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भाकृअनुप
ICAR

Annual Report 2005-06

वार्षिक प्रतिवेदन 2005-06

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) & Director General
Indian Council of Agricultural Research,
visiting NBAIM Campus

NBAIM Family

NBAIM

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NATIONAL BUREAU OF AGRICULTURALLY
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Understanding and conserving our national heritage of agriculturally important microorganisms

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Director, NBAIM

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Contents

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Front View of NBAIM & DSR

Executive summary

National Bureau of Agriculturally Important Microorganisms (NBAIM) was established in the IXth Plan in the year 2001 under the Indian Council of Agricultural Research. The Bureau has the mandate to act as nodal institute 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. The Bureau started functioning at Old NBPGR Building, New Delhi, and on June 1, 2004 it moved to NISST building, Mau Nath Bhanjan, U.P. At present Bureau has no Regional Stations/ Base Centres.

Major programmes of the Bureau include isolation, identification of agriculturally important microorganisms (AIMs), exploration and collection, microbial germplasm introduction, distribution and exchange, characterization, evaluation, documentation and conservation of AIMs. Among the various projects Bureau has accomplished after the implementation of Xth Plan period, notable ones are the "Digitization of microbial data" with CABI, UK funded by NATP and ICAR funded Network Project on 'Application of Microorganisms in Agriculture and Allied Sectors'.

The Bureau has been sanctioned 20 Scientists position and 30 technical staff, excluding temporary posts under time-bound projects/ schemes. The Bureau has started on "strong footing" at new location and now strengthening its linkages with other ICAR/ CSIR/ DBT Institutes, State Agricultural Universities (SAUs), and International Microbial Resource Centres covered under the umbrella of WFCC and OCDE. NBAIM is growing as a dynamic and vibrant organization and in future it will lead the national body for all R&D research on microbial genetic resources with state of the art infrastructural facilities for identification, characterization and conservation of agriculturally important microorganisms. NBAIM has a well maintained National Microbial Repository, National Repository for Patentable Microbes and a Cryopreservation Facility. NBAIM is developing infrastructural facilities for microbial database and microbial information management system.

Since last two years NBAIM has contributed significantly towards strengthening the activities in terms of infrastructural and human resource development. The perspective plan for the Bureau upto 2025 AD aimed to critically evaluate the stepwise need for developing infrastructural facilities, well-equipped laboratories and trained manpower. For this purpose, we will take the help of national and international organization for funding and expertise (e.g. FAO, GBIF, OCDE, WFCC etc.), which will certainly give push to various R&D programmes and activities of the Bureau.

A mid-term appraisal of the perspective plan has greatly contributed in analyzing the achievements made so far and also modifications required in certain items of the earlier plan. This was necessitated owing to the changing global scenario of microbes and its utilization. Thus certain new perspectives have emerged which now would be an integral part of the "microbial resource" programme. The recommendations of First and Second Research Advisory Committee have also been duly considered for revising the perspective plan.

Among the achievements made so far, within a short span of 2 years, since its establishment at Mau, the notable ones include collection of over 2517 accessions including many useful microbes.

Over 2517 microbial accessions, strains, promising agriculturally important microbes were isolated from different locations in the country. Areas mainly targeted are Assam, Arunachal Pradesh, Rajasthan, parts of

western and eastern Uttar Pradesh and Indo Gangetic plains. About 1100 agriculturally important fungi are being repatriated from CABI-UK. A large number of AIMs are available in different National Universities/ Institutes and also within ICAR system which has yet to be brought to the culture collection of the NBAIM. A number of new techniques were developed/ standardized for detection of AIMs from infected plant parts.

Some important AIMs accessions, having significant importance in crop productivity have been characterized, and their evaluation is under way. The data of collected AIMs assigned to NBAIM starting from 2002 has been digitized and is in a retrievable format. Some useful AIMs available at NBAIM consists of (i) Biocontrol Agents: *Trichoderma* spp., *Paecilomyces thermoascus, Paecilomyces lilacinus, Beauveria* spp., *Gliocladium verens, Verticillium* spp.; (ii) Biopesticides: *Beauveria* spp., *Metarhizium* spp., *Paecilomyces* spp., *Verticillium* spp., *Nomurea* spp.; (iii) Growth Promoters: *Pseudomonas fluorescens*, P. *syringae, Rhizobium* spp., *Bradyrhizobium* spp.; (iv) Potential Enzymes/Antibiotics/Toxins producers: *Fusarium pallidoroseum, Fusarium oxysporum, Pencillium citronum, Penicillium frequentens,* isolates of *Aspergillus;* (v) Entomopathogenic: *Beauveria* spp., *Metarhizium* spp., *Verticillium* spp., *Nomuraea* spp.; (vi) Egg Parasitic Fungi: *Paecilomyces lilacinus, Verticillium chalamydosporium*, Mycoparacitic fungi, *Gliocladium* spp.; (vii) Bacteria possessing nematicidal and insecticidal properties: *Bacillus circulans, Bacillus brevis, Paenibacillus alvei, Brevibacillus laterosporus*; (viii) Biofertilizers: Species of *Rhizobium, Azospirillum* and *Azotobacter.*

The DNA fingerprinting protocols using different molecular markers techniques for some important plant pathogenic fungi such as *Fusarium, Macrophomina* and *Rhizoctonia solani* have been developed, which is of great importance. About 100 strains of Fusaria pathogenic to chickpeas, lentils and oilseeds have been fingerprinted under All India Coordinated Network Project on *Fusarium. Macrophomina* isolates have been characterized at the molecular level and eight ITS gene sequences were submitted to GenBank. Several strains of *Pseudomonas* and *Bacillus* have been characterized by using molecular techniques.

A network project on 'Application of Microorganisms in Agriculture and Allied Sectors' with NBAIM as the nodal center was approved during the mid term appraisal of Xth five year plan, and was formally launched on August 27, 2006. The total budget outlay for the project is Rs. 1600.69 lakhs and is operational at 63 different Institutes/ SAUs/ Universities in the country.

In foreseeable future following new perspectives will be included:

- Mapping of microbial diversity through Geographical Information System;
- Integration of bioinformatics and development of softwares for effective management of microbial holdings;
- Development of "Quality Management System" based on OCDE and WFCC microbial resources centres guidelines;
- policies regarding the deposit of "Genetically Manipulated" AIMs and AIMs falling under IPR and patentable "National Importance" and its long term conservation of AIMs which are important for the country;
- Development of microbial gene bank
- Linkages with farmer, industry and academia;
- Utilization/ testing of important AIMs at different ICAR Institutes/NRCs, which will alter result in distribution of AIMs to the farmers.

The "passport data" of each deposit has been developed. The evaluation of some agriculturally important microorganisms will be carried out as with time this Bureau will grow, in future.

Augmentation of AIMs through repatriation from different national and international agencies was initiated and will be done in phase-wise manner.

Repeatable and reliable protocols for cryopreservation of various agriculturally important microorganisms will be developed for *in vitro* repository, which would be useful for the long-term conservation of microbes. It is envisaged to carry out experiments to develop cryopreservation protocols in some of the most important genera that are useful for increasing crop productivity. Attempts will be made to cryopreserve existing microbial genetic resources of *in vitro* repository utilizing the developed protocols for its long-term conservation. Initiatives are to be taken for cryopreserving actinomycetes and other slow-growing microorganisms. Microbial species where cryopreservation is not possible, emphasis will be laid on lyophilization technique or mineral-oil method of deposit. The viability of cryopreserved AIMs would be evaluated.

Information on "Microbial Repository" would be integrated by the ARIS cell into a national database to get desired and reliable output in the form of reports. Effective Microbial Bioinformatics Cell monitoring the important administrative work of the Bureau would be done by developing a suitable database. Linkages through electronic methods would be developed to integrate other "National Repository" of the country.

The implications of IPR policies revised guidelines under WTO/ CBD/ ITPGR related to management of microbes and biosafety are being analysed and technical inputs will be provided to the Council to take action for compliance to these regulations.

The activities of human resource development will continue in various aspects of dissipation of knowledge for:

- Study of biodiversity of AIMs
- Identification, preservation and conservation of AIMs
- New protocols and technologies
- Quality microbial management system with special emphasis on biosystematics
- DNA fingerprinting
- Various conservation strategies
- Molecular detection of microbes and on policy issues related to IPR

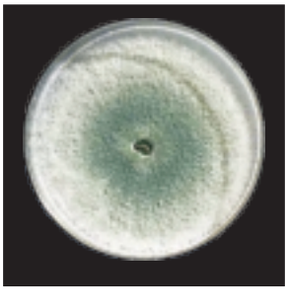
The Bureau has link itself to different M.Sc. and Ph.D. programmes within ICAR system and also with some important National Universities/ Institutes.

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, including obligate parasites on host plants under controlled condition etc. Microbiology Division has the objective 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 will isolate and collect AIMs from different agro-climatic zones of India.

Besides these, the Bureau has HRD component in which training programs will be organized in the field of molecular identification of AIMs and tools for development of microbial technology and its implementation. In addition, the Bureau has six cells namely Agriculture Research Information System (ARIS), IPR & Biosafety Cell, Technical Cell, Culture Collection Cell, Planning Monitoring and Coordination Cell, Hindi Cell and NATP Externally Funded Project Cell.

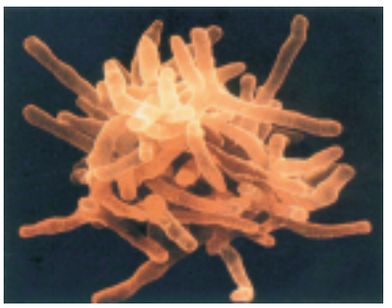


Mandate

"To act as a nodal centre 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"

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).



Detailed Research Activities

Exploration and collection of AIMs

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

Identification, characterization and documentation of AIMs

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

NBAIM Central Lab

Electrophoresis Lab

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 promotion microorganisms, bio-indicators and for bio-degradation, bio-remediation, bio-composting.

Salient Achievements

Germplasm conservation

- The number of cultures in the long term repository of NBAIM culture collection is 2517.
- All the microbial data available at NBAIM have been digitized and put in retrievable form.
- *Macrophomina phaseolina* isolates were characterized at molecular level, ITS region sequenced, gene sequences deposited at GenBank.
- Probes for identification of *Macrophomina* have been developed.
- Fluorescent pseudomonads were characterized at molecular level and DNA fingerprints were developed for identification of various genotypes.

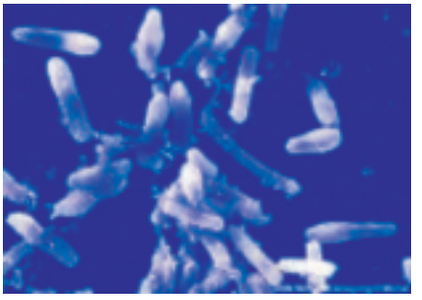
Major on going Research Projects

Externally Funded Projects :

- Diversity and Conservation of Agriculturally Important Microorganisms and their Potential as Biocontrol Agents" APCESS Project, ICAR, New Delhi.
- Comparative PCR based Genomic Fingerprinting of *Macrophomina phaseolina* and Development of Strain Specific Probe" funded by DBT, 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* group of Plant Pathogenic Fungi", ICAR Network Project, New Delhi.
- Effect and Evaluation of Bio-active Compounds from Agriculturally Important Cyanobacteria", funded by DST, New Delhi.

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 microorganisms 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.



Pseudomonas fluorescens

Culture Collection

Following are the major groups of cultures available at the Culture Collection of NBAIM

Source	Bacteria	Fungi	Actinomycetes	Yeast
Plant	93	1212		
Soil	225	641	36	10
Insect		21		
Air flora		18		
Any other	76	185		
Total	394	2077	36	10

List of Microbial cultures deposited at NBAIM (2005-06)

S. No.	Name	No. of Cultures
1.	University of Agricultural Science, Dharwad, Karnataka	52
2.	Directorate of Oilseed Research, Hyderabad	15
3.	Directorate of Oilseed Research, Hyderabad	41
4.	University of Agricultural Science, GKVK, Bangalore	154
5.	Indian Institute of Horticulture Research, Bangalore	27
6.	NRC on Rape Seed Mustard, Rajasthan	27
7.	Allahabad Agricultural University, Allahabad	08
8.	Rajendra Agri. University, Muzaffarpur	05
9.	J. L. N. Krishi Vishwavidyalay, Jabalpur	15
10.	Aligarh Muslim University, Aligarh	40
11.	Indian Agricultural Research Institute, New Delhi	10

Project on Diversity and Conservation of Agriculturally Important Microorganisms and their Potential as Biocontrol Agents

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

Fluorescent pseudomonads are an ecologically important group of soil bacteria that are well accepted as plant growth promoting rhizobacteria (PGPR). Growth promotion occured as a result of direct stimulation of the plant, inhibition of plant pathogens, and/or induction of defense host mechanisms against pathogens. Fluorescent pseudomonads, in general, are considered to be cosmopolitan species with broad distribution in the environment. Phosphate solubilization is an important attribute of PGPR and helps to release bound phosphorus and make it available to the crop plants. Leguminous plants endowed with the ability to fix atmospheric nitrogen in association with rhizobia require higher levels of P. Study was carried out to determine the relationship between the genotype of fluorescent pseudomonad and its ability to solubilize inorganic phosphorus. Genetic profiles were generated using RAPD methods and 16S rDNA- Restriction Fragment Length analysis (RFLP).

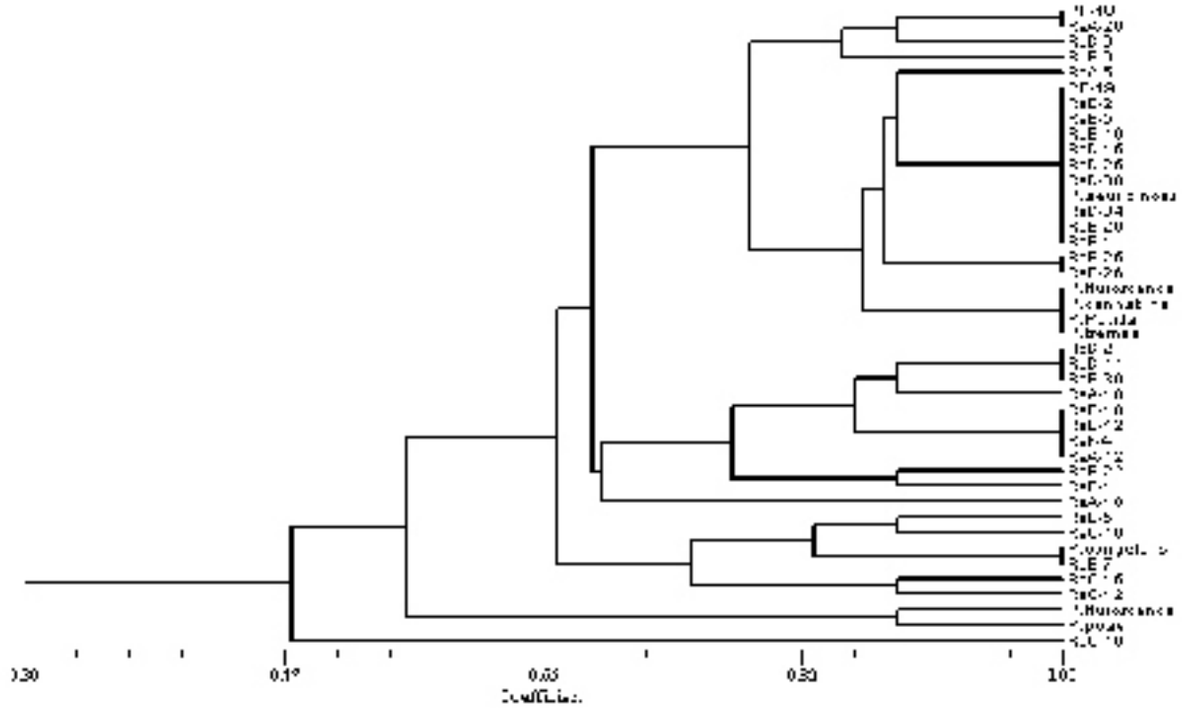
Amplification of 16S rDNA was carried out by polymerase chain reaction using universal primers 16S rRNA For (AGA GTT TGA TCC TGG CTC AG) and 16S rRNA Rev (ACG GCT ACC TTC TTA CGA CTT). Amplified PCR products were digested with 3U of restriction endonuclease: *HaeIII, AluI, RsaI* and *MspI*. The data matrix was generated and used to calculate Jaccard's similarity coefficient for each pairwise comparison. Dendogram was construced from the similarity matrix by the unweighted pair group method with airthmetic mean (UPGMA). Of the 32 isolates screened for phosphate solubilization, 23 were able to solubilize tricalcium phosphate with varying efficiency. The radius of clearing zone ranged between 5.0 to 8.6 mm with isolate RsD-2 showing the maximum value (Table 1). Of the two reference strains tested, *P. aeruginosa* (NCIM-5031 (PF19) could not solubilize phosphorus.

The 18 random primers selected for further testing, yielded 183 reproducible bands. Amplification products yielded complex genomic fingerprints consisting of fragments ranging in size from 80 to 2000 bp. From the 32 isolates and two reference strains of *Pseudomonas fluorescens* and *P. aeuriginosa*, 10 different genome clusters were defined at the 74% similarity level shown in Fig.1. Clusters A, B and E were the major groups and contained 10, 4 and 6 isolates, respectively. These clusters included isolates from different locations. For example, cluster A included isolates from Varanasi, Aligarh, Mau and Tamilnadu. Interestingly, all the isolates of this cluster could solubilize phosphorus. Conversely isolates from single location were distributed in different clusters. For example, the 11 isolates obtained from Varanasi were distributed in clusters A, C, D, E, F, G and J. Isolate RsA-20 from Aligarh, U.P showed 100 % homology with an isolate from Vishakhapatnam (RsE-2). All the isolates in clusters C, H, I and J and one isolate each from clusters B and D failed to solubilize phosphorus.

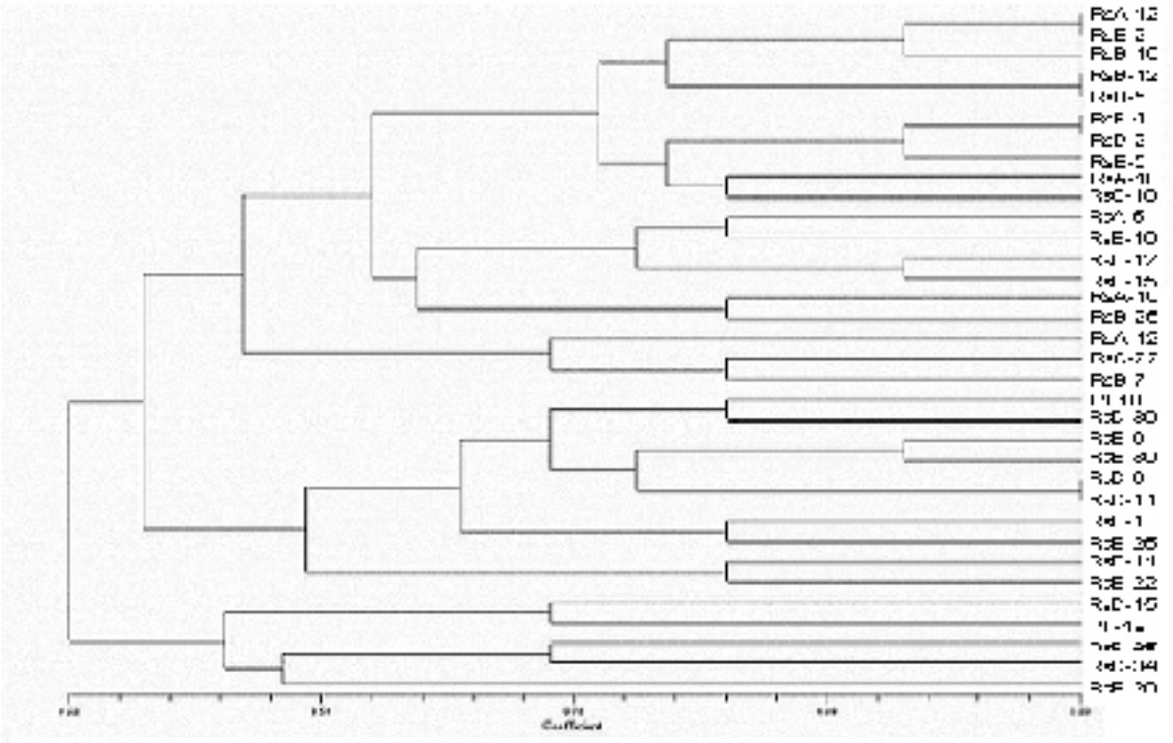
To determine the distribution of genotypes among the known species of fluorescent pseudomonads the phylogenetic analysis was done with 16S rDNA-RFLP analysis of 32 isolates along with 10 reference species. The combined pattern obtained with the four endonucleases defined 9 distinct genomic groups among 32 isolates and 10 reference strains at a similarity coefficient of 75% shown in fig. Of these, the cluster B was the largest and contained 12 isolates and 6 reference strains, two of *P. aeuriginosa* and one each of *P. fluorescens*, *P. cannabina*, *P. putida* and *P. tremae*. The second largest group, C, included 8 isolates from different RAPD genotypic clusters A and E (Table 1). Genomic cluster A contained four isolates from three different locations, Vishakhapatnam, Aligarh and Mau. Isolate RsB-7 from Varanasi soil showed 100% homology with reference strain of P. congelens and were part of the cluster F along with an isolate from Tamilnadu RsC-10. The results revealed that the 16S rDNA-RFLP based genomic grouping does not confirm to the RAPD- based clustering of genotypes.

