeting of the project "Application of Microorganisms in Agriculture and Allied Sectors" on 7th January 2007, at NBAIM under the Chairmanship of Dr. Mangala Rai, Secretary (DARE) and Director General, Indian Council of Agricultural Research, New Delhi.
- Third Research Advisory Committee Meeting was held on 09th January 2007.
- Third Institute Management Committee Meeting was held on 10th January 2007.

Attended

- National Symposium on "Researches in Fungal Biology Emerging Trends" Department of Botany, Punjabi University, Punjab on 30-31 January 2007.
- International Conference on Mushroom Biology at National Research Centre for Mushroom, Solan on 10-11 February 2007
- UK, CABI - ICAR Work plan Meeting at NASC Complex, Pusa, New Delhi on 08.03.07.
- Mega project on fisheries meeting at NASC Conference hall on 27th of February, 2007.
- First Training-cum-Workshop on "IP and Technology Management in ICAR System at NAARM, Hyderabad from May 28-30, 2007.
- Attended the Staff Research Council Meeting at IVRI, Izatnagar, Bareilly, Uttar Pradesh on 14.05.07.

Distinguished Visitors

- Dr. Mangala Rai, Secretary (DARE) and DG, Indian Council of Agricultural Research, New Delhi.
- Dr. Gautam Kalloo, Former Deputy Director General (CS), Indian Council of Agricultural Research, New Delhi.
- Dr. S. Ayyappan, Deputy Director General (Fisheries), Indian Council of Agricultural Research, New Delhi.
- Dr. T. P. Rajendran, Assistant Director General (PP), Indian Council of Agricultural Research, New Delhi.
- Mrs. Shashi Mishra, Former Additional Secretary (DARE) and Secretary, Indian Council of Agricultural Research, New Delhi.
- Dr. A. N. Mukhopadhyay, Former Vice Chancellor, Assam Agricultural University.
- Dr. Y. R. Sarma, Former Director, Indian Institute of Spices Research, Calicut.
- Dr. Ramesh Sonti, Centre for Cellular and Molecular Biology (CCMB), Hyderabad.
- Dr. B. L. Jalali, Former Director of Research, CCSHAU, Hissar, Haryana.
- Dr. Mathura Rai, Director, Indian Institute of Vegetable Research, Varanasi.
- Dr. Rakesh Tuli, Director, National Botanical Research Institute, Lucknow.
- Dr. R. P. Tewari, Director, National Research Centre for Mushroom, Solan.
- Dr. A. N. Rai, Vice Chancellor, Mizoram University, Mizoram.
- Dr. R. J. Rabindra, Project Director, Project Directorate of Biological Control, Bangalore.
- Ms. Vandana Dwivedi, Joint Advisor, Planning Commission, New Delhi.
- Commissioner, Azamgarh Mandal, Azamgarh, Uttar Pradesh.
- Sh. P. Guruprasad, District Magistrate, Mau Nath Bhanjan, Uttar Pradesh.
- Superintendent of Police, Mau Nath Bhanjan, Uttar Pradesh.
- Sh. K. Ilango, District Forest Officer, Mau Nath Bhanjan, Uttar Pradesh.
- Sh. D. N. Moghe, Director, Doordarshan, Mau Nath Bhanjan, Uttar Pradesh.

**Dr. Mangala Rai, Secretary (DARE) and DG, ICAR ,
Dr. S. Ayyappan, DDG (Fishereies), Prof. Dilip K Arora, Director
NBAIM and other members of Review Meeting of AMAAS**

NBAIM Personnel

Director

Dilip K. Arora

Scientific Staff

R. C. Tripathi	Senior Scientist
A. K. Singh	Senior Scientist
A. K. Saxena	Senior Scientist
A. B. Dash	Senior Scientist
Rajeev Kaushik	Scientist (SS)
Anurag Chaurasia	Scientist
Mahesh Yandigeri	Scientist

Research Associates/ S R F

Research Associates	02
Senior Research Fellow	25

Administrative Staff

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S. N. Yadav	Senior Clerk
Manish Kumar Jain	Junior Stenographer
Shyamji Shukla	Senior Clerk
Ashok Kumar	Junior Clerk
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Anchal Srivastava	T-1

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Manoj Kumar	Grade II
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Asheesh Kumar	Grade I
Pratap Singh	Grade I
Ajay Vishwakarma	Grade I

