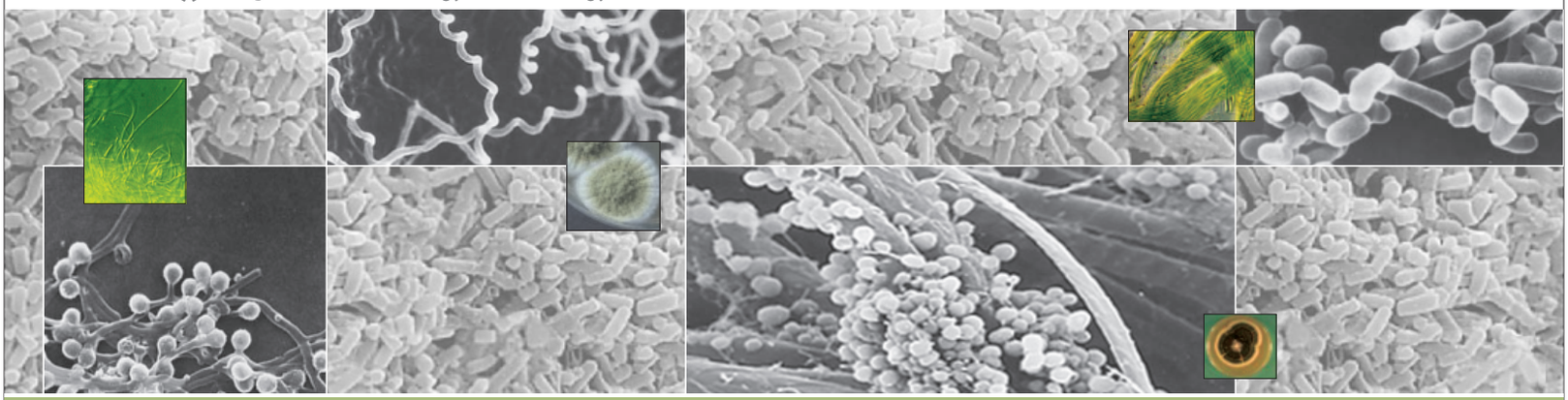
NEWSLETTER

NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT MICROORGANISMS

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो



January - June 2009 Volume - VIII

Development of Diagnostic Kit for *Bacillus*

Bacillus and *Bacillus* derived genera are employed in industry as a source of enzymes, in agriculture as inoculants (PGPR) and biocontrol agents. They are also implicated in bioremediation. The insecticidal property of *Bacillus thuringiensis* has been exploited largely. Rapid identification techniques are needed for the *Bacillus* sp. with agriculturally important traits. The sequencing of 16S rDNA fragment for larger number of isolates is costly preposition. Hence, a molecular probe for rapid identification of *Bacillus* based on 16S rDNA RFLP pattern is being designed. Amplification of 220 bp region of 16S rDNA of *Bacillus* with nested primer pair, followed by sequencing could help in the identification at species level and its derived genera. This small hypervariable region contains all the information for delineation of the species. To prove the hypothesis further, complete 16S rDNA and 220 bp fragment were amplified from 20 different species of *Bacillus*, collected from different geographical areas. All the sequences were BLAST searched and identical identity of the species was obtained from either sequencing complete or partial sequencing. Another approach was also used to design the probe for identification of genus *Bacillus*. An oligonucleotide 50 mer probe for identification of genus *Bacillus* was designed from the internal conserved regions of 16S rDNA following alignment of 16s rDNA sequences of *Bacillus* and its derived genera and related genera (Fig. 1). The probe is non-radioactively labeled and is in the process of validation for its sensitivity and specificity.

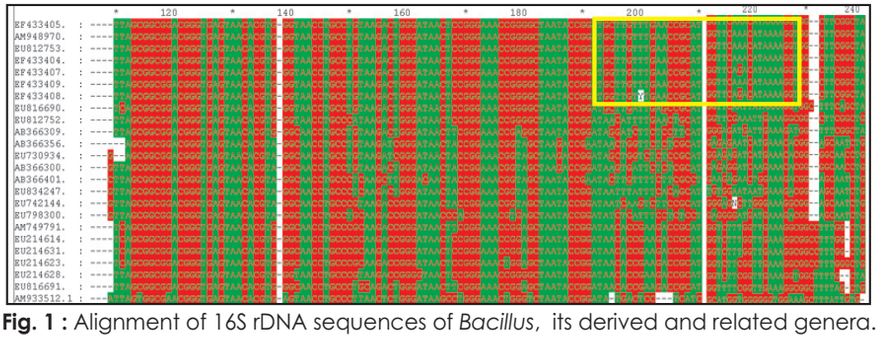


Fig. 1 : Alignment of 16S rDNA sequences of *Bacillus*, its derived and related genera.