Distribution of phosphate solubilizing Fluorescent *Pseudomonads* among RAPD based genotypes and 16S rDNA-RFLP based genomic groups

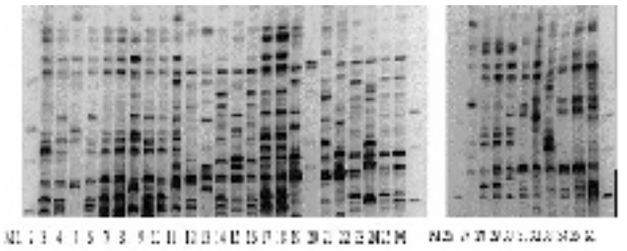
Isolate	P-solubilization (zone of solubilization in mm)	RAPD-based genotype	RFLP-based genomic groups
PFVi-1	5.2	A	B
PFVi-2	5.8	A	B
PFVi-3	6.8	B	B
PFVi-4	5.6	E	A
PFV-1	6.2	A	C
PFV-2	7.9	A	C
PFV-3	6.3	A	C
PFV-4	NS	J	B
PFV-5	7.4	G	B
PFV-6	5.7	G	D
PFV-7	6.1	F	D
PFV-8	7.3	F	B
PFV-9	5.0	E	C
PFV-10	NS	C	B
PFV-11	7.6	D	F
PFAL-1	6.4	A	C
PFA-1	6.9	B	B
PFA-2	NS	C	E
PFA-3	7.2	D	C
PFA-4	5.4	A	A
PFA-5	6.4	A	C
PFT-1	5.9	A	F
PFT-2	5.4	B	G
PFT-3	NS	B	G
PFT-4	NS	D	I
PFM-1	8.6	A	C
PFM-2	6.8	E	A
PFM-3	8.0	E	C
PFM-4	NS	H	B
PFM-5	NS	I	B
PFM-6	5.6	E	B
PFM-7	NS	I	B
NCIM	7.2	E	A
NCIM	NS	H	B



Dendrogram of genetic relationships among 16S rDNA genotypes of fluorescent *Pseudomonas* identified by PCR-RFLP analysis

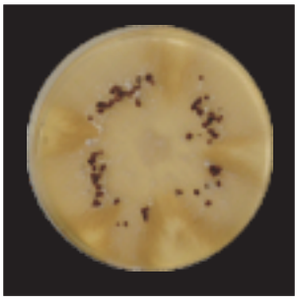


Dendrogram of fluorescent *Psudomonas* isoltes derived from RAPD fingerprints by using 18 different random primers.



ERIC- PCR of fluorescent *Pseudomonas* isolates from different locations in India. M- 1 KB Molecular marker, Lanes 1-35, various isolates including the reference strains.

Thirty five isolates obtained from the soil samples collected from Mau and Delhi were characterized at the molecular level using the conserved motifs in bacterial repetitive (ERIC) elements. PCR amplification revealed that that ERIC like DNA sequences are widely distributed in fluorescent Pseudomonas group. Distinct patterns were obtained among the 35 isolates using ERIC-PCR. An analysis of dendrogram revealed that a major division at 60% similarity coefficient values separated the isolates into two main clusters.



R. Solani

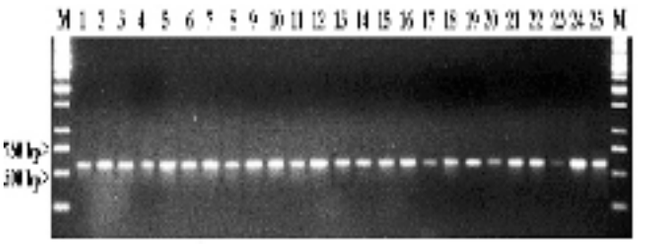
Project on Comparative PCR Based Genomic Fingerprinting of *Macrophomina phaseolina* and Development of Strain Specific Probe

PI : Dilip K. Arora
RA : Rakesh Kumar
SRF : B. Kishore Babu

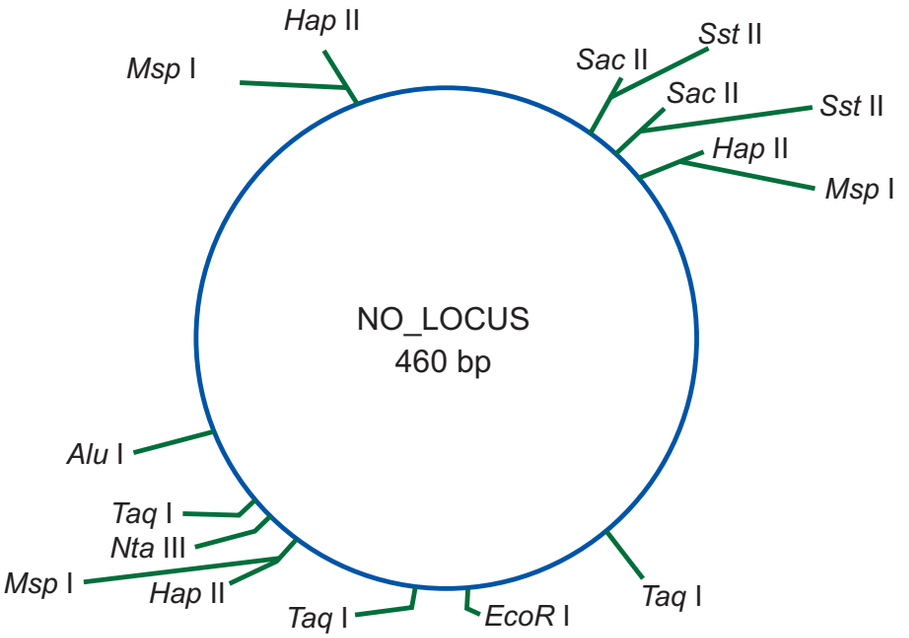
Macrophomina phaseolina (Tassi) Goid is one of the most damaging seed and soil borne pathogen, infecting about 500 plant species in more than 100 families throughout the world. Under favorable conditions the fungus causes many diseases like damping off, seedling blight, collar rot, stem rot, charcoal rot and root rot in various economically important crops. The identification of isolates of *M. phaseolina* is usually based on morphological criteria, but due to wide variations in the phenotype of the isolates these criteria are often not reliable. In addition pathogenic variability among the isolates or sensitivity to chlorate are some other characters utilized to categorize the isolates. Moreover, the use of these techniques is advantageous only for grouping rather than for identification and are laborious, time consuming and not reliable. Therefore, a rapid diagnostic test that can exactly identify and detect *M. phaseolina* isolates both in vitro and in vivo conditions are required. Nuclear rDNAs, particularly in the ITS region, are good targets for phylogenetic analysis in fungi. Oligonucleotide specific primers or probes targeting the ITS region have been demonstrated to selectively detect several agriculturally important fungi like *Trichoderma, Hypocrea, Fusarium, Verticillium, Phytophthora* and *Mycosphaerella* etc. However, no such markers are available for specific detection of *M. phaseolina*. Screening of the GenBank for ITS sequences of *M. phaseolina* revealed the existence of only 6 sequences that showed some degree of variation among them. Sequence variation in the rRNA genes allows the use of these genes as targets for differential amplification. PCR amplification of ITS region, followed by RFLP and sequencing analysis of the ITS sequences for identifying the conserved and variable motifs was thus carried out with an aim to develop specific primers and oligonucleotide probe within the ITS region for specific identification of *M. phaseolina* and to evaluate the efficiency of specific primers and probe to detect the pathogen under in vitro conditions.

The genomic DNA of 25 isolates of *Macrophomina phaseolina* was subjected to PCR amplification of ITS region by using primers ITS-1and ITS-4. The amplified rDNA fragment was used for restriction fragment length polymorphism (RFLP) analysis using 13 restriction enzymes: *Eco*R I, *Nla* III, *Sau*3A I, *Alu* I, *Cla* I, *Sac* II, *Sst* II, *Hap* II, *Msp* I, *Taq* I, *Apa* I, *Hind* III and *Sac* I. The variability within the amplified regions was also analyzed by cleaving the fragment with different restriction enzymes. The restriction enzymes *Eco*R I, *Nla* III, *Sau*3A I, *Alu* I and *Cla* I gave identical pattern for all isolates and produced two fragments of different sizes at single cut. The enzymes *Sac* II, *Sst* II, *Hap* II, *Msp* I and *Taq* I have multiple cleavage sites in ITS sequences and produced fragments less than 100 bp in size. The enzymes *Apa* I, *Hind* III and *Sac* I failed to cleave the ITS sequence. The restriction fragment length polymorphism analysis of amplified ITS product with different restriction enzymes could not produce significant polymorphic patterns among the isolates. Thus the technique was not further used to discriminate *Macrophomina* isolates at molecular level.

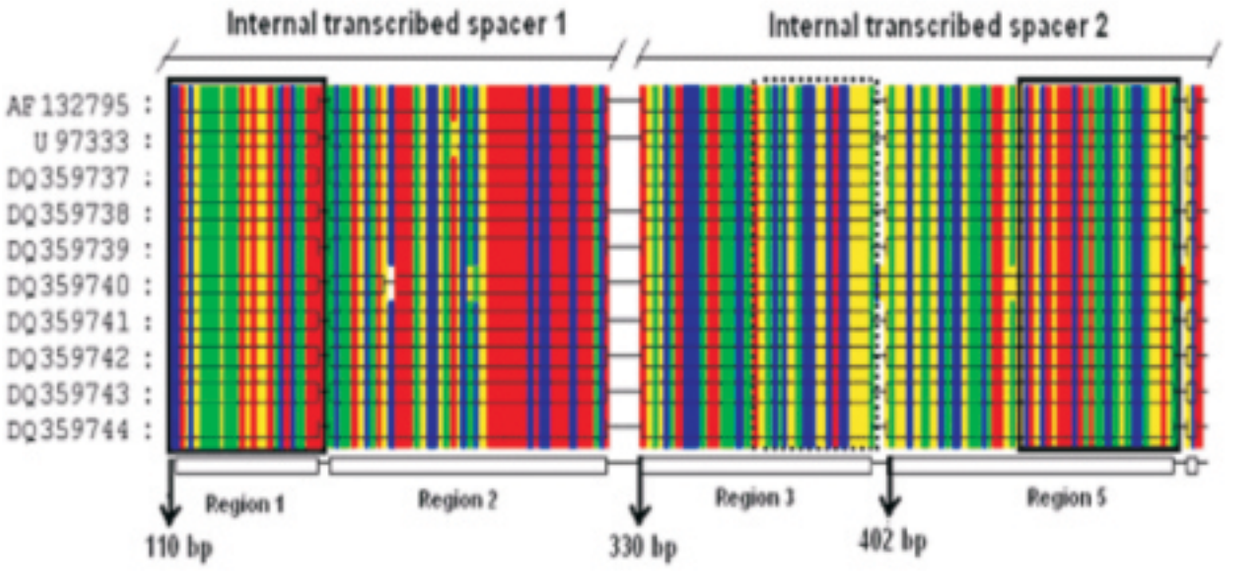
For further characterization, 8 isolates were selected based on different host ranges /geographical origins and subjected to direct sequencing of ITS amplified product by using Big dye terminator method on ABI prism DNA sequencer. Eight gene bank accessions for complete sequence of ITS-1, 5.8s rRNA



PCR Amplification of ITS region by using ITS 1 and 4 primer: Lanes 1 to 25 are amplified products of *M. phaseolina* cultures listed in Table 1, M represents 1 kb ladder.



Restriction map showing different restriction enzyme sites with in the 5.8 S RNA gene, ITS1 and partial ITS2. Total length of the fragment is 460bp.



Development of specific oligonucleotide primers and probe: Alignment of ITS1 and ITS2 sequences from 8 isolates of *Macrophomina phaseolina* and two reference sequences (AF132795 and U97333) taken from GenBank database. Nucleotides are shown in color bars (A-red, G-yellow, T-blue, and C-Green). The regions 1, 2, 3 and 5 are completely aligned, 5.8 S RNA gene is not shown and because of variability the region 4 was omitted. The solid line rectangles indicate specific nucleotide areas used for the development of specific oligonucleotide primers and the dashed rectangle shows the specific region used for designing of the probe. The position of the first nucleotide of region 1 and others were given according to the reference sequence AF132795.

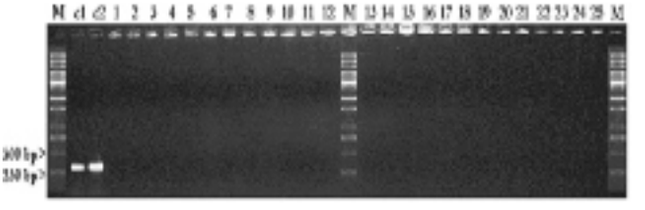
gene and ITS -2 region were obtained. A phylogenetic tree was constructed that showed that all the eight isolates could be clustered into two groups with a similarity of over 90%. The eight sequences from *M. phaseolina* isolates and other two reference sequences retrieved from EMBL and GenBank databases were aligned using the CLUSTAL X (version 1.7) algorithm program. The sequences were visually checked for regions having homologies among isolates of *M. phaseolina* but not among other fungi. The regions, which were conserved among the isolates and specific for *Macrophomina,* were selected to design species-specific oligonucleotides. Sequence comparison of ITS region encompassing ITS1, 5.8s rRNA gene and ITS2 among the isolates of *M. phaseolina* and other related fungal species revealed three regions that were conserved among the *M. phaseolina.* Virtually the complete sequence was divided into five regions from ITS1 to ITS2. The region 4 that showed variability among the aligned sequences of *M. phaseolina* was not considered for further analysis. 5.8 S RNA gene sequence was also excluded from the analysis. After editing and rearrangement of aligned sequences and comparison with the sequences of closely related genera of fungi, region 1 and 5 were selected for the development of species-specific primers for *M. phaseolina.* Two primers MpKFI and MpKRI were designed from the specific nucleotide areas and one oligonucleotide probe MpKH1 (19 mer) was designed from the conserved region, adjacent to 5.8 S gene showed in the region 3. The parameters such as the percentage of G+C content, absence of self-complementarity in oligonucleotides and of complementarity between the primers were analyzed using program Gene Runner (Hastings Software, USA). The designed primers yielded single amplified product of 350 bp with all the isolates of *M. phaseolina* tested. The specificity of the primers was tested on representative species from basidiomycetes, zygomycetes oomycetes, ascomycetes, deuteromycetes and some common soil-borne microbes. The primer pair was found to be specific for *M. phaseolina* as none of the other soil microbes tested could yield any amplification product under identical conditions of amplification.

The oligonucleotide probe MpKH1 was non-radioactively labeled by using DIG-DNA Labeling and Detection Kit. The conditions were optimized for hybridization of DIG-labelled oligonucleotide probe. Detectable hybridization signal was obtained with an oligonucleotide probe at 1 pmol ml^{-1}, pre-hybridization, hybridization and washing steps performed at 55° C, 15 hrs time for hybridization and 50 to 100 ng ml^{-1} of target DNA. Dot blot assay initially carried out with variable quantities of PCR product from two strains of *M. phaseolina* did not show any appreciable differences in signal intensity. In the later dot blot assay carried out with 5ul of PCR product irrespective of their concentrations allowed the detection of all the spots of PCR products from *M. phaseolina* strains. Probe MpKH1 selectively hybridized with two strains of *M. phaseolina* but failed to do so with all representative strains of different groups of fungi. This could be further exploited for ecological monitoring of M. Phaseolina but failed to do so with all representative strains of different groups of fungi. This could be further exploited for ecological monitoring of *M. phaseolina* in complex habitats. This is the first report on development of specific primers and probe for the identification and detection of *M. phaseolina*. At present work is in progress to ascertain the utility of molecular probe for rapid detection of *M. phaseolina* under field conditions.

Another approach followed for the development of species specific probe was to look for a specific amplified fragment using RAPD primers. A 1 kb fragment was identified that was common to all the isolates with a particular 10-mer primer. The fragment was eluted and cloned in pGEMT vector. The sequencing of the fragment is under progress.

Sequences submitted to GenBank

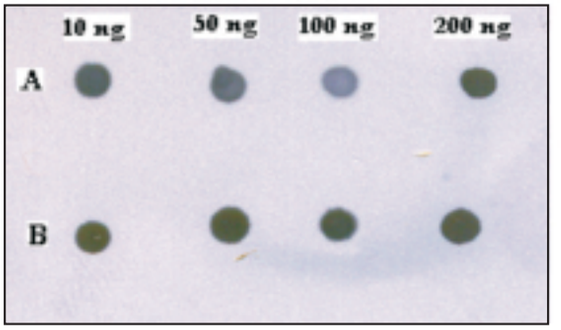
The gene sequences of partial ITS-1 region, complete 5.8S rDNA and partial ITS-2 region were submitted to GenBank and the Gene Bank Accessions Nos. DQ359737, DQ359738, DQ359739, DQ359740, DQ359741, DQ359742, DQ359743, DQ359744 and DQ35974545 for *Macrophomina* isolates KB-1, KB-2, KB-3, KB-4, KB-5, KB-6, KB-7, KB-8 and KB-9 respectively were obtained.



PCR amplification with specific Primers (MpKFI and MpKRI): Lane c1and c2 are positive controls amplified from M. phaseolina, lane 1 to12 are different test microbes. M represents 1 kb ladder.

Genetic diversity of *Macrophomina phaseolina*

The genetic diversity of soil-borne populations of *Macrophomina phaseolina* was also assessed using the RAPD primer- OPA-13. This primer was selected from among twenty 10-mer primers of arbitrary nucleotide sequences tested for amplification of genomic DNA of Mp. Primer OPA-13 produced reproducible RAPD profile with good polymorphism. 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.



Dot blot hybridization of oligonucleotide probe MpKH1 with different concentrations of rDNA amplified product from two different strains of *M. phaseolina*.

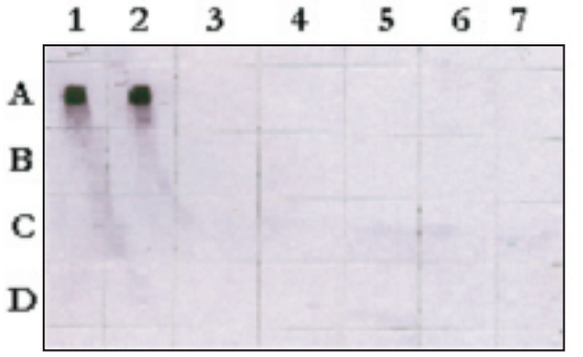


Fig. 6. Dot blot hybridization of oligonucleotide probe MpKH1 with rDNA amplification product from representative strains of different microbial groups. (A1 and A2 *M. phaseolina*; A3 to D3 represent isolates from different fungal groups; D4 and D5 are common soil bacteria; D6 and D7 are Actinomycetes)

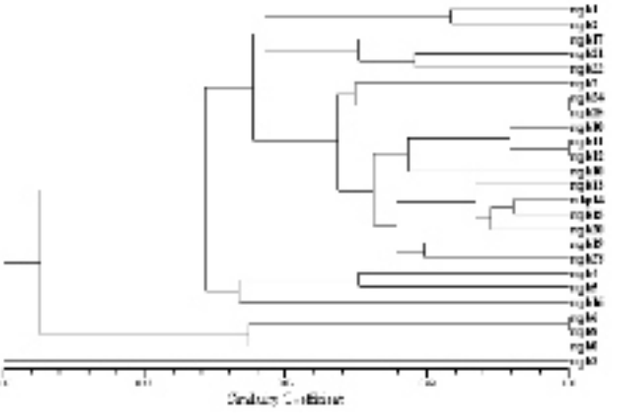
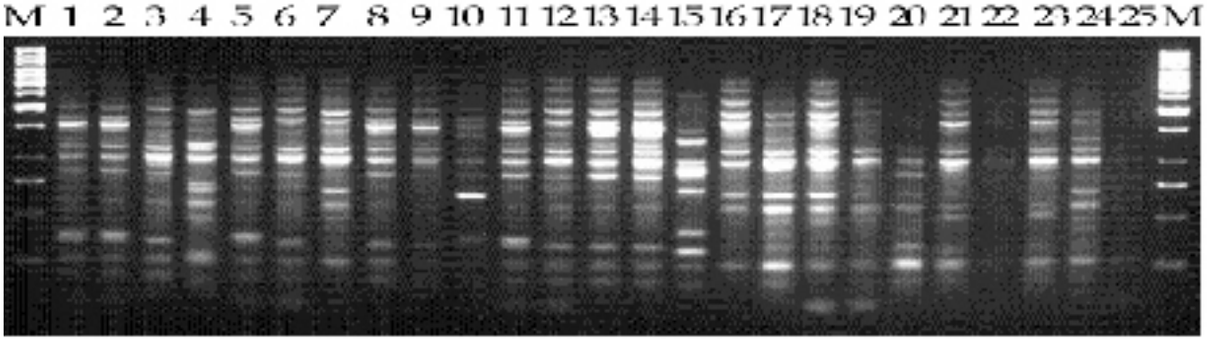


Fig: Dendrogram obtained from 25 isolates of *Macrophomina phaseolina* with UPGMA on Jaccard's coefficient. Branches are labled by isolate numbers. The line below the dendrogram represents the similarity index.