NBAIM Family

Photo Gallery

Bhutan Delegation at NBAIM
Secretary, Ministry of Agriculture, Bhutan (centre)

Dr. R. J. Rabindra, Director, PDBC and
Dr. B. L. Jalali at NBAIM, Mau

Dr. S. P. S. Ahlawat, Director,
NBAGR, Karnal inaugurating the
Microbial Diversity Training at NBAIM

Dr. Mangala Rai, Secretary DARE and D.G. ICAR
Dr. S. Ayyappan, D.D.G. (Fisheries) Dr. T. P. Rajendran, A.D.G. (P.P.)
and Prof. Dilip K. Arora, Director NBAIM
during review meeting of AMAAS

Dr.Vandana Diwedi, Joint Advisor, Planning Commission during
her visit to NBAIM

Mr. B.D. Singh, Commissioner, Azamgarh and DM, Mau
on a Training Programme at NBAIM

राजभाषा खण्ड

- राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो (एन बी ए आई एम) नवीं पंचवर्षीय योजना-अवधि में सन् 2001 में भारतीय कृषि अनुसंधान परिषद (आई सी ए आर) के अन्तर्गत स्थापित किया गया। तभी से ब्यूरो ने कृषि उपयोगी सूक्ष्मजीवों के हर क्षेत्र में यथा - उनके पृथक्करण में, उनके संग्रहण में, उनकी पहचान करने में, उनके वंश-अनुक्रम के गुण-चिह्नांकन में, और उनके अल्पावधि व दीर्घावधि संरक्षण के क्षेत्र में तेजी से प्रगति की है। संक्षेप में, कुछ प्रमुख उपलब्धियाँ इस प्रकार है-
- एन बी ए आई एम (मऊ) के सूक्ष्म संग्रहालय में 2517 सूक्ष्म-वर्द्धन हैं। जिनमें 2077 कवक-वर्द्धन, 394 जीवाणु-वर्द्धन, 36 एक्टीनोमाईसीट्स के और 10 यीस्ट-वर्द्धन, है जो खनिज तेल, शुष्क प्रशीतन/ लायोफिलाइजेशन और ऋणात्मक 80 सेंटीग्रेट ग्लिसरॉल संग्रहालय में रखे हुए हैं।
- भारत के विभिन्न प्रदेशों यथा - उत्तर प्रदेश, हिमाचल प्रदेश, मध्य प्रदेश, अरुणाचल प्रदेश, आसाम, राजस्थान, बिहार, केरल और सुन्दरवन-क्षेत्र में सूक्ष्म-अन्वेषण कार्य-योजना के अन्तर्गत कृषि उपयोगी सूक्ष्मजीवों के संग्रहण और उनके पृथक्करण हेतु दौरे किये गये और इस खोजी मिशन में 725 से ऊपर कवक (फंजाई) के, 65 जीवाणुओं (बैक्टीरिया) के और 27 एक्टीनोमाईसीट्स के सूक्ष्म-जीवों को अलगाया गया।
- एन बी ए आई एम द्वारा किये गये सर्वेक्षणों में प्राप्त विभिन्न सूक्ष्म-वर्द्धनों में से कई सूक्ष्म प्रजातियों को यथा - स्युडोमोनास फलुरोसेन्स, स्युडोमोनास यूरीजिनोसा, बैसीलस सबटिलिस, बी. ब्रेविस, फ्युजेरियम ओक्जीस्पोरम, हाइपोस्रेला डिस्कोडिया, मैट्रीजियम, एनीसोपली, स्युडोमोनास प्रजाति, ट्राइकोडर्मा हार्जियानम, टी. कोनिंघी और वर्टिसीलियम लेकानी आदि सूक्ष्मजीवों को प्रयोगशाला स्तर पर मृदा-जनित रोग मूलक कवक (फंजाई) को दवाने वाला पाया गया।
- चना, मसूड़ और तिलहन के रोगों की फ्युजेरियम वंशक्रम की लगभग 100 प्रजातियों का नियमानुसार विविध अनुसंधानिक प्रयोगों द्वारा विश्लेषणात्मक ढंग से जैविक अनुप्रतिमुद्रण किया गया।
- विभिन्न फ्युजैरियम प्रजातियों को पहचानने के लिए और दो सम-परिवेशी (होम लिविंग) जीन टपोईसोमिरेज-II और सेलोबायोहाइड्रोलेस-सी के परस्पर आनुवंशिक संबंधों के मूल्यंकन हेतु 28-एस राइबोसोमल डी0एन0ए0 का पी0सी0आ0 और आर0एफ0एल0पी0 युगल विश्लेषण किया गया।
- मैक्रोफोमिना फेजियोलीना की पहचान के लिए आई टी एस रीजन के संरक्षित अनुक्रम से दो जाति-विवेचक प्रवेशिकाएँ और एक ओलीगोन्युक्लीटाइड प्रेरक उपरकण तैयार किये गये। आंशिक आई टी एस-I रीजन का जीन अनुक्रम, तैयार 5.8-एस राइबोसोमल डी0एन0ए0 और आंशिक आई टी एस- II रीजन एन बी ए आई एम के जीन बैंक में रखे गये।
- कुक्ष महत्वपूर्ण जीवाणु वर्गों जैसे- बैसीलस, फ्लूरोसेंट स्युडोमोनास और ऐरेसिया की आण्विक पहचान के