Methylotroph Diversity of Chilka Lake

Out of eighty Methylotroph isolates, 25 were selected as representative isolates on the basis of PCR-RFLP and sequenced (Fig. 2). The sequence data was analyzed by BLAST and the nearest match from the NCBI Genbank data obtained. Sequences were deposited in Genbank. DNA sequencing and phylogenetic analysis revealed that all the isolates obtained from the Chilka Lake showed 98 to 100% similarity with the sequence within the NCBI Genbank. Among the isolates, majority showed sequence similarity to the genus *Methylobbacterium* (53.50%) followed by *Hypomicrobium* (15%), *Methylophillus* (7.6%), *Mehthyloversatiles* (7.6%), *Acenetobactor* (3.84%),*Azospirillum* (3.84%), *Mycobacterium* (3.84%) and *Pseudomonas* (3.84%). The closest phylogenetic neighbours according to the 16SrRNA gene sequence data for the 25 isolates 1-25 were *Methylobacterium radiotolerans, M. extorquens, M. hispanicum, M. organophilum, and M. brachiatum, M. mesophilicum, M. lusitanum, M. zatmanii, Hyphomicrobium facile, Methyloversatilis universalis, Mycobacterium brisbanense, Acinetobacter* sp., *Pseudomonas* sp. and *Azospirillum lipoferum.*

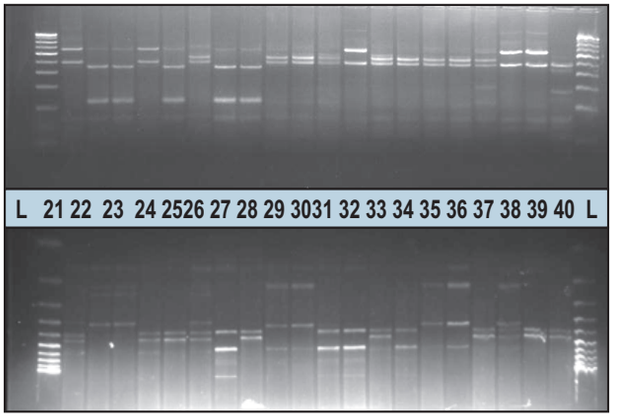


Fig. 2 : RFLP analysis of 16 SrRNA fragments of 40 selected isolates from the Chilka Lake isolates with *HaeIII and Msp I*

Diversity Analysis of Bacteria in Pulikat Lake, Chennai

Aerobic, alkaliphilic bacteria were isolated and characterized from water and sediment samples collected from Pulikat salt lake, India, having pH 7.5. The total number of microorganisms in the sediment and water samples were found to be $10^2 – 10^5$ cfu g^{-1} and $10^2 – 10^3$ cfu ml^{-1}, respectively. Out of 51 selected morphotypes, 8 could grow at 20%, while 4 at 25% NaCl concentration. 16SrDNA PCR-RFLP analysis with *Alu*I identified 29 clusters among the 51 isolates. (Fig. 3) The representative isolates were identified through sequencing as *Bacillus halophilus*, *B. megaterium, B. subtilis*, *B. pumilus*, *Bacillus* sp., *Halomonas sp., H. aquamarina*, *H. pacifica*, *Micrococcus* sp., Uncultured *firmicutes* and *Virgibacillus* sp.

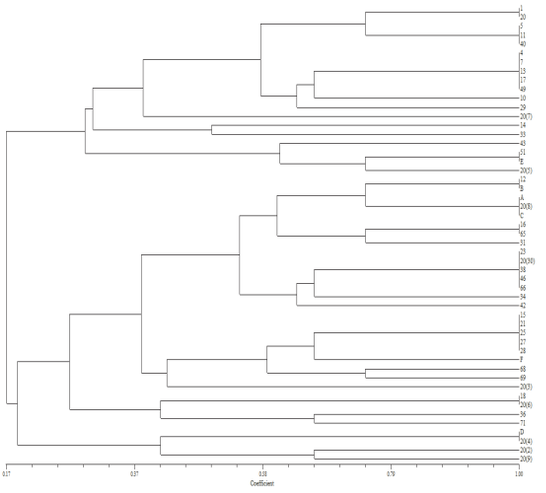


Fig. 3: Dendrogram showing similarity among the isolates based on RFLP analysis with *Alu*1.

Diversity Analysis of Bacteria Isolated from Leh Region

From the soil samples of Leh, the bacterial count ranged from 1.5 to 4.2 x 10^4 g^{-1} soil. Bacteria which were able to grow at 4 °C showed production of copious amount of exopolysaccharide. Among the isolates, 6 bacteria were found to be psychrotolerant and could not grow beyond 15 °C. 16S rDNA-RFLP analysis with *Hae* III and *Hha* I revealed greater diversity among the isolates. Combined dendrogram based on RFLP analysis revealed the existence of 20 clusters among the isolates (Fig. 4).

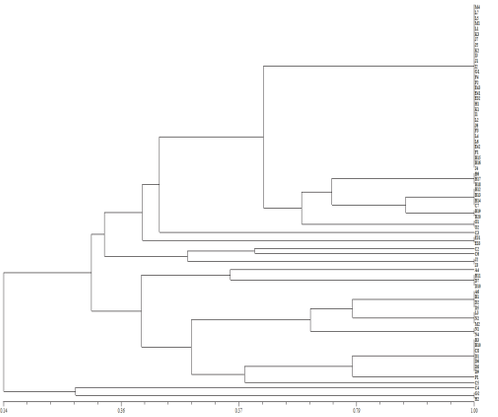


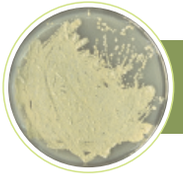
Fig 4. : Combined dendrogram showing similarity among the isolates from Leh (*Hae* III *and Hha* I)

Identification of Alkali-Thermotolerant Bacteria Isolated from Manikaran Thermal Spring

Three promising alkali-thermotolerant xylanase producing isolates were sequenced and the sequences were deposited in the GenBank. DNA sequencing and phylogenetic analysis revealed that all the isolates obtained from Manikaran thermal springs showed 97 to 100% similarity with the sequences within the GenBank. The closest phylogenetic neighbours according to the 16S rRNA gene sequence data for the three isolates H-7, H-9, and R-9 were *Paenibacillus ehimensis, Bacillus cereus and B. subtilis*, respectively. 16S rDNA sequences of identified bacteria were submitted to GenBank with the accession numbers: EU 661710 (*P. ehimensis*), EU 661711 (*B. cereus*) and EU 66172 (*B. subtilis*).