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*.

Research Achievements

Development of Sustainable Management Strategies for the Control of *Parthenium* Weed in U.P. using Biotechnological Approaches

PI : Dilip K. Arora
Co-PI : Alok Srivastava
JRF : Girijesh Patel, Rajat Pratap Singh
PA : Sudhir Upadhyay, Neeraj Soni

Objectives

1. 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.
2. Development of fermentation strategies and its optimization for mass production of the active compounds.
3. 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.
4. 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.
5. The effect of metabolites and active compounds on other plants and weeds will be studied.
6. 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.*
7. 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 U.P. infested with *Parthenium.*

Survey of *Parthenium*

Survey of the wasteland, cropfields, road sides and sides of railway track from Lucknow city to Varanasi, Gorakhpur, Gazipur, Ballia, Mau, Deoria Jaunpur, Azamgarh and some part of Bihar was made at a regular interval of 4 months since August 2005, for determining growth and spread of the weed *Parthenium hysterophorous* L. At most of the identified sites a dense continuous flora of *Parthenium* was noticed. No natural barrier or bio-agent was appeared to deter the growth and spread of the weed on either sides of the track. At moisture-laden spots, particularly *Parthenium* plants grew vigorously with well spread canopy up to 1.5 meter height. The plants completed life cycle by producing abundant foliage, flowers and seedlings. *Zygogramma* beetles were also observed on *Parthenium* at some places defoliating the *Parthenium* plants along the 700 km route, but some plant species also appeared to compete with the *Parthenium* weed such as *Cassia tora, Malvestrum, Munja, Cassia sericea* etc.

The Inoculum Dose Response of Selected Micro herbicides:

Different genera of fungal and bacterial strains were tested for its effect on growth and vigour of *Parthenium* through direct application, and application of its metabolites obtained under different nutritional and environmental conditions in batch cultures. The pathogenic and saprophytic microorganisms were isolated from the rhizosphere of *Parthenium*. The identified and purified strains were mass cultured on wheat bran at 28± 2°C; 75% moisture content for 21 days incubation period. The population of microorganisms was determined by dilution plating. Different concentrations of the cultured microorganisms were prepared by mixing the

predetermined amount of culture with sterilized talc. Germination of *Parthenium* seeds in pots containing different inoculum (ca. 10^4- 10^8 *cfu* g^{-1}) of the microbes was recorded. The seeds were sown in plastic pots (20 cm x15 cm) filled with sand mixed with microbial inoculum. The moisture level was maintained at 75% of WHC and Gt90 was recorded.

In another set of experiments all the isolates were tested for its effect on growth reduction and vigour of *Parthenium* seedlings. The talc containing different concentrations of microorganism (ca. 10^4- 10^5 *cfu* g^{-1}) were sprayed on wet foliage of *Parthenium* seedlings (2-3 week old plants) grown in experimental plots at SMM Town PG College Ballia. After 15 days the plants were again sprayed with the microorganisms. The growth (length, FW and DW) of *Parthenium,* and population of microbes on leaves were recorded up to 8 weeks after the treatment. The pots were arranged in CRD.

In third set of experiment the secondary metabolites of the microorganisms tested for growth reduction was raised in batch cultures. The broth cultures were grown on incubator shaker (50 rev min^{-1}, 28 °C) for 15-28 days. The metabolites were obtained by vacuum filtration. These metabolites were tested for its effect on growth of *Parthenium*

Formulation for Mass Production and Delivery of Microherbicides:

Locally available materials were used for mass culturing of potential microorganisms. The materials *viz:* maize meal, wheat bran, rice bran, soybean cake, sugarcane press mud etc. are used as substrate for growth of microorganisms. The substrate was mixed with sterilized coarse sand (9:1), pH was maintained by adding $CaCO_3$ and inoculated with potential microorganisms obtained earlier. After incubation of 21 days at 28± 2 °C the inoculum was homogenized by mechanical grinder and viable population was recorded by dilution plating. The suitable concentrations (ca.10^4-10^8 cfu g^{-1}) were obtained by mixing the homogenized inoculum with appropriate amount of talc.

These formulations were tested for different delivery methods under pot experiments *viz:* spraying on wet and dry surface, drenching of soil with water, and mixing in soil at seedling stage.

Survey and Isolation of Microorganisms :

Survey of different study sites as mentioned earlier was made and the soil samples and roots and shoots of *Parthenium* and other indigenous weeds were collected in sterilized polythene bags. Soil samples were collected from the different fields. Random samples from different regions of the fields was collected from the upper layer (0-15 cm) of soil with the help of a core sampler, kept in the sterilized polyethylene bags and mixed thoroughly in laboratory before plating. The rhizoplane microorganism of *Parthenium* and other weeds were isolated from the roots collected from different sites. The plants were uprooted with the help of sterilized spatula, kept in polyethylene bags and brought to the laboratory. The roots were segmented (10 mm) and diseased pieces were washed thoroughly four to five times with sterilized distilled water (SDW) surface disinfected with 1% NaOCl solution and 95% ethanol for 2-3 minutes and again washed twice in sterilized distilled water (SDW). The root segments were placed on acidified potato dextrose agar plates (APDA), pH 5.6, 3 pieces $plate^{-1}$) and incubated at 28± 2 ºC. After 4 days of incubation the developing colonies around the root piece were transferred on fresh APDA plates. The fungal isolates were isolated, purified and identified on the basis of cultural, morphological characters, growth rate on selective media, spore type and other physiological and biochemical properties. The isolates were maintained on APDA slants at 4º C, until use.

Pathogenicity Test

The pathogenecity test of isolates was done by pot screening procedure of Haware and Nene (1982). Inoculums of the pathogens were prepared in sand-maize-meal medium. The sand-maize-meal (9:1) was placed in 500 ml Erlenmeyer flasks (100 g), sterilized and inoculated. The flasks were incubated for 15 days at 28± 2 ºC. The inoculum (5% w/w) was mixed thoroughly with sterilized moist sandy soil and filled in earthen posts (10 cm x 7 cm; 300 g pot^{-1}). The surface disinfected seeds (5 seed pot-1) were sown in the pots at a depth of 4 cm. The pots were kept in greenhouse for 28 days. Soil moisture was maintained to -5 kPa by adding predetermined amount of SDW. The plants were uprooted and assayed for the disease symptoms. The isolates that showed positive results in pathogenicity test, were again tested for killing/ growth regulating activities.

Screening of metabolites

The metabolites showing reduction in germination of *Parthenium* seeds were fractioned through column

filteration and different fractions were evaluated for presence of active compounds. In preliminary screening the fractions with toxins and alkaloids showed potential to retard the growth of *Parthenium*. These fractions were being concentrated by lyophilization or through rotary vacuum evaporator and will be characterized by HPLC, NMR and IR spectroscopy for functional group analysis.

Frequency and density of *P. hysterophorus* and other weed in herb and *Cassia tora* zones

Species	Non *Cassia tora* zone				*Cassia tora* zone			
	AF	RF	AD	RD	AF	RF	AD	RD
Parthenium hysterophorus	90	19.5	87	45	20	10	2.8	9
Achyranthus aspera	30	6.5	1.5	0.80	0	0	0	0
Amaranthus viridus	30	6.5	2.2	1.1	0	0	0	0
Cyanodon dactylon	100	22	70	36	90	45	25	80
Cyprus rotudus	30	6.5	19	10	0	0	0	0
Euphorbia prostrata	10	2.2	0.8	0.4	0	0	0	0
Malvestrum coromandelianum	50	11	4.7	2.4	10	5	0.5	1.6

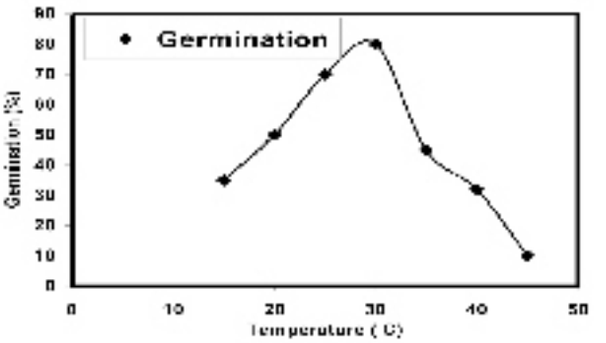
AF: Absolute frequencies RF :Relative frequency, AD: Absolute density , RD: Relative density

Density of *P. hysterophorus* at different study sites

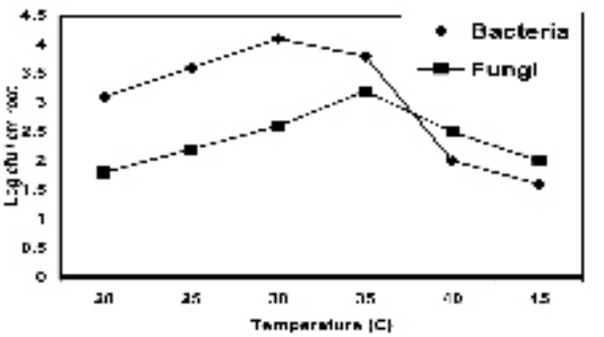
S.No.	Site	Density	Relative Density
1.	Ramana, Varanasi	51/m^2	8.86
2.	Malya, Varanasi	119/m^2	19.18
3	Dafi, Varanasi	87/m^2	17.6
4.	Phephana, Ballia	85/m^2	83
5.	Sohaon, Ballia	63/m^2	43
6.	Haldharpur, Mau	73/m^2	27.06
7.	Salahabad, Mau	87/m^2	11.50
8.	Tamsa river bank, Mau	160/m^2	19
9.	Azamgarh	79/m^2	38
10.	Kusumi Forest, Gorakhpur	36/m^2	11
11.	Nakaha, Gorakhpur	49/m^2	19

Population of different species at different study sites

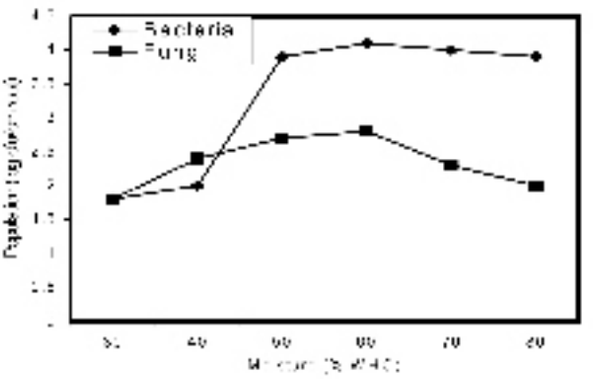
Name of the plant	September 2005				July 2006			
	*1	2	3	4	1	2	3	4
Cleome viscosa	-	+	+	+	-	-	+	-
Calotropis	+	++	-	++	-	+	-	+
Clerodendron sp.	-	-	+	+	-	-	-	+
Cassia	+	+	+	+	+	+	-	+
Sida sp.	++	+	++	+	+	-	+	+
Malvestrum	+	++	+	-	-	+	-	+
Croton	++	++	+	+	+	+	-	-
Eclypha	+	+	-	-	+	-	-	-
Amaranthes	+	-	+	+	+	-	-	+
Solanum xanthocarpum	-	-	+	+	-	-	-	+
Argemone	+	-	-	-	-	-	-	-
Adhatoda vasaka	+	-	+	+	+	-	+	+
Hyptis sp.	-	+	++	+	-	++	+	+
Parthenium	++	+	++	++	+++	+++	+++	+++



Germination of *Parthenium* seeds at different temperature



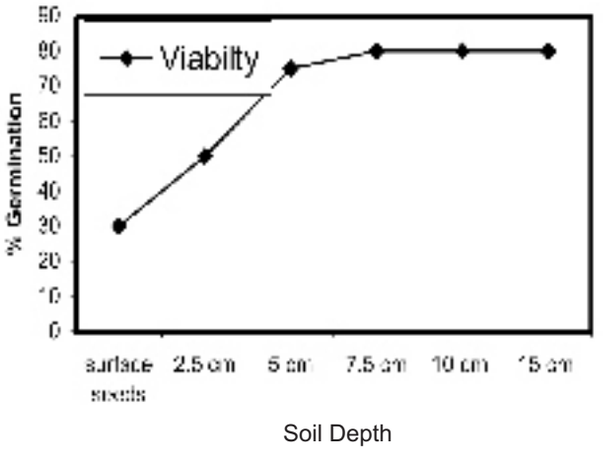
The population of Fungi and bacteria on *Parthenium* root at different temperature



The population of Fungi and bacteria on *Parthenium* root at different moisture.

Composition of the microbial population detected on *Parthenium* roots

Microorganisms	Cfu (%) of total found on roots
A. Bacteria	
Alcaligenes spp.	51 (15.1)
Bacillus spp.	40 (11.2)
Citrobacter spp.	62 (18.8)
Erwinia herbicola.	10 (3.0)
Flavobacterium spp.	21 (5.6)
Pseudomonas fluorescens	67 (19)
P. putida	50 (15)
Nonfluorescent Pseudomonads	70 (19.5)
Serratia spp.	5 (1.5)
Xanthomonas spp.	15 (3)
B. Fungi	
Aspergillus spp.	12 (6)
Epicoccm spp.	7 (3.5)
Cladosporium spp.	5 (2.5)
Fusarium spp.	22 (11)
Humicola spp.	14 (7)
Curvularia spp.	26 (13)
Colletotrichum spp.	19(9.5)
Penicillium spp.	46 (23)
Trichoderma spp.	54 (27)
Rhizoctonia spp.	41 (20.5)
Myrothecium spp	30 (15)
Sclerotium, spp	28 (14)
Phoma spp	13 (6.5)
Helminthosporium spp.	19 (9.5)
Chaetomium spp.	24 (12)
Macrophomina phaseolina	12 (6)
Alternaria spp.	8 (4)
Ascochyta spp.	7 (3.5)

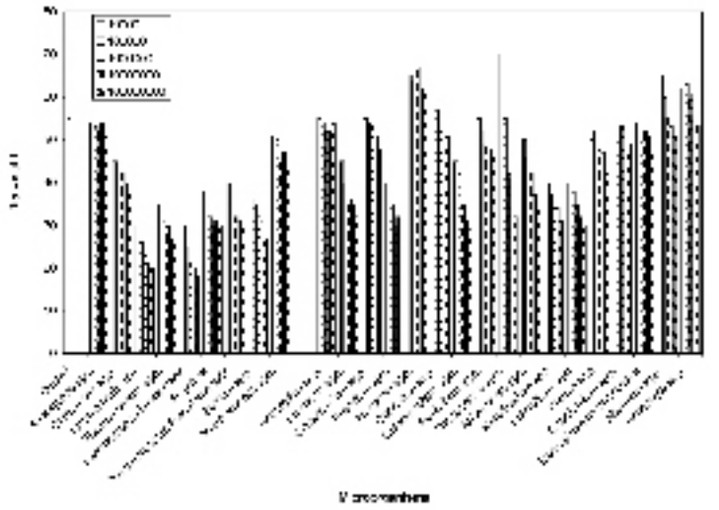


Effect of different concentrations of microorganisms on germination of *Parthenium* seed.

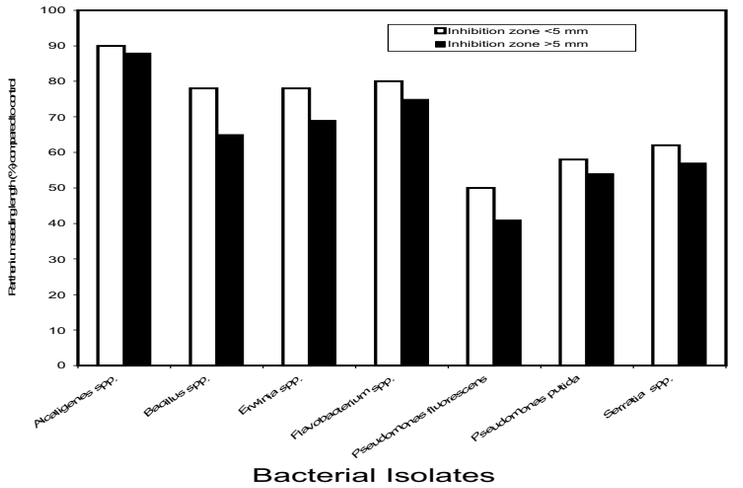
Microorganisms	*Germination of *Parthenium* at concentration				
	10^4	10^5	10^6	10^7	10^8
Control 90	-	-	-	-	
A. Bacteria					
Alcaligenes spp.	90	89	90	87	87
Bacillus spp.	72	68	68	65	63
Citrobacter spp.	85	83	83	80	80
Erwinia herbicola.	75	65	65	60	58
Flavobacterium sp.	88	83	83	83	80
Pseudomonas fluorescens	65	60	55	55	48
P. putida	70	70	68	65	65
Nonfluorescent Pseudomonads	70	65	60	60	58
Serratia spp.	90	90	90	88	88
Xanthomonas spp.	87	84	84	80	76
B. Fungi					
Aspergillus spp.	90	88	86	86	83
Epicoccm spp.	78	73	71	65	65
Cladosporium spp.	76	76	75	76	70
Fusarium spp.	60	56	56	50	48
Humicola spp.	74	73	73	73	71
Curvularia spp.	86	83	83	80	81
Colletotrichum sp.	70	65	60	56	52
Penicillium spp.	87	87	84	81	79
Trichoderma spp.	86	75	75	60	55
Rhizoctonia spp.	65	60	58	57	51
Myrothecium spp	61	56	56	51	49
Sclerotium, spp	70	68	68	64	64
Phoma spp	86	84	84	83	81
Chaetomium spp.	86	84	83	81	76
Macrophomina phaseolina	75	71	70	66	62
Alternaria spp.	78	76	75	72	68
Ascochyta spp.	87	83	83	83	80
Un-identified *Mycelium*	87	82	82	81	80

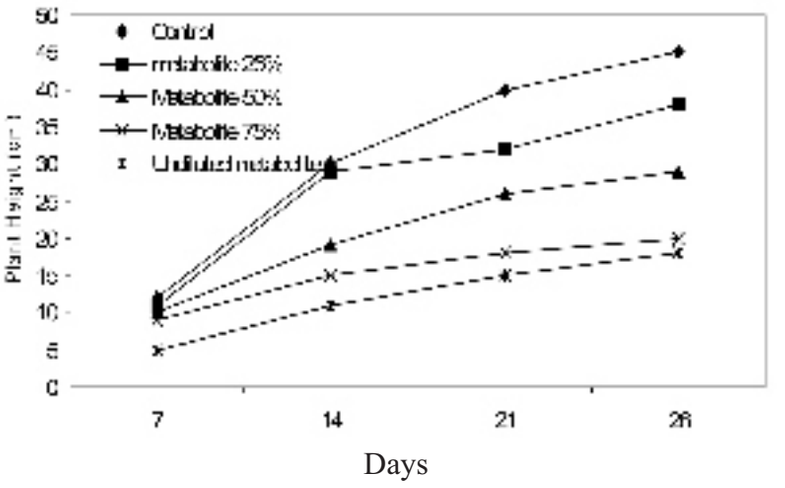
Population of selected rhizobacteria from *Parthenium* seedling inhibiting *E. coli* and significantly (P= 0.05) inhibiting *Parthenium* growth

Bacteria	Total Number tested	Inhibition (%)	
		E. coli	*Parthenium*
Alcaligenes spp.	15	-	-
Bacillus spp.	13	25	7
Erwinia spp.	19	21	10
Flavobacterium	6	40	-
Pseudomonas Fluorescens	76	65	55
Pseudomonas Putida	35	73	24
Serratia spp.	16	55	12