क्षेत्र में एन बी ए आई एम को बढ़त हासिल है।

- 16-एस राइबोसोमल आर0एन0ए0 मात्र एक भाग की सीक्वेंसिंग आधारित प्रजातियों और जीनस वैसीलस की पहचान के लिए एक साधारण प्रक्रिया विकसित की गयी है। 16-एस राइबोसोमल डी0एन0ए0-आर0एफ0एल0पी0 आधारित विशलेषणों पर इंडो-गंगा के मैदानी क्षेत्रों मे बैसीलस के विभिन्न 20 वर्गों की पहचान की गयी।
- फ्लुरोसेंट स्युडोमोनास को आण्विक स्तर पर गुण-चिह्नित किया गया और उनकी विभिन्न जाति-प्रकारों की पहचान के लिए उनकी जीन अनुप्रतिमुद्रा तैयार की गयी।
- विदेशी क्षेत्रों समेत विभिन्न देशी सूक्ष्म-परिवेशी क्षेत्रों से एक्टीनोमाईसीट्स के 200 से अधिक वियुक्त वर्द्धनों को अलगया गया। स्ट्रेप्टोमाईसीट्स प्रजाति के 6 विलगित वर्द्धनों को प्रोटिएज़ का अच्छा उत्सर्जक पाया गया। ये सूक्ष्मजीव 50 डिग्री सेंटीग्रेट ताप पर विकास कर सकते हैं।
- एक्टीनोमाईसीट्स से उनके जिनोमिक डी0एन0ए0 का पृथक्करण आशानुरूप रहा और 16-एस राइबोसोमल डी0एन0ए0 पी0सी0आर0संवर्द्धन हेतु एक्टीनोमाईसीट्स के 56 आईसोलेट्स से अच्छे किस्म के डी एन ए पृथक किये गये।
- कुल 107 जीवाणुओं के, 133 एक्टीनोमाईसीट्स के और 35 कवक सूक्ष्म-वर्द्धनों को राजगिरी ऊष्ण प्रपात से अलगाया गया। उन अलगाए हुए 107 जीवाणु सूक्ष्म-वर्द्धनों से 41 वर्द्धन 45 डिग्री सेंटीग्रेट को और 9 सूक्ष्म-वर्द्धन 55 डिग्री सेंटीग्रेट ताप में टिके रहे। एक्टीनोमाईसीट्स के 133 में से 24 वर्द्धन 45 डिग्री सेंटीग्रेट पर और 19 सूक्ष्म वर्द्धन 55 डिग्री सेंटीग्रेट ताप पर जीवित रहे।
- सेल्युलोज और जाइलेनेज उत्पादकता सामर्थ्य वाले ताप-सह्य जीवाणु की पहचान कर ली गयी है। ये सूक्ष्मजीव जैव-अपशिष्टों के उत्तम अपघटक पाये गये।
- ऐसे जीवाण्विक टीके विकसित किये गये है जो मृदा लवणता के हानिकारक प्रभावों को कम करते हैं और लवण प्रभावित भूमि में गेहूँ की पैदावार बढ़ाते हैं। इस सूक्ष्मजीवों में 8 प्रतिशत की लवण-सांद्रता पर आई0ए0ए0 और घुलनशील फास्फोरस पैदा करने की क्षमता होती है।
- लाल कुँआ, पंतनगर, उत्तराखण्ड स्थित एक पेपर मिल के अपशिष्ट प्रवाह से सिंचित मृदा में सूक्ष्मजैविक अंतरण देखा गया। पेपर मिल अपशिष्ट-जल सिंचित मृदा में जाइलान एवं सेल्युलोज अपघटक जीवाणुओं की संख्या अपेक्षकृत अधिक पायी गयी।
- दसवीं पंचवर्षीय योजना के मध्यावधि मूल्यांकन के दौरान रा0 कृ0 उ0 सू0 ब्यूरो (एन बी ए आई एम) को नोडल सेंटर बनाकर एक नेटवर्क परियोजना- ''एप्लीकेशन आफ माइक्रोआर्गनिज्म्स इन एग्रीकल्चर एंड एलाइड सेक्टर'' (AMAAS) स्वीकृत की गयी और इसका औपचारिक उद्घाटन 27 अगस्त 2006 को हुआ। इस परियोजना के लिए कुल बजट अनुमान 1600.05 लाख रुपये है। वर्तमान में यह परियोजना देशभर मे विभिन्न संस्थानों विश्वविद्यालयों/राज्य कृषि विश्व-विद्यालयों में कुल 63 केन्द्रों पर चलायी जा रही है।
- (AMAAS) परियोजना को कुल छः क्षेत्रों में बॉट कर शोध कार्य चलाया जा रहा है उनके नाम हैं-