Changes on Soil Biological Properties as a Result of Distillery Effluent Irrigation

The short-term changes in soil microbial biomass are useful indicator for understanding the long-term productivity of soil. It is also frequently used as an early indicator of changes in soil physio-chemical properties resulting from soil management and environmental stresses in agricultural ecosystems. The long term irrigation of agricultural fields with anaerobically digested molasses based distillery effluent in Gajraula, Western Uttar Pradesh, caused significant increase in microbial biomass carbon to microbial biomass nitrogen ratio (Cmic: Nmic ratio) from 3.72 to 6.48 in kharif season and from 4.34 to 7.62 in Rabi season. It has been reported that Cmic:Nmic is affected by soil properties such as application of organic effluents, pH, N-fertilization etc. The increase in Cmic:Nmic ratio



suggests shift in bacterial community structure.

The percentage of Cmic in total soil OC showed significant variation in effluent irrigated fields of rice and wheat (1.46 – 2.16 and 2.18 – 3.43) respectively, whereas, in control soil it was 1.18 and 1.51, respectively. The Cmic values obtained in effluent irrigated and control soil were significantly correlated with Corg (r2= 0.54 and 0.56 respectively) (Fig 5 and 6).

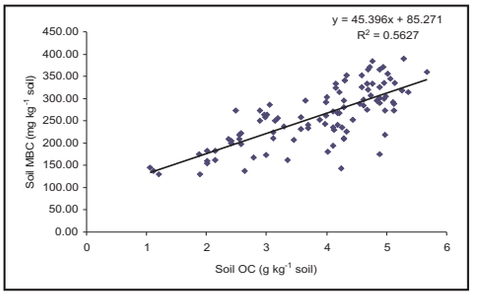


Fig 5. Relationship between organic C and soil microbial biomass C of the control soil

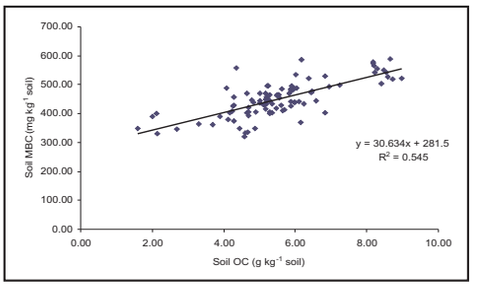


Fig 6. Relationship between organic C and soil microbial biomass C of the effluent irrigated soil

Molecular Characterization of the Actinomycetes Isolates Obtained from Paper Mill Effluent Contaminated Soils

Restriction digestion of 16S rRNA gene using three endonucleases (*Dde* I, *Mbo* I and *Taq* I) has yielded seven to eight distinct restriction patterns for each enzymes (Fig. 7). About two to eight restricted fragments of varying sizes were found in each of the restriction patterns. Cluster analysis of combined 16S rDNA restriction patterns based on Jaccard's similarity index grouped all the 45 isolates under six distinct groups : I, II, III, IV, V and VI. Majority of the isolates were under group I (77.77% of total isolates), and the remaining isolates shared the groups II, III, IV, V and VI (6.66%, 2.22%, 2.22%, 8.88% and 2.22% respectively). CLuster I comprised of 18 isolates from WIF (75.00% isolates of WIF), and 17 isolates from EIF (80.95% of EIF isolates). ARDRA cluster II grouped 3 isolates; two were from WIF (8.33% of WIF isolates) and one isolate from EIF (4.76% of EIF isolates). Cluster III and IV both had only one isolate from EIF (4.76% of EIF isolates). Cluster V had grouped 4 isolates all were from WIF (16.66% of WIF isolates). Cluster VI had only one isolate and it was from EIF (4.76 of EIF isolates). Based on 16S rDNA sequencing the selected isolates were identified as *Streptomyces thermocarboxydus, S. macrosporeus, S. humidus, S. flavoviridis, Streptomyces* sp, *S. variabilis, S. matensis, S. pseudogriseus, S. xylophagus, S. althioticus, Kitasatospora* sp.

To quantify the diversity, the data were subjected to statistical analysis of diversity indices. Shannon index of diversity (H'); Margalef index of richness (R), and Pielou index of evenness (E) calculated for the actinomycetes isolates with their respective sampling sites are presented in Table 1. Among the two different fields studied, EIF recorded maximum actinomycetes diversity (H'=1.539) whereas comparatively WIF has lower diversity (H'=1.362). Margalef index of richness and evenness of community structure (Pielou index) were higher in WIF (R=0.635; E=1.315) in comparison to EIF (R=0.608; E=1.164).

Table 1: Diversity indicies of the actinomycetes obtained from effluent irrigated and fresh water irrigated fields

Sampling sites	Diversity Indices*		
	Shannon index of diversity (H')	Margalef index (R)	Pielou index (E)
WIF	1.362	0.635	1.315
EIF	1.539	0.608	1.164

* The number of isolates showing similar ARDRA profile and differential carbon substrate utilization profile are grouped and used for calculating diversity indices.