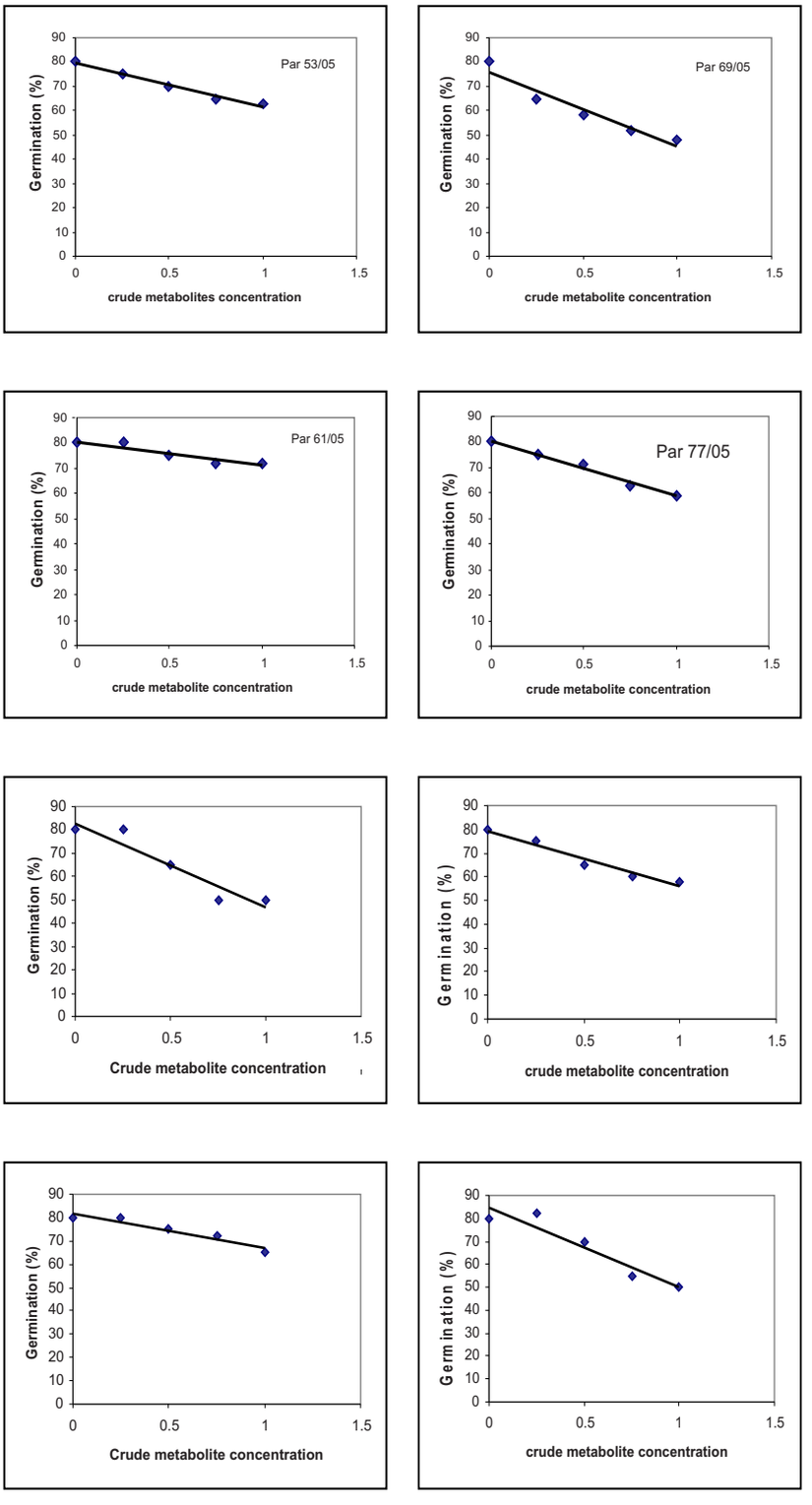


Effect of different concentrations of microorganisms on growth of *Parthenium*

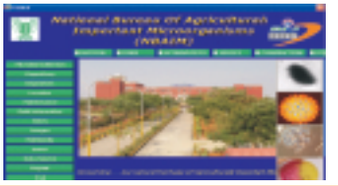




Effect of metabolite of isolate *Par 49-05* on growth.



Relationship between concentration of metabolites and germination of *Parthenium.*



Research Achievements

Collection and Digitization of Agriculturally Important Microorganisms and their DNA Fingerprinting,

PI : D. K. Arora
SRF : Mukesh Kumar Yadav, B. Kishore Babu

Software was developed at NBAIM for the digitization of microorganisms. It has been designated as 'MicroNBAIM'. The software is in Visual Basic with DOTNET as base.

Some of the features are:

1. One can access information on any microorganism available at NBAIM by clicking on the particular group to which it belongs, that is, Bacteria, Fungi, Actinomycetes or Cyanobacteria.
2. The databank has information on items like the geographical location from where isolated, name of the donor (person or Institute), name of the depositor, cultural details of microorganism, the form in which it is preserved and many more.
3. It has 200 fields with report generation component in PDF format.
4. Micro NBAIM includes information's about culture collection, field information, notes, images, sounds, videos, as well as associated information about Collection Management, Transactions such as Loans, Accessions exchanges, etc. MicroNBAIM includes an embedded reporting tool. There are 10 modules representing different fields for microbial digitization.

Modules for Microbial Digitization

1. Culture Collection Module:

This module contains information of Culture Collection details such as Culture Collection Name, Affiliation, Authority, Address, Phone, fax, E-mail etc.

2. Repository:

This module contains information about repository such as Collection Number, Accession Number, Other Accession Number, Scientific Name, Date of Collection, Date of Accession, and Date of Isolation, Type, Source of Isolation, Nature, Importance, GMO Status, Methods of Identification, Depositors, etc with search option on the basis of Scientific Name.

3. Depositors:

This includes Scientific Name, Depositors Name, Address, Phone, Fax, E-mail ID, and Country and Affiliated organization.

4. Maintenance:

It includes scientific name of Microorganism, Isolation media, Specific media, Maintenance media, temperature for Growth, Date of culture renewal, Date of subculture etc.

5. Field Information:

This module provides information of field from where sample was collection for microbial isolation such as locality, temperature, rainfall, ecological note, district etc.

6. Notes:

This modules includes importance of microorganism, Bio-safety and Risk category, Field information such as rainfall, temperature etc.

7. Images:

This module includes image addition, deletion and search picture for particular microorganism.

8. Multimedia:

This module includes Audio, Video and animation associated with microorganism. Includes institute name, contact of person, address, phone, fax, obtained date and search etc.

9. Donor:

Includes institute name, contact person, address, phone, fax, obtained date and search etc.

10. Data Source:

It includes information about available literature such as publication, Author, Year, Vol, Page, Publisher, Key word, Abstract, and Journal etc.

MicroNBAIM has a report design and printing subsystem, Reports are generated in pdf Format. Reports contain information of Collection Number, Accession Number. Scientific Name include genes, Species, Biotype, date of Isolation, date of Accession, date of Collection, Nature, Importance, Locality, Type, Methods of Identification etc.

At NBAIM 550 microorganism have been digitized using MicroNBAIM Software.

Fungi- 400
Bacteria- 150

Development of Molecular Markers for the Identification and Characterization of *Fusarium* groups of Plant Pathogenic Fungi

PI : D. K. Arora
SRF : Sandeep Nair

PCR amplification of 30 strains of *Fusarium udum* for ITS region was accomplished. PCR-based assays were developed to differentiate internal transcribed spacers (ITS) sequence diversity present in *Fusarium.* The ITS primers generated a single amplicon of about 500 base pairs. No substantial polymorphic pattern among the isolates was found by using ITS, although minor variations in the size of ITS was observed among the *Fusarium* species. The results revealed that there is limited diversity among the isolates obtained from various geographical locations. Restriction digestion of ITS with nine different restriction endonucleases (tetra-cutters) revealed that only two enzymes (*Hpa* II and *Rsa*I) could differentiate *Fusarium udum* from *Fusarium oxysporum* fsp. *lentis* and *F. oxysporum* fsp. *Ciceri.*

Another approach which was used for molecular characterization was amplification of genomic DNA using the BOXA1R primer. Genomic DNA of isolates belonging to *Fusarium udum, Fusarium oxysporum* f.sp *ciceri, Fusarium oxysporum* f.sp *lentis* was used. Genetic profiles obtained from PCR amplification showed distinction between the three formae specialis. The profiles generated by the BOXA1R primer are more robust and reproducible compared to those generated by the RAPD primers. The PCR amplification yielded far less artifacts compared to the RAPD primers. In view of these factors, amplification with BOXA1R could turn out to be potential molecular markers. Further investigation is required in this direction to corroborate the results obtained.

RAPD profiling of *Fusarium* spp. isolates was carried out using three 10-mer primers OPB-04, OPB-08 and OPE-12. *Fusarium oxysporum* f.sp *ciceri, Fusarium* f.sp. *lentis* and *Fuasrium udum* exhibited polymorphic banding pattern upon PCR amplification with primer OPB-08, enabling their grouping into different subgroups (Fig 4, 5). Among the 20 fungal isolates, three main groups were obtained based on banding patterns and sizes of PCR-amplified products.

All the isolates of *Fusarium* grouped themselves into the following main groups: group 1 (Foc1, Foc2, Foc6, Foc 7, Foc9, Foc 18, Foc 25; Chickpea), group 2 (F1, F22, F33, F34, F54, F6B, NT7) and group 3 Fol2, Fol3, Fol5, Fol6, Fol7, Fol8 exhibiting 55-78%, 51-86%, 55% genetic similarity, respectively.

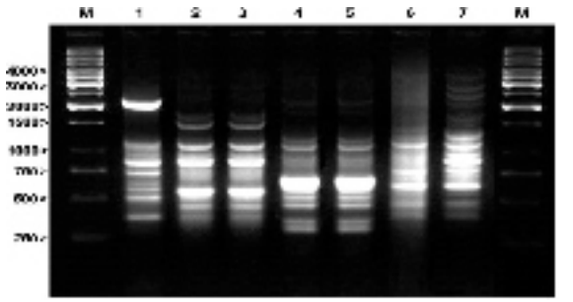
Three main groups were identified ; Group I consisting of *F. udum* isolates, Group II having *F.oxysporum* f.sp. *ciceri* strains and Group III with *F. oxysporum* f.sp. *lentis s*trains. Group I showed a similarity of 57 % to Groups II and III. Group II had a similarity of 65% with Group III.

The RAPD profile with primer OPB-04 also grouped 20 fungal isolates into three main groups. All the isolates of *Fusarium* grouped themselves into the following main groups: group 1 (Foc1, Foc2, Foc6, Foc 7, Foc9, Foc 18, Foc 25; Chickpea), group 2 (F1, F22, F33, F34, F54, F6B, NT7) and group 3 *(*Fol2 Fol3, Fol5, Fol6, Fol7, Fol8) exhibiting 55-78%, 51-86%, 55% genetic similarity, respectively.

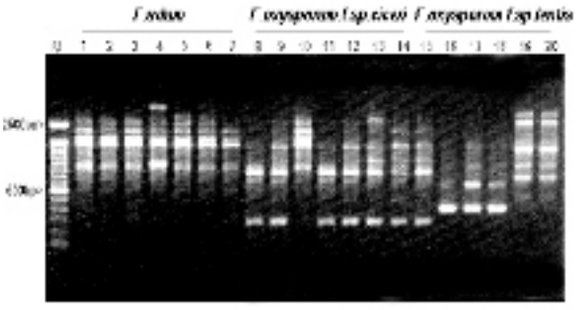
In second main group , F22 and F33 form a sub-group showing 81 % similarity with F 6B, while all these three isolates where 67% similar to F1. In the other sub-group F 34 and F 54 are 82 % similar while both of them are 75 % similar to NT 7. Both these sub-groups exhibit a similarity of 51 %.

In the third main group, Fol 3,Fol 5, Fol 6, Fol 7 and Fol 8 form a sub-group and are 50 % similar to Fol 2.

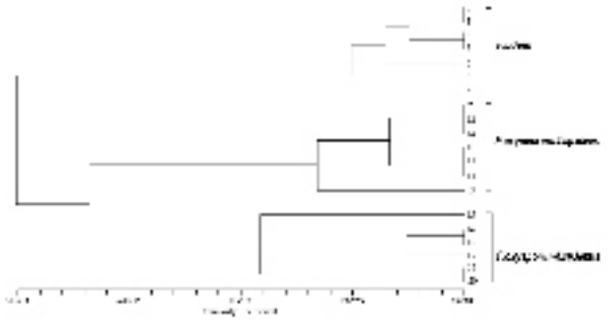
Among the three main groups, group 1 and group 3, showed a genetic similarity of 30 %. Group2 showed a similarity of 19 % with Group 1 and Group 3. Within the first main group , Foc1, Foc2, Foc6, , Foc9, Foc 18 formed a sub-group and were 80% similar to Foc 25, while they showed 53 % similarity with Foc 7 UPGMA analysis of the polymorphism generated by use of OPE-12 primer enabled the grouping of *Fusarium oxysporum* f.sp *ciceri, Fusarium oxysporum* f.sp. *lentis* and *Fuasrium udum* based on their similarity. All the isolates of *Fusarium* grouped themselves into the following main groups: group 1 (Foc1, Foc2, Foc6,



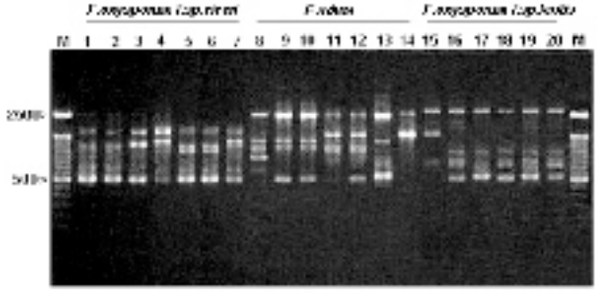
PCR fingerprints obtained with the primer BOXA1R for selected isolates of *Fusarium* udum(Lanes 1 to 3), *Fusarium oxysporum* f.sp.*ciceri* (Lane 4 and 5) and *Fusarium oxysporum* f.sp.*lentis* (Lane 6 and 7) isolated from various hosts namely, *Pigeon pea, Chick pea* and *Lentil* respectively.(M=Marker, 1=F1, 2=F22, 3= F33, ,4=Foc1, 5=Foc2, 6=Fol 2, 7=Fol 3).



RAPD-PCR fingerprints obtained with the primer OPB-08 (for selected isolates of *Fusarium udum, Fusarium oxysporum* f.sp.*ciceri* and *Fusarium oxysporum* f.sp.*lentis* isolated from various hosts namely, Pigeon pea, Chick pea and Lentil respectively). M=Marker, , 1=F1, 2=F22, 3= F33, 4=F34, 5=F54, 6=F6B, 7=NT7, 8=Foc1, 9=Foc2, 10=Foc 6, 11=Foc 7, 12=Foc 9, 13=Foc 18, 14=Foc 25, 15=Fol 2, 16=Fol 3, 17=Fol 5, 18=Fol 6, 19=Fol 7, 20=Fol 8



The dendrogram of RAPD profile with primer OPB-08 was constructed by using UPGMA, for showing the genetic relatedness among the different isolates of *Fusarium udum, Fusarium oxysporum f.sp.ciceri* and *Fusarium oxysporum* f.sp.*lentis*



RAPD of selected isolates of *Fusarium oxysporum* f.sp.*ciceri*, *Fusarium udum* and *Fusarium oxysporum* f.sp. *lentis* ; M=Marker, 1=Foc1, 2=Foc2, 3=Foc6, 4=Foc 7, 5=Foc9, 6=Foc 18, 7=Foc 25, 8=F1, 9=F22, 10= F33, 11=F34, 12=F54, 13=F6B, 14=NT7, 15=Fol2, 16=Fol3, 17=Fol5, 18=Fol6, 19=Fol7, 20=Fol8

Foc 7, Foc9, Foc 18, Foc 25; Chickpea), group 2 (F1, F22, F33, F34, F54, F6B, NT7) and group 3 (Fol2, Fol3, Fol5, Fol6, Fol7, Fol8). Group I is 60% similar to Group II and Group III. The dendrogram exhibits a similarity of 71% between Group II and Group III.

It is clear from the RAPD analysis that *F. oxysporum* f.sp. *ciceri* and *F. oxysporum* f.sp. *lentis* are more closely related to each other than *F. udum.* The three markers were selected on the basis of the polymorphism they generated and the reproducibility of their banding patterns

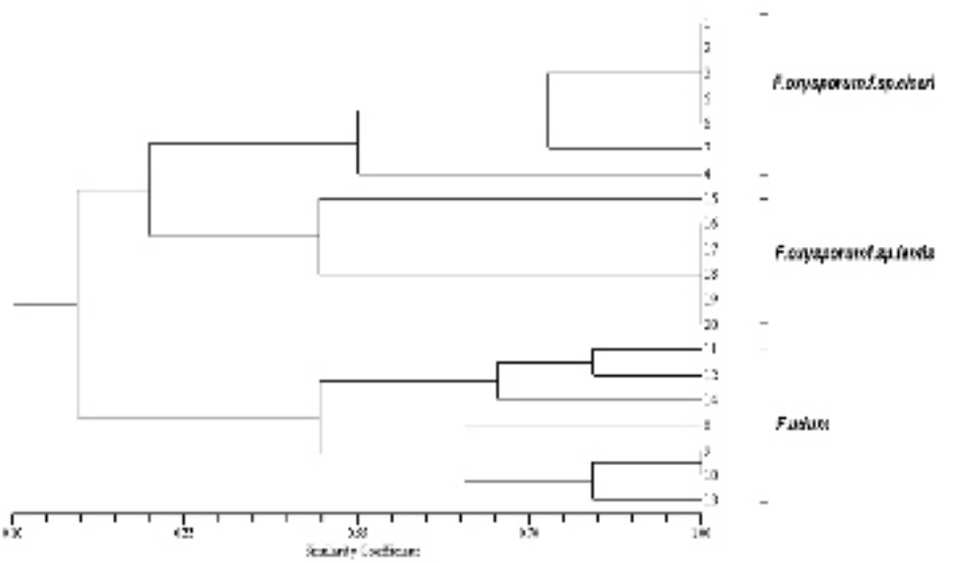
Twenty eight isolates of pathogenic *Fusarium oxysporum* f. sp. *carthami* causing wilt in safflower were characterized by ITS-RFLP for genetic diversity. ITS-PCR-RFLP analysis could group all 28 isolates into 6 clusters. RAPD markers were also used for detecting intra-specific polymorphism among pathogenic *Fusarium oxysporum* f.sp. *carthami.* RAPD patterns with primer OPB-8 showed variations within the species and could group the strains into six major clusters.

Cultural and molecular characterization of *Fusarium udum* vis-a vis other species of *Fusarium*

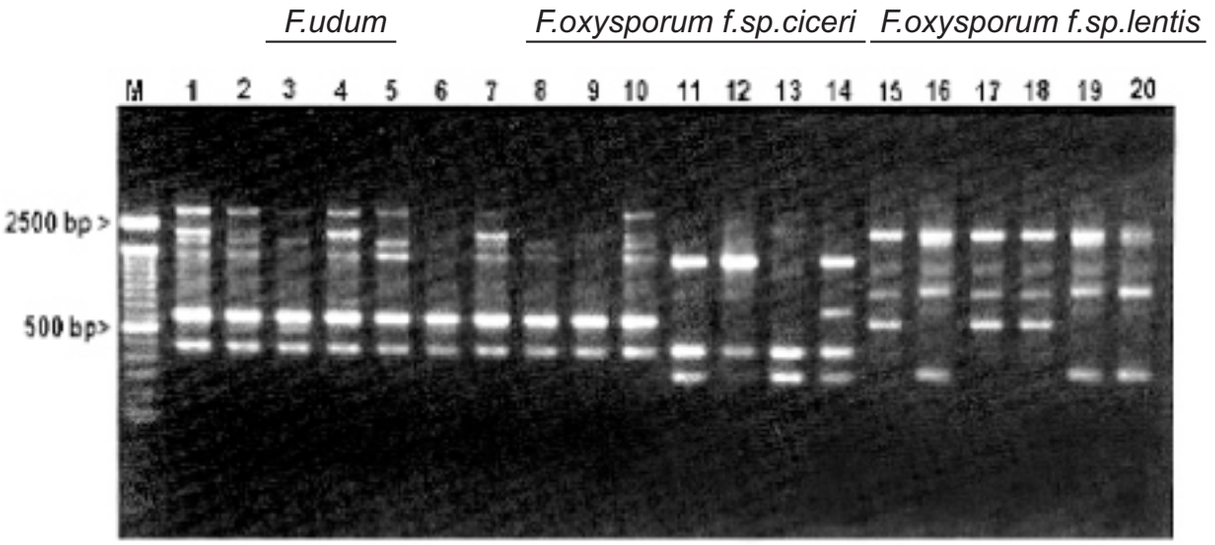
Isolates of *Fusarium udum* deposited by various workers and isolated from different geographical locations were characterized for cultural and molecular characters so as to study the extent of diversity in the country. The list of cultures and their geographical location is given. Lot of variations were observed with regards to pigmentation and number of septa in each spore among the isolates of *F. Udum*.