1. माइक्रोबियल डाइवर्सिटी एण्ड आईडेण्टिफिकेशन;
2. न्युट्रिएंट मेनेजमेंट, पी जी पी आर एण्ड बायोकंट्रोल;
3. एग्रोवेस्ट मेनेजमेंट, बायोरिमेडिएशन एण्ड पी एच टी;

4. माइक्रोबियल मेनेजमेंट आफ एबियोटिक स्ट्रेस;
5. माइक्रोबियल जिनोमिक्स; एवं
6. मानव संसाधन विकास।

- सूक्ष्मजैविक विविधता पर मानव संसाधन विकास को मजबूत करने के लिए राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो में दो ट्रेनिंग प्रोग्राम- ''माइक्रोबियल डायवर्सिटी एनालाइसिस आफ एक्स्ट्रीमोफाइल्स'' (अतिवादी परिवेशों की सूक्ष्मजैविक विविधता) एवं ''माइक्रोबियल कम्युनिटी एनालाइसिस थ्रू मैटाजिनोमिक्स (मैटाजिनोमिक्स के माध्यम से सूक्ष्मजैविक समुदाय का विश्लेषण) चलाये गये।
- बीज अनुसंधान निदेशालय (डायरेक्टरेट आफ सीड रिसर्च) की सहलग्नता में तकनीकि हस्तांतरण को ध्यान में रखते हुए ब्यूरो द्वारा एक किसान मेले का आयोजन किया गया।

कार्य अधि-पत्र

'कषि की संपोषणीय बढ़त को बनाये रखने और तत्सम्बन्धी अनुसंधान एवं मानव संसाधन विकास कार्यों को पूरा करने के लिए, कृषि हितार्थ सूक्ष्मजैविक संसाधनों के अधिग्रहण और प्रबन्धन हेतु राष्ट्रीय और अन्तराष्ट्रीय स्तर पर एक प्रमुख केन्द्र के रूप में कार्य करना।'

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Former Vice Chancellor
Assam Agricultural University

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T. P. Rajendran
Assistant Director General (PP), ICAR

Dilip K. Arora
Director, NBAIM

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Senior Scientist, NBAIM

Dr. R. C. Tripathi, Prof. Dilip K. Arora, Dr. T. P. Rajendran,
Dr. A. N. Mukhopadhyay, Dr. B. L. Jalali, Dr. Ramesh Sonti, Dr. Y. R. Sharma

Important Recommendations:

- Development of separate set up for commencing the Registration of agriculturally important microbes at NBAIM.
- Scientific collaboration with MTCC, Chandigargh and 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.
- 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, symbionts of animals including insects and nematodes.
- In order to facilitate supportive infrastructure for implementing future research programmes lyophilizer of 1000 sample capacity mobile laboratory, cryopreservation unit with liquid nitrogen plant and laboratory facility for diagnostics of fungal pathogens and soil microbes should be developed.