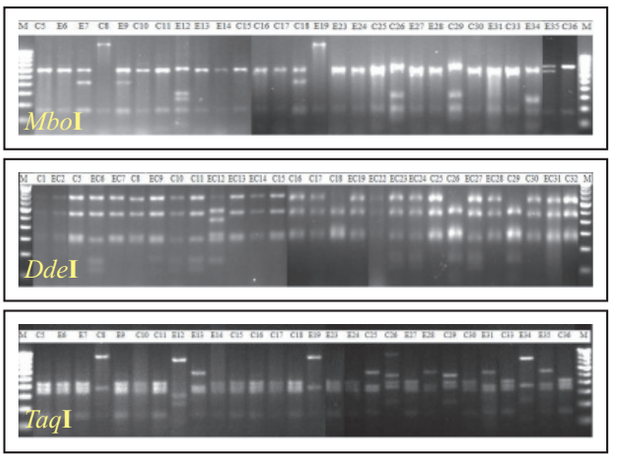


Fig 7. RE pattern of 16S rDNA digested with three restriction endonucleases. M; 100bp DNA ladder, C: isolates from Control field, E: isolates from effluent contaminated field

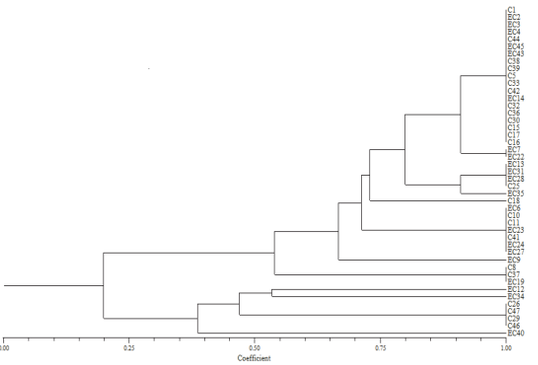


Fig 8. Dendrogram showing clustering of 45 isolates

ISR in Tomato Using *Trichoderma*

The level of chitinase in tomato roots after inoculation with different species/isolates of *Trichoderma* was studied as indicator of induction of systemic resistance. A significant (*P*=0.05*)* increase in activity was recorded from 2-4 days post inoculation. These plant defense reactions can become systemic and protect the entire plant from a range of pathogens and diseases. Apart from the chitinases assay, fresh leaf, stem and root samples from the tomato plants grown in the pots amended with *Trichoderma* isolates and *R. solani* were used for determination of malondialdehyde (MDA), an indicator of lipid peroxidation. A higher level (2-4 fold) of MDA was recorded in stem and leaves after 14 days in treated plants (Fig. 9). No increase in MDA levels were detected in the root samples. The results indicate that selected *Trichoderma* isolates are inducing the level of defensive enzymes in the plants apart from showing potential to increase the growth.

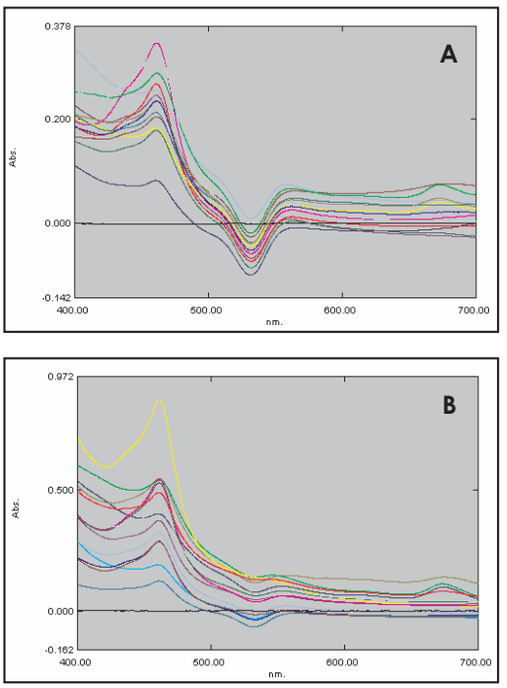


Fig. 9 : Level of MDA in Leaves (A) and Stem (B) of Tomato plants induced with *Trichoderma* isolates after challenge inoculation with *R. solani*

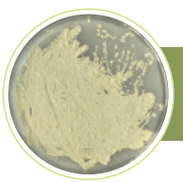
Molecular Characterization of Potential *Trichoderma* Strains

Some *Trichoderma* sp. have an important economic impact, due to their ability to produce hydrolytic enzymes and secondary metabolites to act as biological antagonists against plant pathogens. In present investigation an attempt is being made to characterize few potential isolates of *Trichoderma* isolated from Indo-Ganagatic Plains. Twenty three *Trichoderma* isolated from IGP regions were evaluated for antagonistic potential against *Fusarium melonis, F. cucumeranum* and *F. lycopersici*. Only 10 isolates of *Trichoderma* showed potential antagonism. These *Trichoderma* isolates were subjected to restriction anlysis using *Alu*I, *Msp*I, *Hind* III and *Hae*III. *Hae*III, restriction endonuclease produced a good polymorphism, which could distinguish *Trichoderma* sp. The dendogram at 70 % similarity coefficient separated all the species of *Trichoderma*. *Hind*III also provide 60% of similarity coefficient.

Development of Microbial Consortium for Alleviation of Salt Stress for Growth and Yield of Wheat

In India about 10 million ha of arable land is salt affected. Microorganisms have been implicated in alleviating the effects of abiotic stress by different mechanisms.