RAPD analysis

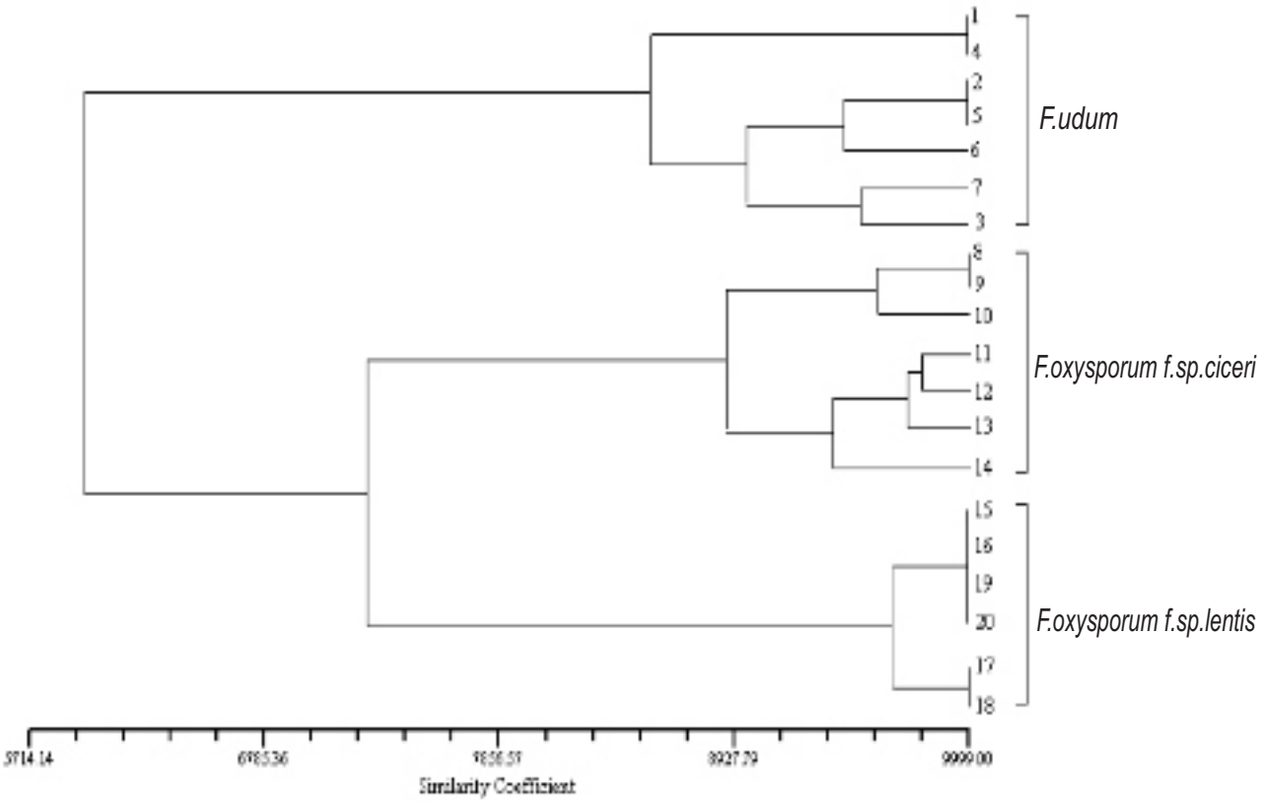
Isolates of *F. udum* along with 16 other isolates of different species of *Fusarium* were amplified using random primers. Initially four primers were tested , that is, OPE-12, OPE-13, OPE-15 and OPE 16. The profiles produced by OPE-12 and OPE-13 were not able to differentiate between *F.udum* and *F. oxysporum* soecies, similarly OPE-16 could not distinguish between *F. oxysporum* fsp. *ciceri* and *F. oxysporum* f. sp. *glydeoli.* Hence OPE-15 that gave the best results and maximum number of polymorphic bands was used to amplify all the 45 isolates. DNA bands ranging from 100 bp to 2000 bp were amplified using OPE-15. The results showed that isolates of *F. udum* from the same location have different RAPD profiles. Conversely isolates from two different locations had identical RAPD profiles. The RAPD profiles of other species of *Fusarium* tested were distinct from that of *F. udum*. Among the isolates of all the *Fusarium* species analysed, there was a similarity of 53%. At this level, two clusters diverged. The isolates of *F. udum* were found interspersed among both the clusters.



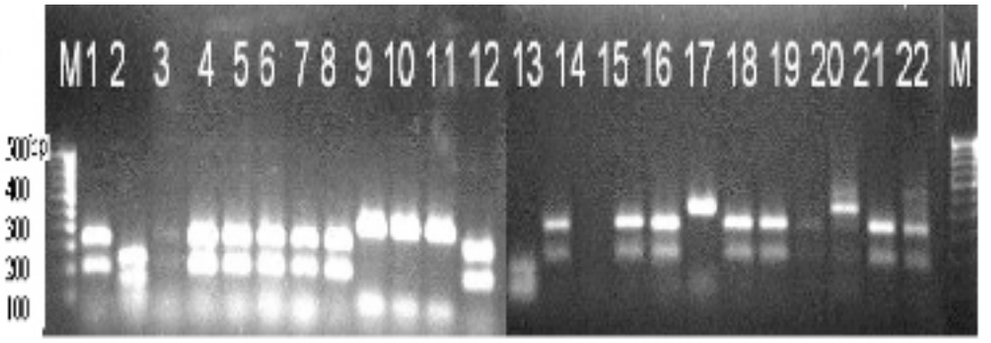
The dendrogram of RAPD profile with primer OPB-04 was constructed by using UPGMA, for showing the genetic relatedness among the different isolates of *Fusarium udum, Fusarium oxysporum* f.sp.*ciceri* and *Fusarium oxysporum* f.sp.*lentis*



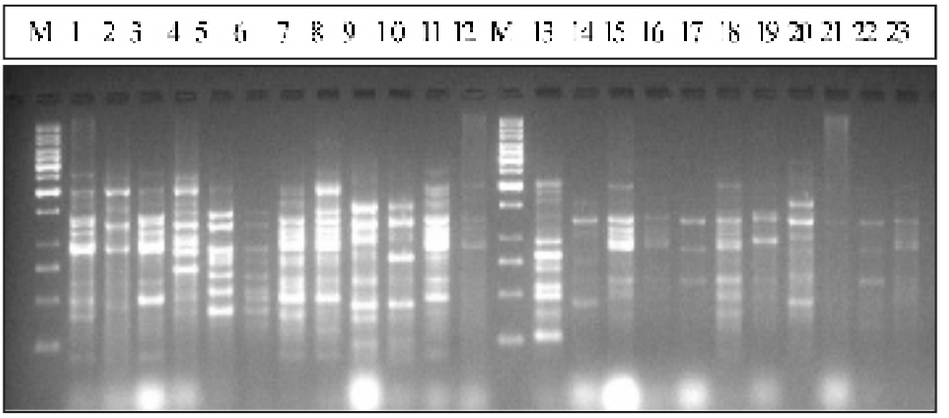
RAPD-PCR fingerprints obtained with the primer OPE-12 (for selected isolates of *Fusarium udum*, *Fusarium oxysporum* f.sp.*ciceri* and *Fusarium oxysporum* f.sp.*lentis* isolated from various hosts namely, Pigeon pea, Chick pea and Lentil respectively). M=Marker, , 1=F1, 2=F22, 3= F33, 4=F34, 5=F54, 6=F6B, 7=NT7, 8=Foc1, 9=Foc2, 10=Foc 6, 11=Foc 7, 12=Foc 9, 13=Foc 18, 14=Foc 25, 15=Fol 2, 16=Fol 3, 17=Fol 5, 18=Fol 6, 19=Fol 7, 20=Fol 8



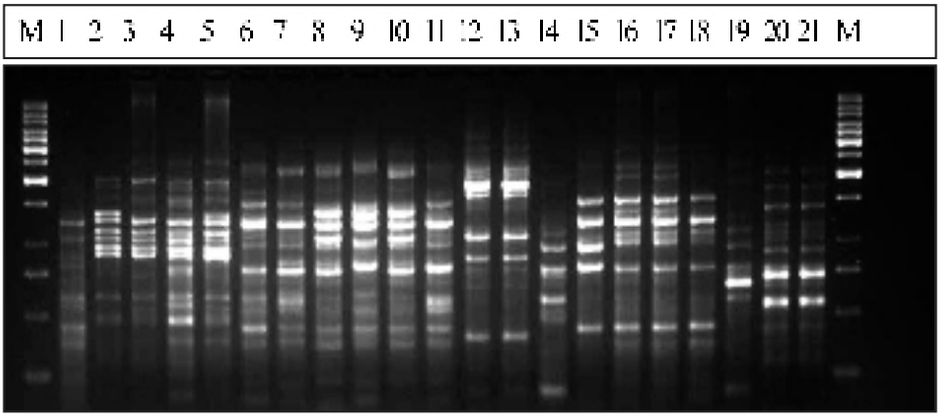
The dendrogram of RAPD profile with primer OPE-12 was constructed by using UPGMA, for showing the genetic relatedness among the different isolates of *Fusarium*



ITS-PCR-RFLP analysis with restriction endonuclease could group 28 strains of *Fusarium oxysporum* f. sp. *carthami* into 6 groups. Lane M, Molecular marker; Lanes1-22, strains of *Fusarium oxysporum* f. sp. *carthami*



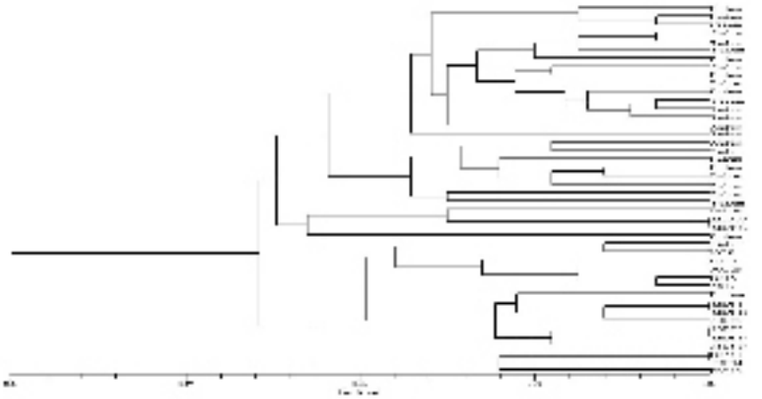
RAPD profiles of *F. udum* isolates using random primer OPE-15. M : Molecular marker - 1 KB ladder, Lanes 1-23 *F.udum* isolates



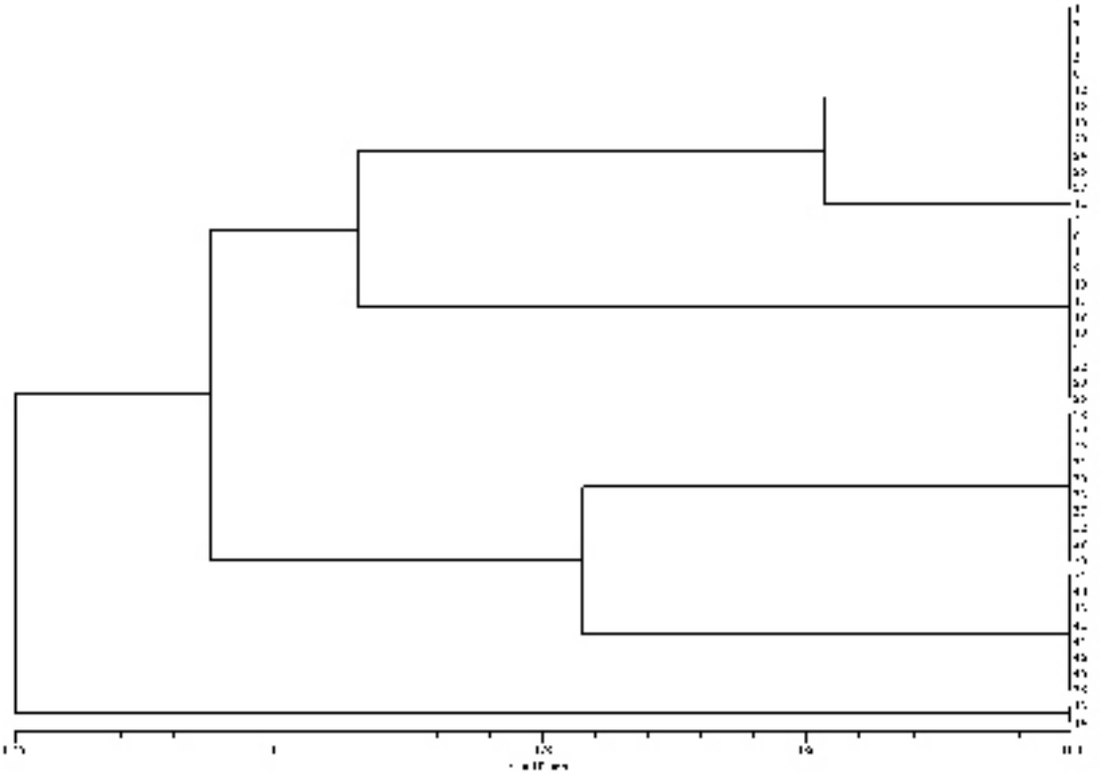
RAPD profiles of *Fusraium* species isolates using random primer OPE-15. M : Molecular marker - 1 KB ladder, Lanes 1-5 *F.udum* isolates, Lanes 6-11: *F. oxysporum* f. sp. *ciceri,* Lanes 12-13: *F. oxysporum* f. sp. *glydeoli.* Lanes 14-19 *F. solani,* Lanes 20-21 *F. pallidioroseum*

Geographical location and NBAIM Accession number for isolates of *Fusarium udum* selected for the study.

S.No	Name of the species	Geographical Location	State	NBAIM Accession No.
1	*Fusarium udum*	Rangareddy	Andhra Pradesh	259
2	*Fusarium udum*	Rangareddy	Andhra Pradesh	261
3	*Fusarium udum*	Khammam	Andhra Pradesh	175
4	*Fusarium udum*	Khammam	Andhra Pradesh	177
5	*Fusarium udum*	Guntur	Andhra Pradesh	182
6	*Fusarium udum*	Guntur	Andhra Pradesh	187
7	*Fusarium udum*	Warangal	Andhra Pradesh	262
8	*Fusarium udum*	Gulbarga	Karnataka	118
9	*Fusarium udum*	Gulbarga	Karnataka	119
10	*Fusarium udum*	Bidar	Karnataka	151
11	*Fusarium udum*	Bidar	Karnataka	153
12	*Fusarium udum*	Kolar	Karnataka	222
13	*Fusarium udum*	Kolar	Karnataka	223
14	*Fusarium udum*	Tumkur	Karnataka	251
15	*Fusarium udum*	Tumkur	Karnataka	252
16	*Fusarium udum*	Hassan	Karnataka	232
17	*Fusarium udum*	Hassan	Karnataka	233
18	*Fusarium udum*	Bangalore rural	Karnataka	157
19	*Fusarium udum*	Bangalore rural	Karnataka	158
20	*Fusarium udum*	Bangalore	Karnataka	228
21	*Fusarium udum*	Bangalore	Karnataka	229
22	*Fusarium udum*	Mandya	Karnataka	230
23	*Fusarium udum*	Dharwad	Karnataka	168
24	*Fusarium udum*	Krishnagiri	Tamilnadu	206
25	*Fusarium udum*	Pudukotti	Tamilnadu	204
26	*Fusarium udum*	Pudukotti	Tamilnadu	208
27	*Fusarium udum*	Thiruchi	Tamilnadu	207
28	*Fusarium udum*	Kanpur	Uttar Pradesh	29
29	*Fusarium udum*	Kanpur	Uttar Pradesh	F-40
30	*Fusarium udum*	Kanpur	Uttar Pradesh	F-53



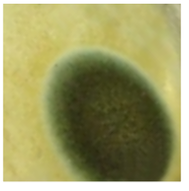
Dendrogram showing relationship between *F. udum* and other species of *Fusarium* based on RAPD profile with primer OPE-15.



Dendrogram showing relationship among *F. udum* isolates and other *Fusarium* species based on RFLP analysis of ribosomal ITS with restriction endonuclease Hae III.

28S rDNA-RFLP Analysis

The 28S rDNA of 10 pathogenic *F. udum* and 10 other strains of *Fusarium* species were amplified using the primers ITS-1 and P3. All the strains produced a single amplified product ranging from 1100 to 1200 bp. The amplified product was digested with a tetracutter restriction endonuclease *Hae* III. Among the isolates of *F. udum*, variations were found and the ten representative strains showed three distinct patterns. However RFLP analysis of 28S rDNA with *Hae* III alone could not distinguish *F. udum* distinctly from other species. The polymorphic fragments were scored and a dendrogram was constructed showing similarity among the isolates. Isolates of *F. udum* showed 58% similarity with isolates of other species. All *F. udum* isolates formed two clusters at a similarity coeffecient of 75%. The results indicated that 28S rDNA is highly conserved and could not distinguish isolates of the same species.



Project on Molecular and Functional Diversity of Microorganisms Isolated from Extreme Environments

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

Major objectives

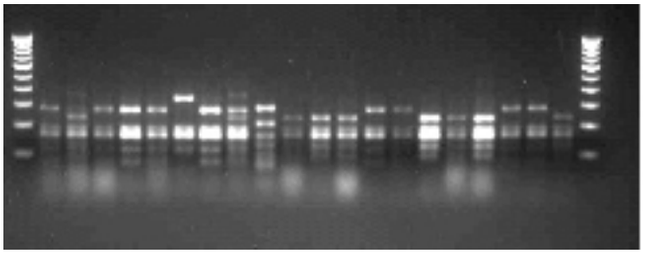
1. To isolate, identify and characterize microbial strains from extreme environments (psychrophiles, thermophiles, halophiles and osmophiles).
2. Molecular fingerprinting of the isolates.
3. Characterization of novel microorganisms for their utilization in biodegradation of agriultural residues, bioremediation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

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.

Progress of Research

The soil samples were collected from the rhizosphere of wheat growing at two locations at NBAIM campus, Mau and Sidhauna, Varanasi. Bacteria were isolated from the soil samples employing both specific (King's B, Jensen's N free) and non-specific (Nutrient Agar, Soil extract agar) media.



RFLP analysis of PCR amplified 28 S r DNA with restriction endonuclease Hae III. M: Molecular marker - 1kb ladder, Lane 1-10 *F. udum*, Lane 11,12,14, 16 and 20 *F. oxysporum ciceri,* Lane13 *F. oxysporum glydeoli*, Lane 15 and 17 *F. solani*, Lane 18 and 19 *F. pallidioroseum*

A total of 60 isolates were obtained, 30 from NBAIM soils and 30 from Sidhauna, Varanasi. Isolates were selected based on variation in the cultural characterisitics. Among the media employed, maximum diversity in morphophytes could be obtained on Nutient agar medium.

Intrinsic resistance to salt: All the isolates obtained were subjected to NaCl salt stress by amending various concentrations of NaCl (2%, 4%, 6%l, 8%, 10%, 12%) in Nutrient Agar medium. Control plates were also maintained with 0.05% NaCl. The plates were incubated for 48 hour at 30° C and the growth on NaCl amended plates were compared with control plates. Of the 30 isolates obtained from NBAIM campus fields, 10 showed intrinsic resistance to 10% NaCl salt and only one isolate could grow at 12 % NaCl. Amongst the isolates from Sidhauna, Varanasi, none could grow at a concentration of 10% or above. Only one isolate out of 30 screened could grow at 8% NaCl stress. The results revealed diversity among the bacteria in two soils with respect to intrinsic resistance to salt. These salt tolerant bacteria will be further analysed for the accumulation of various osmolytes.

Isolation and characterization of salt tolerant Arthrobacter species from saline soils

Soil samples were collected from the rhizosphere of wheat growing in salt affected soils of Mau to isolate Arthrobacter spp. Appropriate dilutions were plated on Trypticase soy agar medium amended with 2% NaCl and methyl red. A total of 32 bacterial colonies were isolated and observed under the microscope for rod-coccus cycle. Eleven isolates that showed rod-coccus cycle, a typical character of Arthrobacter were selected and screened for salt tolerance. Of the 11 isolates, four could tolerate 10% NaCl concentration. They were further screened for temperature tolerance and 6 isolates- G1,G2, G3, G7, G8 and G10 were able to grow at an incubation temperature of 48^{0}C.

Intrinsic resistance of *Arthrobacter* isolates to salt stress.

Isolate Number	% NaCl 2	6	8	10
G1	+	+	+	+
G2	+	+	-	-
G3	+	+	+	+
G4	+	+	-	-
G5	+	+	+	-
G6	+	+	+	+
G7	+	+	-	-
G8	+	+	-	-
G9	+	+	+	-
G10	+	+	+	+
G11	+	+	+	+

Number of isolates obtained on different media from the soil samples collected from the rhizosphere of wheat growing at NBAIM campus, Mau and sidhauna, Varanasi.