Institute Management Committee

Chairman

Dilip K. Arora
Director, NBAIM

Members

T. P. Rajendran
Assistant Director General (PP), ICAR

B. D. Kaushik
Head, Division of Microbiology, IARI

M. N. Khare
Former Professor & Dean, JNKVV, Jabalpur

S. J. Singh
Former Head of Division,

Member Secretary

T. N. Vidyadharan
A.A.O., NBAIM

Important Recommendations:

- A detailed road map for the XIth Five Year Plan should be developed and submitted to the Council. Achievement/ output of the Xth Plan is to be prepared and duly showing the future requirement spill over from Xth Plan (development of infrastructure, ancillary facility, staff etc.) should also be reflected in the XIth Plan proposal.
- IMC suggested training the scientists/ office staff of NBAIM in appropriate institutions, so as to be helpful for the strengthening of the mandated activities of the Bureau.
- Regarding the Cadre strength, IMC advised that the cadre strength chart should be prepared in the related disciplines of ARS (Plant Pathology, Microbiology and Plant Biotechnology).
- Cadre strength of technical staff should be made discipline / services-wise as well as need-based like Instrumentation assistant, biochemical technical assistant, molecular biology laboratory assistant, data collection assistant, computer operator, farm machinery and power etc.
- IMC recommends having a security system in the NBAIM for the security purposes as the valuables in certain key laboratories to safeguard unauthorized entry to the culture collections as well as the molecular laboratories in public interest.
- The IMC proposed and suggested that the Institute should have the facility of Electron Microscope/ Confocal microscopy.
- The Bureau should create Sports activities to sponsor teams for participation in ICAR Zonal Sports Meets.
- The committee also recommended to have

an Internal Communication system with WLL wireless system in the NBAIM for fast communication between various installations in the campus.

- IMC also recommended that as and when any infrastructure development is be made, it should be common and permanent in nature that may be taken up jointly by NBAM and DSR.
- IMC has also agreed upon for having the facility of construction of 3rd floor of laboratory building, renovation of generator room, renovation of Type-V quarters, repairing and renovation of residential quarters, renovation of water sump well, electric feeder line, renovation/ repairing of hostel, renovation/ repairing of mess, renovation of internal campus roads, fitting of additional hand pumps, children park, community park, community centre, car parking, scooter parking and the 300 and more seating capacity conference hall which is not available at present and necessary for the use of both the Institutes.
- The IMC strongly recommended to install a Water Treatment plant for both research purpose and to provide the pure drinking water in the campus as well as to maintain the purity of water quality to avoid health problems.
- IMC agreed upon for developing the facility of a Museum of Microbes in the XIth Five Year Plan, where entire activities will be displayed in a Central place to exhibit the activities of the Bureau to the visitors.
- Creation of Police Security post outside campus was discussed and agreed upon and requested the Director to take up with the appropriate district authorities.

Members of IMC
From left to right: Sh. T. N. Vidyadharan, Dr. S. J. Singh, Dr. T. P. Rajendran, Prof. Dilip K. Arora, Dr. B. D. Kaushik, Dr. R. C. Tripathi

Society Membership Form

Reg. No. 183/2005-06

Society of Agriculturally Important Microbial Genetic Resources

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Society of Agriculturally Important Microbial Genetic Resources

MEMBERSHIP APPLICATION

Recent Photograph

Name (In full)	
Father's Name	
Date of Birth	
Profession	
General field of specialization	
Specific field of specialization	
Research field of specialization	
Permanent Address	
Correspondence Address	
Phone / Mobile / Fax	_____ _____ _____
E-mail / URL	_____ _____
High Degree obtained	
Membership required	☐ Life member ☐ Associate Member ☐ Annual Member ☐ Student Member
Members of other Scientific Society	

Date:

Place: Signature

National Bureau of Agriculturally Important Microorganisms
Kusmaur, P. B. No. 06, Post Kaithauli, Mau, Uttar Pradesh 275 101, INDIA
G +91-547-2530080(extn.), email-saimgr_2005@yahoo.com

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