About 65% yeild losses of wheat is reported in moderately saline areas. Thus an attempt was made to alleviate the effect of salt stress by inoculating wheat crop with rhizobacteria to improve its growth and yield in saline soils. The microorganisms and wheat cultivars were initially screened for salt tolerance. A total of 130 bacteria were isolated from the rhizosphere of wheat growing in the salt affected soils and screened for salt tolerance at graded concentrations of NaCl. Of the 130 isolates, about 42 isolates were able to tolerate NaCl stress upto 8% while only two isolates showed tolerance to 12% NaCl. Nine strains that performed well as single inoculant in the field were tested for compatibility with each other so as to develop the microbial consortium. Of the nine strains, isolate no. 13 was inhibitory to most of the other strains; isolates 8 and 47 were most sensitive, while 5, 8 and 18 were compatible with all isolates. Based on the results eight different consortia were prepared and field evaluated. A differential response to microbial consortium inoculation was observed on the plant growth parameters both 60 and 90 days after sowing. Shoot length was significantly influenced due to inoculation of T1 as compared to control and other treatments. Dry biomass of shoot was significantly higher for treatment T7 [a consortium of *Bacillus aquimaris* (isolate 8), *B. aquimaris* (isolate 44) and *B. pumilus* (isolate 3)] as compared to uninoculated control. Root dry weight was significantly influenced due to treatment T8 both at 60 and 90 DAS. The grain yield and biomass yield, in general, was significantly influenced due to inoculation of microbial consortium. Maximum grain yield of wheat was achieved in treatment T3 (3085 kg ha-1) [a consortium of *B. pumilus* (isolate 121), *Pseudomonas mendocina* (isolate 40) and *Arthrobacter* sp. (isolate 18), closely followed by T8 (3057 kg ha^{-1})



Diversity of Actinomycetes

(A) From Indo-Gangatic Plains

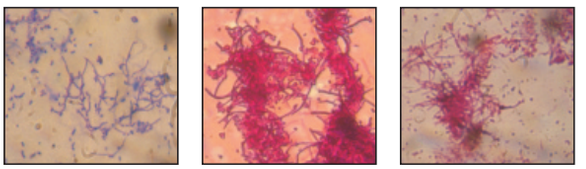
Actinomycetes isolated from Lucknow, Allahabad, Kanpur region, and Central Punjab region were identified and characterized for diversity analysis. Isolates were characterized on the basis of morphological, physiological and biochemical characteristics and sugar utilization patterns. Based on biochemical analysis 62 isolates were identified up to genera level as *Streptomyces* (38 isolates), *S. antibioticus* (9), *Nocardia* (13), *Streptosporangium* (2). Based on 16S rDNA sequencing isolates were identified as *S. viridodiastaticus*, *S. heliomycini*, *S. albogriseolus*, *S. griseorubens* and *Streptomyces* sp.

(B) From Extreme Environments

Extreme environments represent a unique ecosystem and may harbor novel microbial flora. Extremophiles can be grouped according to the conditions in which they thrive. Actinomycetes are known to survive in extreme climatic conditions such as thermophilic, psychrophilic, barophilic, halophilic, acidophilic and alkaliphilic conditions. A total of 217 isolates of actinomyctes were isolated from Manikaran thermal springs, cold climates of Leh and Laddakh regions, hot desert of Rajasthan, Sambhar Lake (Rajasthan) and Pulicat salt lake (Tamilnadu). Isolate ACY 160, ACY 161 and ACY164 were found to be promising protease (157 IU) and amylase (86 IU) producers. Based on synoptical keys for identification of actinomycetes genera, we tentatively identified cultures belongs to genera as *Streptosporangium* (5), *Microbispora* (10), *Streptomyces* sp. (40), *Micropolyspora* (3), *Sporichthya* (4), *Nocardia* (19), *Microtetraspora* (8) respectively. Molecular diversity of 23 isolates was carried out by phylogenetic analysis using universal primers of 16S rDNA and metabolic gene "Nitrile hydratase" followed by restriction analysis with *Mbo*I by using Neighbor- Joining algorithm.

(C) From Saline soils

Exploratory soil sampling survey was carried out for the salt affected regions of district Mau, Uttar Pradesh. The pH of the soil samples ranged from 7.2 to 11.8 cfu count of the actinomycetes varied from 0.1 to 9 x 10^3 g^{-1} soil. Acid fast staining was carried out to differentiate the Mycobacteria, Nocardia and Streptomyces genera of actinomycetes (Fig. 10). The further study is in progress.



Non acid fast isolate | Acid fast isolate | Partial acid fast isolate

Fig. 10 : Photomicrograph of actinomycetes isolates.

Exploring Cyanobacterial Biodiversity in Extreme Habitats for Potential Applicability in Agriculture

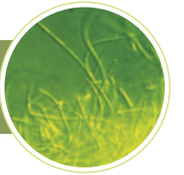
Fifty samples of cyanobacteria have been collected from hyper saline conditions (pH range 8-10) from Mau, U.P., the isolates were done on BG II (N^+ or N^-). The isolates were identified as *Oscillatoria, Anabaena, Hapalosiphon, Scytonema* and *Nostoc* species on morphological basis. Antibacterial and antifungal properties of the isolated organisms have been evaluated and they are found potentially effective against *Bacillus* and *Pseudomonas* strains and *Fusarium, Sclerotium rolfsii* and *Alternaria* sp. HPLC results indicated that these cyanobacterial strains are producing phenolic acids namely gallic, ferulic and cinnamic acids.

Modified and simplified protocol for the isolation of genomic DNA from 12 different species of cyanobacteria (*Anabaena doliolum, Phormidium fragile, Aulosira fertilissima, Tolypothrix tenuis, Anabaena oryzae, Plectonema boryanum, Hapalosiphon intricatus, Cylindrospermum musicola, Oscillatoria acuta, Microchaete uberima, Calothrix* sp.) has been developed and validated in terms of quality and quantity of DNA and RFLP.