Media	Number of isolates obtained	
	NBAIM	Sidhauna, Varanasi
Nutrient Agar	14	18
King's B	5	3
Jenson N Free	9	8
Soil extract gar	3	1

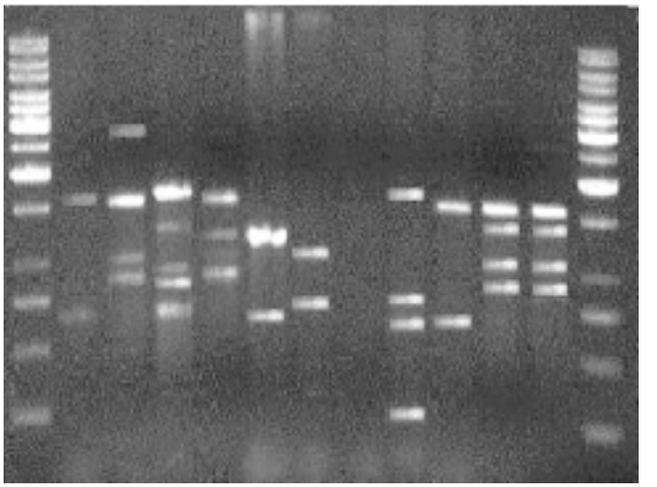
Enzyme production: All the isolates were tested for their ability to produce amylases, catalases and proteases. While all the isolates were able to produce catalase only five isolates showed production of amylases and four isolates showed caseinase activity.

Screening of *Artrobacter* isolates for enzyme activities

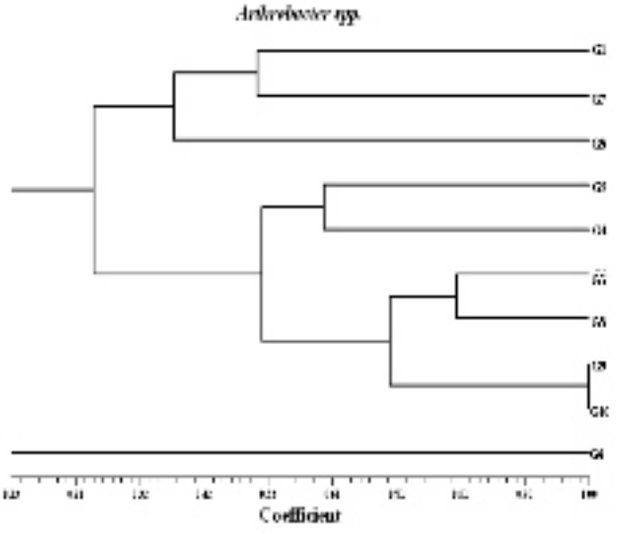
Isolate Number	Amylase activity	Catalase activity	Caseinase activity
G1	-	+	-
G2	+	+	-
G3	-	+	+
G4	-	+	-
G5	+	+	+
G6	+	+	+
G7	+	+	-
G8	-	+	-
G9	+	+	+
G10	-	+	-
G11	+	+	-

RAPD analysis of *Arthrobacter* strains

Isolates of *Arthrobacter* were subjected to RAPD analysis to look for the diversity among them. One random primer with sequence 5' GTCCACACGG 3' was initially used to develop RAPD profiles. Two clusters diverged at 29% similarity level whereas isolate G6 formed an off shoot. The major cluster had 6 isolates while the minor cluster had 3 isolates. Among all the isolates, G9 and G10 showed 100% homology among themselves.



RAPD profile of *Arthrobacter* isolates using random primer with sequence 5' GTCCACACGG 3'



Dendrogram showing relationship among *Arthrobacter* isolates

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

PI : A. K. Saxena
CO-PI : Rajeev Kaushik
Anurag Chaurasia

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.

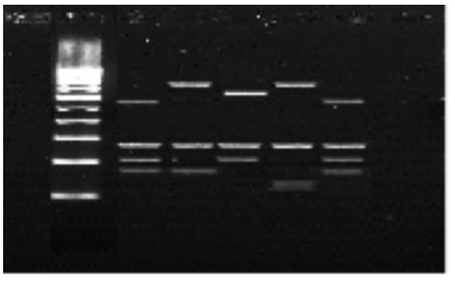
Rationale

The genus *Bacillus* is a large, heterogeneous group of Gram positive, aerobic, endospore forming, rod shaped bacteria. Since endospore formation is a universal feature of these bacteria, spore morphology has traditionally been given considerable weightage in their classification and identification. 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.* In India no systematic study has been carried out to look for the distribution of different *Bacillus* and *Bacillus* derived genera.

Fluorescent pseudomonads are an ecologically important group of soil bacteria that are well accepted as plant growth promoting rhizobacteria (PGPR). Growth promotion occurs as a result of direct stimulation of the plant, inhibition of plant pathogens, and/or induction of defense host mechanisms against pathogens. Bacteria belonging to genera *Bacillus* and *Pseudomonas* have been studied in detail both at phenotypic and genotypic levels. Fluorescent pseudomonads, in general, are considered to be cosmopolitan species with broad distribution in the environment. This group of pseudmonads is big and includes several genotypes that needs to be characterized for their full exploitation in agriculture as PGPR and as biocontrol agents.

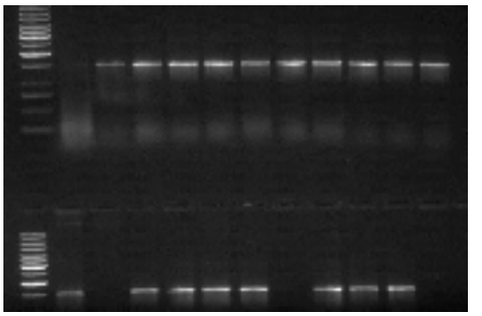
Progress of Research

Five strains each belonging to *Bacillus subtilis, Bacillus licheniformis, Bacillus cereus, Bacillus circulans* and *Bacillus megaterium* were initially selected for molecular characterization. Genomic DNA was isolated from each strain and was used as a template for PCR amplification. Amplification of 16S rDNA was carried out with 50ng of pure genomic DNA using the forward (PA) and reverse (PH) primers. The sequence of the primers used were PA: 5' AGAGTTTGATCCTGGCTCAG 3', PH: 5' AAGGAGGTGATCCAGCCGCA 3'. A strong amplification band of about 1540 bp from each *Bacillus* strain was obtained. Restriction fragment length polymorphism (RFLP) analysis was carried out using four tetra cutter endonucleases *Msp*1, *Alu*1, *Hae*III and *Rsa*1.The restriction endonuclease *Alu*I was selected as it could distinguish closely related strains of *B. subtilis* and *B. licheniformis*. Amplified restriction digestion of rDNA analysis (ARDRA) from the five strains showed distinct variations among the strains.



Restriction digestion of *Bacillus* sp. 16S rDNA with Alu1 M: 100 bp DNA ladder, Lane 1: *Bacillus subtilis*, 2: *B. licheniformis*, 3: *B. cereus*, 4: *B. circulans*, 5: *B. megaterium*

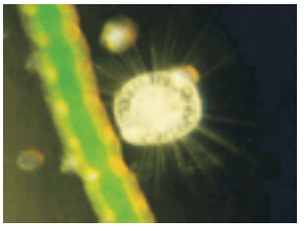
Isolation of *Bacillus* species was carried out from the soil samples collected from the northern Indo-Gangetic plains (near Dehradun) following enrichment technique. One gram of soil sample was added to 10 ml. of nutrient broth and heated in a hot water bath at 80^0C for 15 minutes in triplicates. The broths were incubated at different temperatures- 30, 37 and 55^0C 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 and were purified on Nutrient agar medium amended with methyl red. Genomic DNA was isolated from all the isolates along with 25 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. Fluorescent *Pseudomonas* was also isolated from the soil samples collected from Indo-Gangetic plains 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.



Amplification of 16S rDNA gene sequence from few isolates of *Bacillus* sp.

Isolation of *Bacillus* species and fluorescent *Pseudomonas* from soil samples collected from regions adjoining Dehradun (Indo-Gangetic Plains)

	Bacillus spp.			Fluorescent *Pseudomonas*
	30 oC	37oC	55oC	
Sample Number	1	2	3	-
1A	2	4	5	-
5A	2	2	5	-
6A	2	1	3	-
7B	3	4	1	2
8A	1	5	2	-
9A	3	2	1	1
10B	2	2	4	1
11B	2	3	2	-
12A	1	4	0	-
13	1	2	3	1



Project on Microbial Diversity Analysis of Soils Contaminated with Industrial Effluents in Northern Plains of Indo-Gangetic Regions

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

To analyze the microbial diversity of Indo Gangetic Plains (IGP), a survey was conducted at the farmer's field contaminated with effluents of paper mills and distilleries in Karnal and Meerut region under Rice-Wheat Cropping System. Rhizospheric soil and plant samples of Wheat, Maize and Jatropha curcas were collected. From the rhizosphere of maize, wheat and Jatropha 60 bacterial isolates were obtained. From the endorhizosphere, 51 bacterial strains were isolated. Further studies on their biochemical and molecular characterisation are in progress.

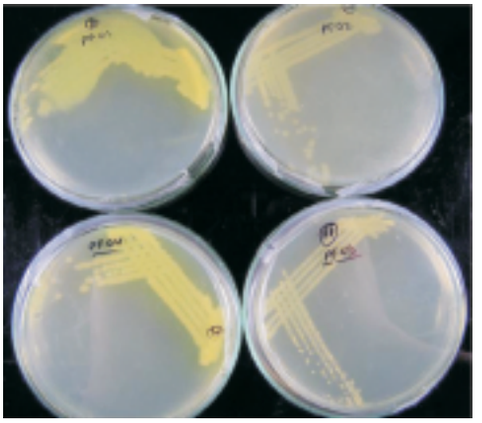
From the rhizosphere of maize and Jatropha, bacterial isolations were made on King's B agar medium. Out of large number of bacterial colonies screened, 34 isolates were selected and designated as fluorescent *pseudomonads* based on their fluorescence under UV and biochemical characterization. The results of biochemical characterization of the isolates are given in Table . All the isolates were Gram negative, catalase positive and could not grow on media containing methyl red. Variations among isolates were observed for gelatin hydrolysis, oxidase, nitrate reduction, casein hydrolysis and TSI test. Presumptive identification of rhizobacterial isolates was done on the basis of Bergey's Manual of Determinative Bacteriology. The results confirmed that all the isolates belong to fluorescent *Pseudomonas* group. Strains were further screened for plant growth promoting and biocontrol attributes such as production of IAA, siderophore, ammonia and HCN.

Irrigation channel carrying paper mill effluent for irrigation

A field irrigated with paper mill effluent

Biochemical Characteristics of Fluorescent *Pseudomonas* isolates

Isolate	Pigment	Gram Reaction	Catalase	Oxidase	Gelatin Hydrolysis	Growth at 43°C	Nitrate Reduction	Urea Hydrolysis	Methyl Red	Casein Hydrolysis	Sucrose or lactose	Glucose
PF01	GF	-	+	+	+	+	+	+	-		-	+
PF02	GF	-	+	+	+	+	+	+	-	+	+	-
PF03	GF	-	+	+	+	+	-	+	-	-	-	-
PF04	GF	-	+	+	+	+	+	+	-	-	+	-
PF05	GF	-	+	-	-	-	-	+	-	-	+	-
PF06	GF	-	+	+	+	-	-	+	-	-	-	-
PF07	GF	-	+	+	+	+	-	+	-	-	-	-
PF08	GF	-	+	-	+	+	+	+	-	+	-	-
PF09	GF	-	+	-	+	+	+	+	-	-	+	-
PF10	GF	-	+	+	-	+	-	+	-	+	-	-
PF11	GF	-	+	+	+	+	-	+	-	-	+	+
PF12	GF	-	+	+	+	+	-	-	-	-	-	-
PF13	GF	-	+	+	+	+	-	+	-	+	-	-
PF14	GF	-	+	-	+	+	+	+	-	-	-	+
PF15	GF	-	+	-	+	+	+	+	-	-	-	-
PF16	GF	-	+	+	+	+	-	+	-	-	-	-
PF17	GF	-	+	-	+	-	-	-	-	-	-	-
PF18	GF	-	+	-	-	+	+	+	-	-	-	-
PF19	GF	-	+	-	+	+	+	+	-	-	-	-
PF20	GF	-	+	+	+	+	-	+	-	+	+	-
PF21	GF	-	+	+	+	+	+	+	-	+	-	-
PF22	GF	-	+	+	-	+	+	+	-	+	+	-
PF23	GF	-	+	+	+	+	-	+	-	-	+	-
PF24	GF	-	+	+	-	+	+	+	-	-	-	-
PF25	GF	-	+	-	+	+	-	+	-	-	+	-
PF26	GF	-	+	+	-	+	-	+	-	-	+	-
PF27	GF	-	+	+	-	+	-	+	-	-	-	-
PF28	GF	-	+	-	+	+	+	+	-	+	+	+
PF29	GF	-	+	-	+	+	+	+	-	-	+	-
PF30	GF	-	+	+	+	+	-	+	-	+	-	-
PF31	GF	-	+	+	+	+	-	+	-	-	-	-
PF32	GF	-	+	+	+	-	-	-	-	-	+	-
PF33	GF	-	+	+	+	+	-	+	-	+	+	-
PF34	GF	-	+	-	+	+	+	+	-	-	-	-



Colony morphology of Fluorescent *Pseudomonas* Isolates

Plant Growth Promoting Attributes of Fluorescent *Pseudomonas*

IAA production: All the isolates were tested for their ability to produce IAA both in the presence and absence of tryptophan. It was observed that all the isolates produced IAA in the absence and presence of tryptophan. However, in the absence of tryptophan IAA production ranged from 6 to 47 µg mg^{-1} protein and in its presence IAA concentration ranged from 14.5 to 99.2 ug mg^{-1} protein. The strains PF01, PF05, PF10, PF12, PF14, PF22, PF25, PF32 and PF34 produced IAA significantly higher than all other strains and ranged from 82.0 to 99.2 µg mg^{-1} protein. The results clearly indicated that there was tremendous increase in IAA production in presence of tryptophan. Certain strains like PF32 and PF14 showed higher IAA production even in the absence of tryptophan. Strain PF28 did not show any impact of tryptophan addition. In general no correlation was found in the production of IAA between the isolates with or without tryptophan.

P-solubilization: All the isolates of fluorescent *Pseudomonas* showed variations among themselves with regards to their ability to solubilize phosphorus in Pikovskaya's medium with tri-calcium phosphate as insoluble source of phosphorus. Of the 34 isolates, 25 could solubilize phosphorus to various extents. The radius of clearing zone ranged between 1.6 to 6.5 mm with PF10 showing the maximum value. It was closely followed by PF19, PF26 and Pf02.

Siderophore production: Siderophore production was observed by the formation of orange yellow halo zone around the growth of fluorescent *Pseudomonas* after 48 h of incubation. Variations were observed among the 35 isolates with regards to their ability to produce

Production of IAA (µg mg^{-1} protein) by fluorescent *Pseudomonas* isolates

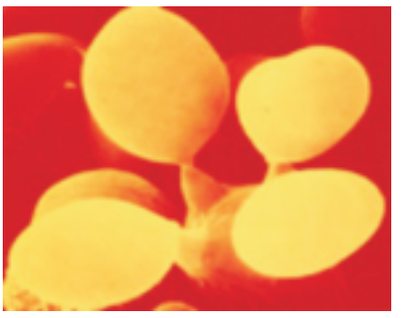
Isolate	IAA production (µg mg-1 protein) Without Tryphtophan	With Tryphtophan
PF01	11.0	82.0
PF02	06.0	46.2
PF03	15.6	26.0
PF04	18.0	59.0
PF05	14.5	92.9
PF06	15.4	66.2
PF07	12.7	43.2
PF08	09.5	14.5
PF09	13.5	42.0
PF10	12.5	72.0
PF11	22.5	61.7
PF12	34.7	94.0
PF13	13.2	34.2
PF14	38.7	95.9
PF15	22.1	64.1
PF16	25.7	45.8
PF17	19.3	34.6
Pf18	11.6	45.6
PF19	36.1	57.9
PF20	09.8	64.0
Pf21	33.7	72.0
PF22	27.2	89.2
PF23	11.4	63.4
PF24	15.1	54.1
PF25	32.7	99.2
PF26	24.4	52.0
PF27	14.0	68.1
PF28	37.5	44.0
PF29	15.1	64.2
PF30	27.4	47.1
PF31	19.7	24.7
PF32	47.1	96.7
PF33	06.0	65.6
PF34	24.2	82.0

Siderophore.. Two isolates PF19 and PF20 were negative for the production of siderophores. Isolate Pf30 was found to be the best siderophore producer as was evident from the size of the halo zone. Of the 34 isolates, seven showed very small halo zones indicating their ability to produce siderophores in small quantity.

HCN production: Variations were found among the isolates regarding their ability to produce HCN. All the isolates except PF02, PF05, PF12, PF15, Pf16, Pf20,

PF22, PF25 and PF32 could produce HCN to various Levels. Isolates PF01, PF06, PF11, PF14, PF17, PF19, PF21, PF26, PF31 and PF34 were very efficient in HCN production, followed by PF03, PF10, PF13, PF18, PF23, PF30, PF33, which were moderate HCN producers. Nine isolates namely PF04, PF07, PF08, PF09, PF24, PF27, PF28 and PF29 were poor producers of HCN.

Ammonia production: Production of ammonia is another attribute of fluorescent *Pseudomonas* that is responsible for the plant growth promotion indirectly by suppressing pathogens. Variations were observed among the 34 isolates regarding the ability to produce ammonia. All the isolates could produce ammonia to various levels. Four isolates PF05, PF12, PF16 and PF30 were very efficient in ammonia production.



Project on Exploration, Germplasm Collection and Characterization of Antagonistic Microorganisms of Soil Borne Fungal Pathogens in Indo-Gangetic Plains of India

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

Objectives

1. Germplasm collection of antagonistic microorganisms from soil and plants from different locations of Indo-Gangatic plains of India
2. In vitro, screening of fungal and bacterial isolates against soil-borne pathogens.
3. Identification and characterization of antagonistic microorganisms by biochemical and molecular tools.

Achievements

Survey was conducted at different locations in Indo-Gangetic plains namely district Dehradun and Haridwar, Utranchal during 2005-06 Rabi season. About 50 soil, plant and plant part samples were collected from fields and forest area. Different fungi, bacteria and actinomyctes were isolated from these materials by using standard protocols.

Collection of samples and detail of microorganisms isolated

Location	Sample description	Fungi	Bacteria	Actinomycetes
Lacchiwala, Distrcit Dehradun	Soil/rhizospheric soil of Teak, Eucalyptus, Dead leaves and Twigs	09	05	02
Jalalia Peer, District Dehradun	Soil/ rhizospheric soil of Barley, Wheat, Garlic, Onion Barseem	14	04	01
Sahaspur District Dehradun	Soil/ rhizospheric soil of Mango, Wheat, Barseem	06	07	02
Kalsi, District Dehradun	Soil/ rhizospheric soil of Rye, Wheat, Onion	11	03	04
SelaKuai, District Dehradun	Infected leaves and twigs collected from Mango, Kinnoo, Orchards, Soil Rhizospheric soil, Humus/ litter of Tropical pine plantation	20	06	02
Rishikesh	Soil/ rhizospheric soil from Sal forest Teak forest, Wheat	12	13	04

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

Coordinator : D. K. Arora

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 for necessary approval and funding.

Rationale of the Network Project

Microorganisms present in the soil play an important role in nutrient solubilisation, mobilization and recycling. They have very wide potentialities by controlling soil-borne pathogens, stimulating plant growth, increasing nutrients availability and accelerating decomposition of organic materials, and are anticipated to increase crop production as well as maintain sound environments for crop production. Its resident microbial flora chiefly determines the health of the soil. Since the discovery of Rhizobium as a symbiotic Nitrogen fixer at the turn of last century, several new nitrogen fixing microorganisms like *Azotobacter* and *Beijerinckia* were isolated. In 1974, a new associative nitrogen fixer, *Azospirillum* was described by Doberiener from Brazil. Since 1988 many new endophytic nitrogen fixing bacteria like *Acetobacter diazotrophicus*, *Herbaspirillum*, *Azoarcus*, *Azomonas* have been discovered. Besides these nitrogen fixing bacteria, phosphate-solubilizing microorganisms (*Bacillus megaterium* var. *phosphaticum*, *Bacillus polymyxa*, *Bacillus circulans*) were isolated and tested for various crops. The discovery of Plant Growth Promoting Rhizobacteria in 1974 by Kloepper and Schroth has opened a new vista for use of microorganisms in agriculture. The Indian Council of Agricultural Research (ICAR) launched projects like 'All India Coordinated Research project on Biological Nitrogen Fixation', 'Network project on Biofertilizers' and 'All India Coordinated Research Project for Pulses and Soybean' to continuously encourage the use of biofertilizers in the country and to develop superior inoculant strains for various crops. In the process, strains of *Rhizobium*, *Azospirillum*, *Azotobacter*, phosphate solubilising microorganisms, AM mycorrhiza were developed. Carrier based inoculants are commercially supplied through various ICAR Institutes and State Agricultural Universities (SAUs). Bureau of Indian Standards (BIS) specifications are also available for *Rhizobium* and *Azotobacter*. In 20 years of field experiments under the All India Coordinated Research Project on Biological Nitrogen Fixation of the ICAR in various agroecological zones of India, it was found that inoculation with *Azotobacter* and *Azospirillum* in cerals gave 10-15% yield increase with benefits equivalent to about 10-15 kg N ha-1. Because of the lead taken by Microbiologists in the field of legume-*Rhizobium* associations, a joint collaborative programme under Indo-US (STI) programme on "*Rhizobium* Strain Improvement, maintenance of *Rhizobium* Germplasm and Improvement of Carrier and Inoculation techniques" was also awarded. Under this programme (1984-1991), the culture collection was augmented with strains of rhizobia from India and abroad. Looking at the importance of culture collections, Department of Biotechnology (DBT) funded for the establishment of facility for culture collections of Blue Green Algae and *Rhizobium* at IARI. In 1986, National Facility for Blue Green Algae (now called Culture Collection Unit for Blue Green Algae) was established as an independent unit. National facility for *Rhizobium* Germplasm collections was established at the Division of Microbiology, IARI. A 'National Facility for Marine Cyanobacteria' was also established at Trichy. Looking at the importance of microorganisms in agriculture, ICAR established a 'National Bureau of Agriculturally Important Microorganisms' (NBAIM) at Mau Nath Bhanjan, U.P in 2000-01. NBAIM will act as nodal centre for the acquisition and management of indigenous and exotic microbial genetic resources for food and agriculture, and carry out related research and HRD for sustainable growth of agriculture. Till now very limited and isolated efforts were made to tapping of microbial diversity, identification, evaluation and

preserving them for different applications. The present project aims at coordinated efforts leading to exhaustive surveys, isolations, and identification, molecular diversity, bioprospecting and conserving the microbial diversity from various niches for varied applications.