Complete Genome Sequencing of *Mesorhizobium ciceri* Ca 181

2000 transformed colonies with inserts from Luria Agar plates amended with ampicilin + X-Gal + IPTG picked and transferred to LB freezing buffer to store the transformed clones at -70 °C. 1344 plasmid DNA from clones isolated and 576 clones have been sequenced till now through forward and reverse sequencing. 1152 sequences have been compared with other organisms through blast analysis. Maximum matches were with the *Mesorhizobium loti, Sinorhizobium meliloti, Bradyrhizobium* and *Sinorhizobium medicae*. Around 400 sequences found to be unique.

The genome sequence assembly was done by using software DNA Star (SeqMan NGen Pro v1.2). Total 12 (6 forward and 6 reverse) sequenced plates were used for the assembly of the Bacterial genome. In the genome assembly the electropherogram sequenced data reads were used whose range of read length is 350-800 bases. After the assembly, total 185 contigs were formed and 8 singletons were left, the largest contig size is 1316 bp which was formed by the alignment of 6 reads. In the assembly there were 4 contigs above 1 Kb and 170 contigs with size below 1 Kb. Out of the 4 contigs one had maximum match with *Mesorhizobium loti*, one with *Sinorhizobium meliloti* and two contigs did not match with available database.



Genes Identified in the Genome of *Mesorhizobium ciceri*

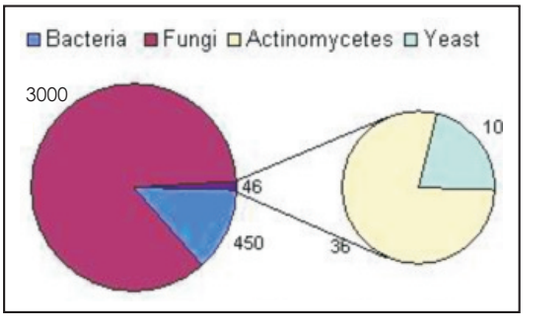
Succinoglycan biosynthesis regulator	Isocitrate lyase protein
Cytochrome-c oxidase	Histidine kinase
Pilus assembly protein,CpaD	glutathione-dependent formaldehyde-activating GFA
o-acetylserine	ABC transporter
alanyl tRNA synthase cystein synthase	Thymedylate synthase
D-Alanine aminotransferase	ATP dependent C1p like protein
Haloacid dehydrogenase	Ribulose 1,5-bisphosphate
GDP- mannose 46- dehydratase	Cystein desulfurase Nifs like aminotransferase
AsmA protein	Fumarate hydratase
Phosphoenolpyruvate-protein phosphotransferase	Ferrodoxin iron-sulphur binding protein
30S ribosomal protein S11 DNA-directed RNA polymerase alpha subunit	Seryl-tRNA synthetase
6-pyruvoil tetrahybridopterin synthetase	Xylose ABC transporter
Dehydrofolate reductase	Transposases protein
urease accessory protein G	Phenylalanin-tRNA lipase beta chain
Ribonucleoprotein	Phosphoheptose isomerase
Nitrogen reguration protein Uroporephyrin decarboxylase	Molybdopterin oxidoreductase

National Agriculturally Important Microbial Culture Collection (NAIMCC)

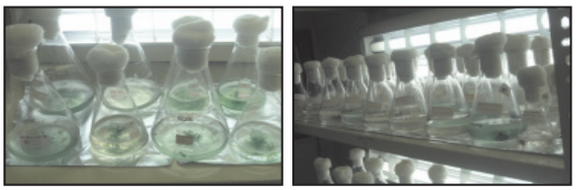
The culture collection preserves and conserves the microbial diversity of the country. The collection is enriched every year through isolation efforts made at the Bureau, deposits from organizations or research workers throughout the country and repatriation of cultures of Indian origin. The collection has wide diversity of fungi and includes more than 700 species belonging to 250 genera. Likewise the bacterial collection has more than 100 species belonging to 35 genera. NAIMCC has developed state-of-the art short term conservation of AIMs based on culture and mineral oil techniques. Using these techniques, AIMs can be conserved for 5-10 years. NAIMCC has high capacity lyophilizers for long term preservation of AIMs (20-25 years) under vacuum at -60 °C. NAIMCC exchange the cultures on MOU basis with different National Institutes/Organizations. NAIMCC has a good collection of very useful microbes which could be used as PGPR Biofertilizers, Biopesticides, Biodegrader and in Biocomposting, Bioremediation industries etc.

NAIMCC has conserved bacterial isolates from extreme environments for the first time reported in India such as "*Bacillus humi, B. drentensis, B. asahii, B. cohnii, B. pumilus, B. niacini, B. djibeloresis, B. fumarioli, B. senequalensis, B. oleronius, and B. sporothermodurans, B. thuringiensis, Halomonas* sp., *Marinobacter alkaliphilus, Marinobacter hydrocarbonoelasticus, Halomonas variabilis, Alteromonadales bacterium, Nitrinicola lacisaponensis, Chromohalobacter salexigens, Marinobacter aquaeolei.*

Now the cyanobacterial cultures have been included in NAIMCC as part of culture collection. 30 important cyanobacteria has been identified, characterized and incorporated. Many more are in the process of addition.



Pi-diagram of NBAIM culture collection


Cyanobacterial Cultures being maintained at NBAIM

NAIP Projects

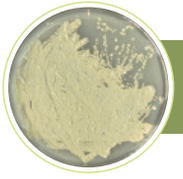
Bioprospecting of Microbial Genes and Allele Mining for Abiotic Stress Tolerance

NBAIM is a consortium partner in this NAIP Mega Project and its objectives of the project are:

- Prospecting novel genes, promoters and alleles for economically important traits using indigenous bioresources with emphasis on less studied species.
- Functional validation of the new genes in model systems and different genetic backgrounds
- Transfer of the validated genes and alleles to recipient species cutting across biological barriers.
- Generation of genomic resource base to facilitate gene prospecting and allele mining.
- Prospecting for new genes and alleles for abiotic stress tolerance (moisture stress, salinity and sodicity, soil acidity, adverse temperature and submergence/anoxia).

Diversity Analysis of *Bacillus* and Other Predominant Genera in Extreme Environment and its Utilization in Agriculture

NBAIM is a consortium leader of this NAIP project and the objectives of the project are:



- Diversity analysis and identification of *Bacillus* and other predominant genera from extreme conditions of salinity, drought, acidity and mangrove.
- To understand the mechanisms of adaptation in *Bacillus* and mining of relevant genes.
- Study of the diversity of *Bacillus* and other predominant genera associated with plant species under extreme environments and evaluating their role as ameliorating agents for crops grown in deteriorated environments.
- Selection of novel strains of *Bacillus thuriengiensis* and other *Bacillus* species with insecticidal properties and isolation of novel cry and other insecticidal genes.

Georeferenced Soil Information System for Land Use Planning and Monitoring of Soil and Land Quality for Agriculture

NBAIM is a consortium partner in this NAIP Project and its objectives of the project are:

- Microbiological evaluation of soils.
- Quantitative estimation of fungi, bacteria and actinomycetes of soils.
- Active biomass of soils by estimation of dehydrogenase and urease.

New Projects on Application of Microorganisms in Agriculture and Allied Sectors

Arbuscular Mycorrhizal Fungi for Biofertilization in Horticultural Crops under Theme (PGPR, Nutrient Management and Biocontrol)

The objectives of the project are:

- Collection of Arbuscular mycorrhizal cultures from different centers.
- Molecular characterization of Arbuscular mycorrhizal fungi.

Evaluation of Endophytic and Rhizosphere Fungi for Growth Promotion and Biocontrol under Theme (PGPR, Nutrient Management and Biocontrol)

The endophytic microorganisms penetrate plants tissue mainly by the root. However, aerial parts such as stomata, flowers and cotyledons also can serve as entrance. Endophytic filamentous fungi represent an important genetic resource for biotechnology. These fungi are being exploited for their potential to produce important secondary metabolite for applications particularly in the pharmaceutical and food industries. Novel antibiotics, immunosuppressant and anticancer compounds are only a few examples of what has been found after isolation, culture, purification and characterization of some endophytes in the recent past. The objectives of the project are:

- Isolation of endophytic fungi from different crops.
- Molecular characterization of endophytic fungi.

Utilization of Actinomycetes to Alleviate Salt Stress for Cereal Crops under Theme (PGPR, Nutrient Management and Biocontrol)

Microbes have been implicated in alleviation of effects of abiotic stresses by various mechanisms like production of osmolytes, sugars, sugar alcohols, exopolysaccharides etc. Such microorganisms not only alter the environment around the rhizosphere of crops but also maintain the ratio of various nutrients. Actinomycetes are found in neutral to saline soils. Most of actinomycetes are tolerant to alkaline conditions and in alkaline soils, 95% population may be actinomycetes. Most of the actinomycetes possess inherent capacity to tolerate salt stress (especially Streptomycetes genera) by synthesis of the compatible solutes like alanine, proline, glycine betaine and -glutamine in response to stresses. Thus keeping these points in consideration, an attempt was made that they can be utilized to alleviate the salt stresses and increase the crop yields under salt affected soils. The objectives of the project are:

- Isolation and screening of actinomycetes from different salt affected area of India for salt tolerance.
- Characterization of the isolates for the accumulation of sugars, sugar alcohols, amino acids and other osmolytes.
- Evaluation of the actinomycetes isolates under pot/ field experiments and study of plant microbial interactions during salt stress.
- Development of consortia of actinomycetes cultures to alleviate the salt stress for wheat and other millets.

NBAIM as coordinating center in other ICAR Network Projects

Conservation, Characterization and Documentation of Different Species of *Alternaria, Colletotrichum* and *Cercospora*

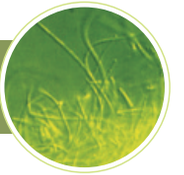
The objectives of the Project are:

- Conservation, characterization and documentation of different species of *Alternaria, Colletotrichum* and *Cercospora*.
- Development of database for Indian isolates of *Alternaria, Colletotrichum* and *Cercospora.*

Conservation, Characterization and Documentation of Different Species of *Fusarium*

The objectives of the Project are:

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



Establishment of Microbial Genomic Resource Repository (MGRR)

"Microbial Genomic Resource Repository" (MGRR) is established at NBAIM under the ICAR Newtork Project on "Application of Microorganisms in Agriculture and Allied Sectors" (AMAAS). MGRR is the national facility to conserve the genetic material (DNA) of Microorganism. The genomic DNA is being stored using standard storage protocol.