Sustainable agriculture involves the successful management of agricultural resources to satisfy changing human needs while maintaining or enhancing the environmental quality and conserving natural resources. The continuous decline in soil organic matter levels due to continuous cropping without recycling enough crop or animal residues, and insufficient application of nutrients has led to serious nutrient imbalances, impaired soil health and declining factor productivity. There is currently a gap of nearly 10 million tones of nutrients between what crops take out and what is added through fertilizers and manures. Thus there is an urgent need to recycle all available organics in a more efficient way and improve and expand biofertilizer usage. 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. The current aim of scientists all over the globe is to look for methods of retaining applied nutrients, and make it available to plant roots since not more than 30-40% of the applied nutrient is taken up by crops, particularly in rice. Therefore, the best achievement could be to minimize the energy and cost intensive chemical nutrient application to crops. It is essential to study the influence of microbes such as nitrogen fixers, P-solubilisers, VAM and PGPR in the retention of absorption and release of nutrients to plants to augment crop yields. Development of model systems and sensors for effective N and P utilization and newer probes to assess microbial diversity and microbial enzymes in model systems would generate information on the role of organics and biofertilizers in integrated nutrient management in sustainable agriculture.

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. There is need to develop technology to utilize the consortia in combination with organic manures, crop residues and small doses of chemical fertilizers to sustain production of crops.

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. On an average fertilizer usage in dry land crops varies between 5-40 kg ha^{-1}. In rain fed areas where soil moisture conditions are uncertain, farmers are unable to take any risk of cultivating improved varieties or applying higher doses of fertilisers. Hence, the crop yields in these regions are extremely low, but there is a great potential for cultivating leguminous plant species with low cost appropriate inputs and inoculation which can eventually boost the economy and social life of the resource poor farmers. 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. Elevated soil temperature (>35°C)

resulting in soil desiccation during various stages of crop growth, decrease the population of N_2-fixing organisms, therefore the complete potential of nitrogen fixing systems is rarely realized in dry land soils. 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. *Rhizobium* inoculation has been reported to alleviate salinity stress in legumes like chickpea. Based on careful seelction of the rhizobial partners and the hosts it is possible to select for high performing symbiotic combinations under salt stress. Predominant are alteration in the availability of nutrients so as to maintain Na: K ratio in the plant, production of antioxidants to prevent injury to the plant because of salt stress, and production of growth promoting substances. 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 on a network mode in India as the crops are varied and with diverse climate and soil factors. There is a need to consolidate the research efforts on PGPR for reduced use of chemical fertilizers and plant protection chemicals. Apart from applied research, the basic mechanisms involved in rhizosphere competence, interaction among the microbial communities and cropping systems needs to be understood.

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. In India lot of work has been done on agrowaste management and ICAR also sponsored a 'All India Coordinated Research project on Organic matter decomposition'. Technologies like rapid composting employing cellulolytic and lignolytic microorganisms, development of phospho-enriched compost from crop residues and leaf litter, vermicompost , production of enzymes were developed under this project. In India about 355.67 507.836 m ton of crop reisdues are generated every year. Three fourth of the residues are produced by three crops viz. rice, wheat and oilseeds. When crop residues are applied directly to the soil plant system for crop production, it creates hazards related to nutrient management. Therefore there is a need to develop cost-effective, ecofriendly and appropriate technology to maximize economic value of nutrients contained in agrowastes for sustainable agriculture.

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. Soil and plant-associated environments harbor a wide variety of microorganisms that play an integrated role in plant growth and disease management. 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*. Work on biological control in India really gained a momentum in the 1980s with efforts of Departments of Plant Pathology from G. B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal and Tamilnadu Agricultural University, Coimbatore, Tamilnadu. At present, there are strong centers like Project Directorate of Biological Control (ICAR), Bangalore, Karnataka engaged in plant disease

biological control and in recent years, numerous publications have appeared in the literature in this field of research. Many private and government agencies started production of fungal bioagents especially from Tamilnadu, Karnataka, Andhra Pradesh and Maharashtra at commercial level but because of recent stringent registration guidelines set by Govt. of India hindered the pace as small scale producers were not able to register their products because of high registration costs involved.

The future holds tremendous possibilities for efficient use and manipulation of *Trichoderma* and other biological agents for management of crop diseases under varied agroclimatic conditions. Recently, CIB (Central Insecticide Board), Government of India has developed and fine-tuned the regulatory requirements for the mandatory registration of biological control agents.

The conventional diagnostic tests currently available for the diagnosis of *Phytophthora* and *Fusarium* in plants and the detection of *Phytophthora* in soil are time consuming and expensive. Often diagnosis is not done to species level, which necessitates the time consuming process of producing pure cultures and inducing various diagnostic structures. Improved diagnostic tests are needed to encourage more widespread testing by growers in the production of disease free planting material and the identification of disease free plantin sites to avoid or better manage *Phytophthora* and *Fusarium.* Under this three-year project that involves collection of reference material, obtaining DNA sequence information from *Phytophthora* and *Fusarium* in India, the design and testing of genus specific DNA based diagnostic methods, and an effort to use the latest developments in molecular genetics to significantly improve detection and identification of these pathogens. The outcomes of this research will form the basis for the introduction of DNA based diagnostic tests suitable for routine use. Detection of these pathogens is an important tool towards the production of disease free plants, and in the identification of disease free planting sites as well quarantine responsibility of NBAIM in global exchange of germplasm. Detection of plant pathogen with quarantine status can have severe economic consequences. The scientific basis for DNA based microbial diagnostics must continue to be investigated and explored; there is a good possibility that the various systems developed will be used extensively in dairy industries, foods of animal origin and disease diagnosis. The outcome of this coordinated project will become a diagnostic model to select number of agriculturally important microbes in sequel in forth coming days. Overall, the present project aims to develop a fast real time animal microbe diagnosis with out culturing. It also eliminates post PCR gel analysis thus it will pave the way for 'portable lab' mode diagnostic approach in the field with-in-real time with out compromising accuracy and specificity of the diagnostics.

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. The field of microbial genomics has moved away from the primary initial focus on pathogens genomes to include the sequencing of diverse prokaryotes that occupy a range of environmental niches, and which are responsible for an array of environmental processes. Every genome that has been sequenced to date has provided new insight into biological processes, activities, and potential of these species that was not evident before the availability of the genome sequence. 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 microorganisms 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 microorganisms. 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 will have 6 components:

1 Microbial diversity and identification

2 Nutrient management, PGPR and Biocontrol

3 Agrowaste management, Bioremediation and Post harvest processing

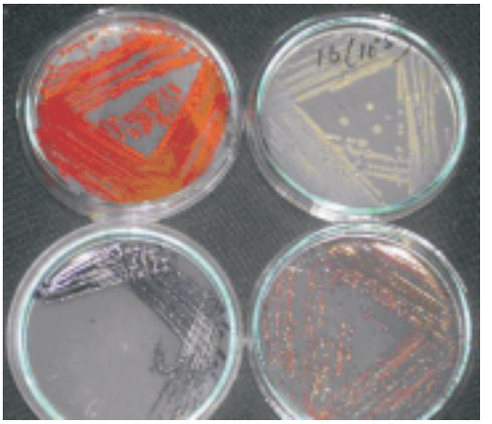
4 Microbial management of abiotic stress

5 Microbial genomics

6 Human resource development

The overall objectives of the project are:

- Deciphering the structural and functional diversity of agriculturally important microorganisms and to develop microbial map of the country.
- Improving nutrient use efficiency through microbial interventions for sustainable crop production and maintenance of soil health.
- Characterization of plant growth promoting rhizobacteria and to develop bioconsortium for enhanced growth and yield of important crop plants.
- Formulation of microbe or microbe-based preparations for biocontrol of phytopathogens, insect pests and weeds.
- Development of microbe-based technologies for agrowaste management and biodegradation for sustainable crop production.
- Harnessing microbial activities for bioremediation of organic and inorganic environmental pollutants.
- Management of abiotic stresses using microorganisms.
- Development of microbe mediated processes for product development and value addition in agriculture.
- 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.



Library, Information and Documentation

Books

Administration	45
Bacteriology	13
Biochemistry	07
Bioinformatics	06
Bioinstrumentation	01
Biotechnology	56
Botany	05
Environmental Sciences	14
IPM	12
Microbiology	189
Molecular Biology	39
Phycology	12
Plant Pathology	77
Plant Virus	03

Periodicals

Annual Review of Microbiology (Vol 47 to 56 & 59)
Annual Review of Phytopathology (Vol 30 to 41)
Applied and Environmental Microbiology (Vol 1 to 11)

A Glance of NBAIM Library

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 Institute of Science
- Fungal Genetics and Biology
- The Journal 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

Research Papers / Reviews / Books

- R. Saikia, R. Kumar, D. K. Arora, D. K. Gogoi, P. Azad (2006) *Pseudomonas aeruginosa* inducing rice resistance against *Rhizoctonia solani*: production of salicylic acid and peroxidases. Folia Microbiol. 51 (5), 375-380
- Ratul Saikia, Mukesh Yadav, Bhim Pratap Singh, Dip K. Gogoi, Tanuja Singh and Dilip K. Arora (2006) Induction of resistance in chickpea by cell wall protein of *Fusarium oxysporum* f. Sp. *Ciceri* and *Macrophomina phaseolina.* Current Scienice Vol. 91.
- Ratul Saikia, Mukesh Yadav, Saju Varghese, Bhim Pratap Singh, Dip K. Gogoi, Rakesh Kumar and Dilip K. Arora (2006) Role of Riboflavin in induced resistance against *Fusarium* wilt and charcoal rot diseases of chieckpea. Plant Pathology Journal 22(4).
- Bhim Pratap Singh, Ratul Saikia, Mukesh Yadav, Rakesh Singh, V. S. Chauhan and Dilip K. Arora, (2006) - Molecular characterization of *Fusarium oxysporum* f.sp. *Ciceri* causing wilt of chickpea. African J. of Biotechnology (vol 5(6), pp. 497-502)
- Ratul Saikia, Alok K. Srivastava, Kiran Singh and Dilip K. Arora (2005)- Effect of Iron Availability on Induction Systemic Resistance to *Fusarium* Wilt of Chickpea by *Pseudomonas* spp. Mycobiology. 33 (1): 35-40.
- Ratul Saikia, Saju Varghese and Dilip K. Arora (2005)- Influence of minerals amendment on disease suppressive activity of *Pseudomonas fluorescens* to *Fusarium* wilt of chickpea. (In Press: Microbiological Research).
- Ratul Saikia, Bhim Pratap Singh, Rakesh Kumar and Dilip K. Arora (2005)- Detection of pathogenesis related proteins - chitin. and ß-1, 3-glucanase in induced chickpea. Current Science. 39(4), 659-663
- T. Singh, R. Saikia and D. K. Arora (2005)- Partial Purification of Lectin from Mycoparasitic species of *Trichoderma.* (The Plant Pathology Journal).
- Dilip K. Arora, Ratul Saikia, Ram Dwivedi and David Smith (2005)- Current Status, Strategy and Future Prospects of the Microbial Resource Collection. Current Science, 89:1-10.

REVIEWS / BOOKS

Applied Mycology & Biotechnology:

- 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)

Conference/Symposia Attended

- Prof. Dilip K. Arora attended the "Staff Research Council Meeting" as an invited member and delivered a lecture at Central Tobacco Research Institute (CTRI), Rajahmundry, (A.P.) from 01st to 5th August, 2005.
- Prof. Dilip K. Arora attended the "Bio-diversity Act meeting" at NAARM, Hyderabad.
- Prof. Dilip K. Arora was invited as National Expert to develop global biological resource centre OCDE, Paris, France

Committees and Panels

- NBAIM is the Member of the World Federation of Culture Collection (WFCC)

Honours, Awards & Recognition

- Prof. Dilip K. Arora selected as Member of National Academy of Agricultural Sciences (NAAS), 2005-06.
- Prof. Dilip K. Arora selected as National Expert OCDE Committee on Biological Resource Centres; Paris, France (2005)
- Prof. Dilip K. Arora selected as Member of the Editorial Board, Indian Journal of Microbiology.

Workshop/In House training

- National Training Programme in the area of "Microbial Diversity Analysis of Agriculturally Important Microorganisms" was organized from January 3-25, 2006. It was attended by 26 participants. Major themes of training programme were: Microbial community analysis; Diversity of microbes in exotic environments; Utilization of microbial diversity; Microbial diversity tools (biochemical); Bioinformatics and microbial diversity; Microbial bioresource centres; Microbial biodiversity and its utilization; Molecular biological tools to study microbial diversity; IPR/ biosafety/ biopiracy of microbial diversity; Exchange of microbial germplasm; Microbial Gene Bank.

Participants of Microbial Diversity Training

Distinguished Visitors

Dr. Mangala Rai, Secretary (DARE) and Director General, Indian Council of Agricultural Research; Dr. Gautam Kalloo, Deputy Director General (CS&H), Indian Council of Agricultural Research; Dr. T. P. Rajendran, Assistant Director General (PP), ICAR; Dr. Dasho Sangay Thinky, Honourable Secretary, Ministry of Agriculture, Bhutan; Dr. Ugyen, Tshewang, Program Director, National Biodiversity Centre, Bhutan; Dr. S. P. S. Ahlawat, Director, National Bureau of Animal Genetic Resources, Karnal; Dr. A. C. Gaur, Former Professor and Head, Division of Microbiology, IARI, Delhi; Dr. Vishwadhar, Head, Crop Protection, Indian Institute of Pulses Research, Kanpur; ; Prof. L. C. Rai, CAS in Botany, BHU; Dr. T. K. Adhya, Central Rice Research Institute, Cuttack; Dr. Ashok Kumar, Coordinator, School of Biotechnology, BHU; Dr. K. V. Bhat, NRC for DNA Fingerprinting, New Delhi; Dr. B. D. Kaushik, Head, Division of Microbiology, IARI, New Delhi; Prof. D. N. Tiwari, Head, Department of Botany, BHU; Dr. S. P. Singh, Emeritus Scientist, BHU, Varanas; Prof. B. D. Singh, School of Biotechnology, BHU, Varanasi; Dr. Vishwas Chavan, NCL, Pune; Dr. Sudershan Kumar, NBRI, Lucknow; Dr. O. P. Rupela, ICRISAT; Dr. P.W. Ramteke, AAI-Deemed University, Allahbad; Dr. L. R. Kashyap, NRCPB, New Delhi; Dr. R. H. Balasubramanya, Head, Biochemical Processing Division, CIRCOT, Mumbai; Dr. K. Ramasamy, Director, Plant Research Centre, TNAU, Coimbatore; Dr. B. L. Jalali, Former Director of Research, HAU Campus, Haryana; Dr. M. S. Basu, Director, NRC for Groundnut; Dr. V. Krishnamurthy Act. Director, CTRI; Dr. George V. Thomas, Act. Director, IIHR, Bangalore; Dr. H. S. Sen, Director, CRIJAF; Dr. R. J. Rabindra, Project Director, PDBC Bangalore; Mr. Mukesh Meshram, Ex DM, Mau; Mr. P. Guruprasad, DM, Mau

Dr. Gautam Kalloo
Deputy Director General (CS&H) Indian Council of Agricultural Research, visiting NBAIM Research Lab

Dr. T. P. Rajendran
Assistant Director General (PP) Indian Council of Agricultural Research along with IMC members of NBAIM

NBAIM Personnel

Director

Dilip K. Arora

Scientific Staff:

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

Administrative Staff:

T. N. Vidyadharan	Asstt. Admn. Officer
Shashi Kant	Assistant
S. N. Yadav	Senior Clerk
Manish Kumar Jain	Junior Stenographer
Ganatra N. Jaysinh	Senior Clerk
Ashok Kumar	Junior Clerk
Devendra Fuloria	Junior Clerk
Sudesh Kumar	Junior Clerk

Technical Staff:

Suresh C. Panda	Driver T-1
Mahesh Yadav	Driver T-1
Pilloo Meena	Driver T-1
Anchal Srivastava	Technical Grade I
Amit Rai	Technical Grade I

Supporting Staff:

Satish Pal	Supporting Staff Grade IV
Amar Nath Patel	Supporting Staff Grade IV
Bali Ram	Supporting Staff Grade II
Chetan singh	Supporting Staff Grade II
Manoj Kumar	Supporting Staff Grade II
Rukmani	Supporting Staff Grade I
Rekha Gupta	Supporting Staff Grade I
Ram Gopal	Supporting Staff Grade I
Chandra Kishore	Supporting Staff Grade I
Anil Kumar Rana	Supporting Staff Grade I
Ram Avadh Singh	Supporting Staff Grade I
Asheesh Kumar	Supporting Staff Grade I
Pratap Singh	Supporting Staff Grade I
Ajay Vishwakarma	Supporting Staff Grade I

Scientists/ Research Associates/ Senior Research Fellow (Tenurial)

Ratul Saikia	DST-Young Scientist
Sudhanshu Kashyap	Research Associate
Rakesh Kumar	Research Associate
Bhim Pratap Singh	Senior Research Fellow
B. Kishore Babu	Senior Research Fellow
Mukesh Yadav	Senior Research Fellow
Sandeep Nair	Senior Research Fellow
Girijesh Patel	Junior Research Fellow
Rajat Pratap Singh	Junior Research Fellow
Neeraj Soni	Project Assistant
Sudhir Upadhyay	Project Assistant

Infrastructure Development

NBAIM main entrance gate

NBAIM building

NBAIM new laboratory block

Far view of NBAIM / DSR Hostel

NBAIM residence

NBAIM transit quarters

Inside view of NBAIM new laboratory block

Inside view of NBAIM molecular laboratory

Curricular Activities

Celebration of Independence Day

Tree Plantation

Director, NBAIM with the trainees

NBAIM family on excursion

DM, Mau during the Kisan Mela organized by the District Authorities

Visit of School Children

Director, NBAIM with the School Children

Welcome of New Scientists at NBAIM

Photo Gallery

Bhutan Delegation to NBAIM
Secretary, Ministry of Agriculture, Bhutan at 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

Group Photo of Trainees of
Microbial Diversity Analysis

Group Photo during Farewell to
Sh. Shashi Kant, Assistant, on VRS

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

Dr. Gautam Kalloo, Deputy Director General (CS&H), Indian Council of
Agricultural Research, New Delhi during his visit to NBAIM

Farewell to the Russian visiting Scientist
Dr. Galina A. Vydryakova

राजभाषा खण्ड

प्रशासकीय संक्षिप्त विवरण

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो (एन बी ए आई एम) सन् 2001 में दसवीं पंचवर्षीय योजना में भारतीय कृषि अनुसंधान परिषद के अन्तर्गत स्थापित किया गया। एक प्रमुख संस्थान के रूप में कृषि के विकास को बनाये रखने और तत्सम्बन्धी क्षेत्रों में मानव संसाधन विकास कार्य को पूरा करने के लिए इस ब्यूरो के पास राष्ट्रीय स्तर पर खाद्य एवं कृषि क्षेत्रों में देशी और विदेशी सूक्ष्म आनुवंशिकी संसाधनों के अधिग्रहण एवं उनका प्रबन्धन करने का कार्य करने का कार्य-अधिपत्र है। प्रारम्भ में ब्यूरो ने ओल्ड एन बी पी जी आर भवन में कार्य करना शुरू किया और तत्पश्चात 01 जून, 2004 को यह एन आई एस एस टी भवन, मऊनाथ भंजन, जिला - मऊ उत्तर प्रदेश में स्थापित हो गया। वर्तमान में ब्यूरो का कोई क्षेत्रीय/बेस कार्यालय नहीं है।

इस ब्यूरो के प्रमुख कार्यों में कृषि उपयोगी सूक्ष्म जीवों का पृथक्करण उनकी पहचान करना, अनुसंधान और उनका संग्रहण करना, सूक्ष्म जनन-द्रव्य का परिचयन, सूक्ष्मजीवों का वितरण एवं विनिमयन, उनका गुण-चिह्नांकन, मूल्यांकन, प्रलेखन एवं अन्त में उनका संरक्षण करना। दसवीं पंचवर्षीय योजना निरूपण के बाद भी ब्यूरो ने जिन विविध कार्य योजनाओं में योगदान दिया है जिनमें यू के - कैवी के साथ एन ए टी पी वित्तपोषित परियोजना 'सूक्ष्मजीवीय ऑकड़ों का डिजिटांकन' एवं आई सी ए आर वित्त पोषित नेट-वर्क परियोजना 'कृषि और तत्सम्बन्धी क्षेत्रों में सूक्ष्मजीवों का अनुप्रयोग' प्रमुख हैं।

समय-बन्धित परियोजनाओं/स्कीमों के अतिरिक्त इस ब्यूरो को 20 बैज्ञानिकों एवं 30 प्राविधिक वर्ग के पदो की स्वीकृति प्राप्त है।

अपने इस नवीन स्थान पर स्थानान्तरित होने के बाद ब्यूरो मजबूती से आगे बढ़ रहा है और अब आई सी ए आर / सी एस आई आर / डी बी टी संस्थानों, राज्य कृषि विश्व-विद्यालयों, डब्ल्यू एफ सी सी एवं आई सी डी ई के अन्तर्गत आने वाले अन्तर्राष्ट्रीय सूक्ष्मजैविक संसाधन केन्द्रों के साथ अपने अनुबन्धों को मजबूत कर रहा है। एन बी ए आई एम एक सक्रिय और गुंजायमान संस्थान के रूप में आगे बढ़ रहा है और भविष्य में कृषि उपयोगी सूक्ष्मजीवों की पहचान, उनके गुण-चिह्नांकन और संरक्षण के लिए अत्याधुनिक बुनियादी सुविधाओं सहित सूक्ष्मजैविक आनुवंशिकी संसाधनों पर सभी प्रकार के अनुसंधान एवं विकास कार्यों के लिए राष्ट्रीय स्तर पर नेतृत्व करेगा। राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो के पास एक पूर्ण सुसज्जित 'राष्ट्रीय सूक्ष्मजीव संग्रहालय, स्वत्वाधिकार योग्य सूक्ष्मजीवों का एक राष्ट्रीय भण्डार और हिमांकीय संरक्षण सुविधा उपलब्ध है। राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो 'माइक्रोबियल डेटावेस' एवं 'माइक्रोवियल इन्फार्मेशन मेनेजमेंट सिस्टम' के लिए ढ़ाँचागत सुविधाओं का विकास कर रहा है।

ढाँचागत एवं मानव संसाधन विकास कार्यों को मजबूती देने के विचार से एन बी ए आई एम पिछले दो सालों से शानदार योगदान दे रहा है। सन् 2025 तक ब्यूरो की पारिदृश्यिक योजनाओं का उद्देश्य पूर्ण सुसज्जित प्रयोगशालाओं, प्रशिक्षित मानवश्रम एवं बुनियादी सुविधाओं का विकास करने के लिए चरणबद्ध आवश्यकताओं का विवेचनात्मक मूल्यांकन करना है। इस उद्देश्य के लिए, हम राष्ट्रीय और अन्तर्राष्ट्रीय संस्थानों (उदाहरणार्थ - एफ ए ओ / जी बी आई एफ/ ओ सी डी ई / डब्ल्यू एफ सी सी आदि) से वित्त एवं विशेषज्ञता की मदद प्राप्त करेंगे, जो निश्चित रूप से ब्यूरो के अनुसंधान एवं विकास कार्यों को गति प्रदान करेंगे।

परिदृश्यिक परियोजनाओं के मध्यावधि अनुमानों ने अब तक की उपलब्धियों को विश्लेषित करने, और पहले की योजना की कुछ निश्चित मदों में आवश्यक सुधार करने में बड़ा योगदान दिया है। सूक्ष्मजीव और उनके अनुप्रयोग के बदलते हुए वैश्विक परिदृश्य के कारणों से यह अनिवार्य हो गया था। इस प्रकार कुछ नये परिदृश्य सामने आए हैं जो अब 'सूक्ष्मजैविक संसाधन' प्रोग्राम के अभिन्न अंग होंगे। पारिदृश्यिक परियोजनाओं की परिवीक्षा के लिए प्रथम एवं द्वितीय 'रिसर्च एडवाइजरी कमैटी की' अनुशंसाएं भी अभी विचाराधीन है।

इसके मऊ स्थापित होने के बाद, 2 साल के लघु कार्यकाल में अब तक की उपलब्धियों में सबसे उल्लेखनीय है तमाम उपयोगी सूक्ष्मजीवाणुओं समेत 2500 से अधिक सूक्ष्म प्रजातियों का संग्रहण करना।

उत्साहजनक कृषि उपयोगी सूक्ष्मजीवों की 2500 से अधिक प्रजातियों को देशभर के विभिन्न परिवेशों से अलगाया गया। आसाम, अरूणाचल प्रदेश, राजस्थान, पूर्वी और पश्चिमी उत्तर

प्रदेश, और इंडो गंगा के मैदानी भागों को विशेष रूप से लक्ष्य किया गया। लगभग 1100 कृषि उपयोगी कवक सूक्ष्मों को यू के - के वी से प्रत्यार्तित किया गया है। देश की विभिन्न संस्थाओं, विश्व-विद्यालयों और आई सी ए आर में बड़ी संख्या में कृषि उपयोगी सूक्ष्मजीव उपलब्ध हैं जिन्हें अभी एन बी ए आई एम के कल्चर कलेक्शन में लाना है। प्रभावित पादप भागों से कृषि उपयोगी सूक्ष्मजीवों को खोज निकालने की बहुत सी नयी तकनीके विकसित/प्रमापित की गयी हैं।

फसल उत्पादन में खास महत्व वाले कुछ कृषि उपयोगी सूक्ष्म बर्द्धनों को गुण चिह्नित किया गया है और उनका मूल्यांकन विचाराधीन है। सन् 2002 से एन बी ए आई एम के पास संग्रहीत कृषि उपयोगी सूक्ष्मजैविक आंकड़ों का डिजिटांकन किया गया है और यह पुनर्नियोंजन प्रारूप में है। एन बी ए आई एम के पास उपलब्ध कुछ कृषि उपयोगी सूक्ष्मजीवो में (1) जैव नियन्त्रण उत्प्रेरक : ट्राइकोडर्मा सप., पेसीलियोमाईसिस थर्मोएस्कस, पेसीलोमाइसीज लीलासिनस, ब्युवेरिया सप., ग्लियोक्लेडियन वर्नस, वर्टीसीलियम सप., (2) जैव कीटनाशक :विवेरिया सप., मैटारीझियम सप., पेसीलोमाईसिस सप., वर्टीसीलियम सप., नोमुरिया सप.,(3) वृद्धि-वर्द्धक : स्युडोमोनास फलोरेसेन्स, पी सिरिंजे, राइजोवियम सप., ब्रेडिराइजोवियम सप., (4)पुटेंश्यिल एंजाइम्स /प्रतिजैविकी/ जीव-विष उत्पादक : फयुजेरियम पेल्लिडोरोसियम, फयुजेरियम औक्सीस्पोरम, पेन्सिलियम फ्रीक्वेन्टेन्स, पेन्सिलियम सिइट्रोनम, आइसोलेटस आफ एसपरजीलस, (5) कीट रोगमूलक: विवेरिया सप., मेटारीझियम सप., वर्टिसीलियम सप., नोमुरा सप., (6) अण्ड परजीवी कवक : पेसीलियोमाईसीज लीलासिनस, वर्टिसीलियम क्लामाइडोस्पोरियम, माइकोपेरासिटिक फंजाई, ग्लायोक्लेडियम सप., (7) बैक्टीरिया प्रसंस्कृत कृमि-नाशी और कीट-नाशी पदार्थ: बैसीलस आर्कुलान, बैसीलस ब्रेविस, पेनिवासिलस अल्वी, ब्रेविवासिलस लैटरोस्पोरस, (8) जैव उर्वरक : राइजोवियम की प्रजातियॉ, एजोस्प्रिलियम एवं एजोटोवेक्टर. ।

कुछ खास पादप रोग-मूलक कवक जैसे -फ्युजेरियम, मैक्रोफोमिना, राइजोक्टोनिया_सोलानी के लिए अलग-अलग आण्विक चिह्नक-तकनीकों का प्रयोग करते हुए डी एन ए अनुप्रतिमुद्रा संलेख का विकसित किये जा चुके हैं, जो कि बड़े राष्ट्रीय महत्व की बात है। चना, मसूर, और तिलहन की रोग-जनक फ्युजेरिया की लगभग 100 प्रजातियों को एक अखिल भारतीय समन्वित नेटवर्क परियोजना के तहत अनुप्रतिमुद्रित किया जा चुका है। मैक्रोफोमिना की विलगित संरचनाओं को आण्विक स्तर पर गुणचिह्नित किया गया है और जिनमें से आठ आई टी एस वंशक्रमों को जीन बेंक में प्रस्तुत कर दिया गया है। स्युडोमोनास और बैसीलस की बहुत सी प्रजातियों को आण्विक तकनीकों के प्रयोग से गुण-चिह्नित किया जा चुका है।

'कृषि और तत्सम्बन्धी क्षेत्रों में सूक्ष्मजीवों के अनुप्रयोग' एक नैटवर्क परियोजना एन बी ए आई एम को प्रमुख केन्द्र बना कर स्वीकृत की गयी। दसवीं पंचवर्षीय योजना के मध्यावधि समीक्षा और इसके औपचारिक प्रवर्तन किया गया। 27 अगस्त, 2006 को।

दसवीं पंचवर्षीय योजना की मध्यावधि समीक्षा के दौरान 'कृषि और तत्सम्बन्धी क्षेत्रों में सूक्ष्मजीवों के अनुप्रयोग' पर एक नेटवर्क परियोजना के लिए एन बी ए आई एम को एक प्रमुख केन्द्र के रूप में मंजूरी प्राप्त हुई है और इसका औपचारिक प्रत्यावर्तन 27 अगस्त, 2006 को हुआ। इस परियोजना के लिए कुल बजट राशि रू0 1600.05 लाख स्वीकृत की गयी है और इसका परिचालन देशभर के कुल 63 विभिन्न केन्द्रों / संस्थानों / विश्वविद्यालयों / राज्य कृषि विश्वविद्यालयों पर किया गया है।

भविष्य के पूर्वानुमानों के आधार पर निम्नांकित नवीन विचार बिन्दुओं का समावेश किया जायेगा -

- भौगोलिक सूचना-तंत्र के माध्यम से सूक्ष्मजैविक विविधता को सुव्यवस्थित करना।
- जैव - सूचना विज्ञान का समाकलन और सूक्ष्म सम्पत्तियों के प्रभावी प्रबन्ध के लिए कम्प्यूटर साफ्टवेयर का विकास।
- ओ सी डी ई एवं डब्ल्यू एफ सी सी सूक्ष्म संसाधन केन्द्रों के दिशा निर्देशों में 'सक्षम प्रबन्धन व्यवस्था ' का विकास करना।
- 'जेनेटिकली मेनीपुलेटेड' कृषि उपयोगी सूक्ष्मजीवों एवं आई पी आर अन्तर्गत आने वाले और एकस्व योग्य कृषि उपयोगी सूक्ष्मजीवों के संग्रहण सम्बन्धी नीतियां बनाना, कृषि उपयोगी सूक्ष्मजीवों के दीर्घावधि संरक्षण के राष्ट्रीय महत्व को देखना जो देश के लिए महत्वपूर्ण है।
- सूक्ष्मजीव जीन बैंक का विकास।
- किसानों, उद्योगों और उच्चतर शिक्षण संस्थानों में इसकी सहलग्नता स्थापित करना।
- विभिन्न भारतीय कृषि अनुसंधान परिषदीय संस्थानों/ राष्ट्रीय अनुसंधान केन्द्रों में महत्वपूर्ण कृषिउपयोगी सूक्ष्मजीवों का प्रयोग परीक्षण करना जिससे कि कृषकों को कृषि उपयोगी सूक्ष्मजीवों के वितरण में बदलाव आये।
- हर सूक्ष्म-संग्रह के 'पार-पत्र ऑकड़े' विकसित किये गये

हैं, कुछ कृषि उपयोगी सूक्ष्मजीवों का मूल्यांकन समय के साथ पूरा किया जायेगा, जब यह ब्यूरो, भविष्य में आगे बढ़ेगा।

- विभिन्न राष्ट्रीय और अन्तर्राष्ट्रीय एजेन्सियों से प्रत्यावर्तन के माध्यम से कृषि उपयोगी सूक्ष्मजीवों के संबर्द्धन हेतु चलाये गये कार्यक्रमों को चरणबद्ध ढ़ंग से पूरा करना।
- विभिन्न कृषि उपयोगी सूक्ष्मजीवों के खुले भंडारण को हिमांकीय अनुरक्षण के लिए पुनरावृत्तिक एवं विश्वसनीय संलेख तैयार किये जायेगें, जो कि जीवाणुओं के दीर्घावधि संरक्षण के लिए उपयोगी होंगे। फसलोत्पादन बढ़ाने में उपयोगी परीक्षणों को पूरा करने के लिए हिमांकीय अनुरक्षण क्रिया-विधाओं का विकास किये जाने का विचार किया गया है। खुले भंडार के मौजूदा हिमांकानुरक्षित सूक्ष्मजैविक आनुवंशकीय संसाधनों के दीर्घावधि संरक्षण के लिए विकसित संलेखों का प्रयोग करते हुए प्रयास किये जायेंगे। एक्अीनोमाइसीट्स और दूसरे मन्द-बृद्धि वाले सूक्ष्मजीवों के हिमांकीय अनुरक्षण के लिए खुले आमत्रंण अभी लिये जाने हैं। इन सूक्ष्म प्रजातियों को जहॉ हिमांकानुरक्षण सम्भव नहीं होगा, के संग्रह के लिए लाइफोलाइजेशन या खनिज-तैल विधि तकनीकि पर जोर दिया जायेगा। हिमांकानुरक्षित कृषि उपयोगी सूक्ष्मजीवों की जैव क्षमता मूल्यांकित की जायेगी।

समग्र रिपोर्ट्स के रूप में राष्ट्रीय डाटा-वेस में इच्छित एवं विश्वसनीय परिणाम पाने के लिए 'सूक्ष्मजैविक भण्डार' पर सूचनाएं एरिस सैल द्वारा एकीकृत की जायेगी। ब्यूरो के महत्वपूर्ण प्रशासकीय कार्यों के कार्य प्रबोधनार्थ एक प्रभावी सूक्ष्मजैविक जैव सूचना विज्ञान प्रकोष्ठ एक उपयुक्त डाटा-वेस विकसित करके पूरा किया जायेगा। देश के दूसरे 'राष्ट्रीय सूक्ष्म-जैविक आधानों' को एकीकृत करने की दिशा में इलैक्ट्रानिक माध्यमों से अनुबन्धों को विकसित किया जायेगा।

सूक्ष्मजीव प्रबन्धन एवं जैव सुरक्षा से संबंधित डब्ल्यू टी ओ/ सी डी बी/ आईटीपीजीआर के अन्तर्गत आई पी आर नीतियों के निहितार्थ संशेधित दिशा निर्देश विश्लेषित किये जाने है और परिषद को इन विनियमों के अनुपालनार्थ कार्यवाही करने का तकनीकि निवेश प्रदान किया जायेगा

ज्ञान के फैलाव के विविध पहलुओं पर मानव संसाधन विकास की गतिविधियां इस प्रकार जारी रहेंगी -

- कृषि उपयोगी सूक्ष्मजीवों की जैव विविधता का अध्ययन।
- कृ0 उ0 सूक्ष्मजीवों की पहचान, संरक्षण एवं अनुरक्षण।
- नये सूक्ष्मजैविक विधा संलेख एवं प्रोद्योगिकियाँ।
- जैव व्यवस्था विज्ञान पर विशेष जोर से साथ 'आदर्श सूक्ष्मजीवीय प्रबन्धन योजना'।
- डी एन ए अनुप्रतिमुद्रण।
- विविध संरक्षण रणनीतियाँ।
- जीवाणुओं के आण्विक संसूचन एवं आई पी आर सम्बन्धी नीति निर्धारण।

आई सी ए आर सिस्टम के संस्थानों एवं प्रमुख राष्ट्रीय विश्व-विद्यालयों / संस्थानों के साथ एम एस सी एवं पी एच डी कोर्स चलाने के लिए ब्यूरो अपने प्रयास करेगा।

कार्य अधि-पत्र

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

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Important Recommendations:

The RAC meeting was held on January 24, 2006 and the following recommendations were made:

- **Manpower** - Prime focus was on the "Manpower" and the RAC strongly recommended at least 8 Scientist (S1) in different areas of specialization may be deputed to the NBAIM as soon as possible.
- **Infrastructure** - RAC complemented the infrastructure development made by NBAIM. ADG (PP) suggested to form the SRC of the NBAIM and also advised that after critical discussion of different scientific issues, they may be then placed before the RAC (like process of project, half yearly reviews etc.).
- With regard to Scientific Recruitment, RAC members gave emphasis on the recruitment of pro-rata Technicians and more expertise in the NBAIM for the research work.
- RAC suggested that in the software "MicroNBAIM" that has been under preparation, include the Passport Data form in the PDF format for downloading online or in any circumstances the domain of NBAIM cannot be used by anyone online.
- In respect of cataloguing, the RAC suggested to develop catalogue with the proper descriptors, and should act as a Nodal Centre for the Registration of agriculturally important microorganisms.
- It has been suggested by the RAC that the quarterly reports/ cabinet reports should be very clearly spelt out.
- RAC also recommended to develop linkage with the International/ National level institutes.

Institute Management Committee

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Important Recommendations:

The IMC meeting was held on January 25, 2006 and the following recommendations were made:

- Extra Manpower for the NBAIM e.g. Scientist at the level of S-1 and Technical staff.
- NBAIM should have a direct feeder line for 24 hours electric supply in the campus. For this a proposal may be developed with U.P. Power Corporation Ltd.
- A periodical or annual medical check up facility may be established in the Bureau Campus.
- IMC recommends that the Green House is must for the NBAIM. The members suggested to build a third floor on the top of the new laboratory.
- IMC also proposed the Solar Energy System for the non-stop electric power supply for the NBAIM may be established.
- IMC recommended more technician and supporting staff should be proposed for the 11th Five Year Plan.
- IMC proposed the training to the Scientist of NBAIM in different area of specialization.
- IMC recommended extra funding for more infrastructure development and also appreciated the infrastructure development so far made by the NBAIM.
- IMC recommended that NBAIM should develop a Museum of Microbes and in that an "Computerized Animated System" like an information bank may be installed for the visitors.
- The Members of the IMC were of the opinion that the research activities should be planned in phased manner. The priority should be given to collection, identification, cataloguing of the AIMs. Linkage should be established with other institutes in India and abroad.

Culture Collection Form

Form for Submission of Microbial Cultures/Passport Data

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो

National Bureau of Agriculturally Important Microorganisms (NBAIM)

For NBAIM use only

Our Accession Number
Registration Number
Microbe Code
Scientific Staff Code

Name of the depositor Last First Middle	Designation Affiliation Phone E-mail Fax No.
Name of the microorganisms	Type of culture Bacterium Virus Fungus Actinomycetes Other
Details of source of culture Place of isolation (habitat, crop, plant or animals etc.) Collection date Collected by District & State	
Details of isolation Isolated by (person and address) Isolation date Specimen isolated from (eg.leaf, stem, root, soil, egg mass, insect, etc.)	

Growth and maintainance Medium of growth Medium for sporulation Optimum temperature for growth Incubation time Subculture period Special requirements for growth & sporulation, if any	
Identified by	
Geographical origin	
A brief description or distinctive features of the microorganisms	
Any record on RFLP/RAPD pattern or unique markers	
Whether deposited mirroorganisms are	Agriculturally Important, if yes, please provide details/published materials. Industrial/Biotechnological importance Any Other
Taxonomic data	Morphological Chemotaxonomic/Immunological Molecular (DNA fingerprint/Sequence analysis) (Enclose available documents)
Microorganisms is deposited in	Lyophilized form (in ampoules) In Slants/Petri plates Any Other
Nature	Plant pathogenic Pathogenic to animals/humans Hazardous Risk to environment Unknown Any Other
IPR/Patent information, if any	Group
Provide accession number, if deposited elsewhere	
Any Other information	
Signature and date	

- Attach separate sheet, if necessary.
- Photocopies of this form may be used.
- Attach separate form for each isolate.
- Tick appropriate Box

Address:
National Bureau of Agriculturally Important Microorganisms
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Ph: 0547-2500000, Fax: 0547-2506508
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Website: http://www.nbaii.org.in/nbaim/index.htm

Society Membership Form

Reg. No. 183/2005-06

Society of Agriculturally Important Microbial Genetic Resources

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

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