Mandate of MGRR

1. To coordinate assemblage, conservation, quality control and validation of the microbial genomic resources to facilitate their optimal exploitation and utilization.
2. To act as a single window system for import and exchange of microbial genomic resources and facilitate protection of related IPR issues.
3. To conduct and promote basic, strategic, applied and anticipatory research for development and management of microbial genomic resources.

Objectives of MGRR

1. Nationwide Survey and Collection of Information about the genetic resources/DNA.
2. Development of linkages between different research institutions, Universities, and individual researchers for obtaining microbial culture and genetic material.
3. Development of infrastructure facilities for the preservation and maintenance of Genetic Resource.
4. Technology and Protocols development for isolation and long-term preservation of the Microbial Genetic Resources.
5. Development of Databases or Information Bank for Microbial Genomic Resources and linkages with other DNA Bank.
6. Collection of environmental Microbial Samples from different Agro climatic Regions.
7. Technology and protocols development for collection/transportation microbial samples.
8. Exploration of non-culturable micro organisms and direct DNA isolation from environmental samples.
9. Documentation and Electronic Cataloguing of Genetic Resources.
10. Development and Implementation of Genome Projects to explore non culturable micro organisms.

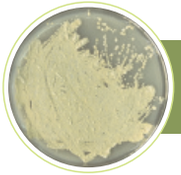
Infrastructure Facilities Generated at MGRR

MGRR is well equipped with all modern sophisticated equipment and instruments such as Robotic DNA Extractor, Pyrosequencer, Molecular Imager Gel Doc XR System, Chemiluminescence Gel Imaging System, DNA Fluorimeter System, Electroporator, Fraction Collector System, Gene Analyzer, Gradient Thermal Cycler, High Throughput Electrophoresis, Pulse Field Gel Electrophoresis System, Ultra Centrifuge, Incubater Shaker, etc.

Robotic DNA Extractor

Pyro Sequencer

High Throughput Electrophoresis



Confocal Laser Microscope

The Centre will Maintain Genetic Materials like:

1. Whole Genome.
2. PAC/BAC/YAC clone vectors competent cells from sequencing projects.
3. A collection of vectors/gene constructs contributed by researchers.
4. Promoter DNA fragments fused to the reporter genes.
5. RFLP probes specific for different microbes.
6. cDNA/EST Libraries.
7. Cloned DNA.

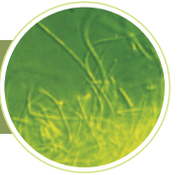
Theme-wise Significant Achievements of AMAAS

Microbial Diversity and Identification

- The AMAAS network project which is operational all over the country yielded a total of 4810 bacteria, 124 cyanobacteria, 310 actinomycetes, 348 fungi and 261 mushrooms from different agro-ecological regions of India. Many new eco-zones are being covered regularly.
- Two value added cyanobacterial products namely, Spiro papad and Spirogel have been prepared and launched in markets and are in high demand in local community of Imphal.
- Some of the rare bacterial species identified were *Chromobacterium violacearum*, *Exiguobacterium* sp., *Arthrobacter* sp., *Bacillus fumarioli*, *Pseudomonas chlororaphis*, *Microbacterium*, *Pantoea*, *Cronobacter*, *Brevibacillus laterosporus*, *Serratia marsecens* and *Beijerinkia*.
- A total of 100 rDNA sequences were submitted to NCBI GenBank.
- One of the bacteria from Garam Pani (a natural hot spring) of Golaghat District, Assam with biocontrol potential was identified as *Brevibacillus laterosporus* (BPM3).
- A novel *Bacillus* sp. with insecticidal property was identified using partial sequencing of 16s rDNA. The bacteria belonged to *B. fumarioli* cluster with swollen sporangia.
- In total 573 wild mushroom specimens were collected from Himachal Pradesh, Uttarakhand and Rajasthan. Tissue cultures from 191 specimens were raised and conserved in the Gene Bank of NRCM, Solan. Two new *Lignicolous volvariella* spp., a new species of *Flammulina*, were isolated and identified using 5.8S rDNA sequencing.
- From different brackish water eco-system, intertidal zones of Mumbai and freshwater ecosystems of Orissa, 370 bacteria, 66 actinomycetes, 55 fungi 21 yeast isolates and 7Archaebacteria has been isolated.
- These microbes possessed several beneficial traits such as agarolytic activity, nucleases, protease, lipase, chitinase, ligninase and cellulase activity, sulfur oxidation, denitrification, salt resistance (2.5-30%), pigment production etc.
- Two sets of new ISR based primers have been developed for rapid and sensitive screening of *Flavobacterium* species from aquatic environment.
- Analysis of diversity from different extreme environments hot springs, cold deserts, acidic soils and salt lakes has led to the isolation of unique microorganisms that are tolerant to high temperature (90^{0}C), high salt concentration (25% NaCl) and low pH (pH 3.0).
- A simple diagnostic approach for identification of *Bacillus* sp. per se and to classify into different species was developed.

Nutrient Management, Plant Growth Promoting Rhizobacteria and Biocontrol

- Potential PGPR's viz *Rhizobium, Bradyrhizobium, Azotobacter, Azospirillum, Bacillus*, Fluorecsent *Pseudomonas* and *Anabaena* were screened at green house level for different crops such as Rice, Wheat, Sorghum, Soybean, Chickpea, Pigeon pea, Black Pepper, ginger, coconut and cocoa.
- Cold tolerant strains of *Pseudomonas fragi* and *Pseudomonas lurida* showing P solubilizing ability at 4 °C were reported for the first time.
- Bacterial and fungal isolates having ability to control disease caused by *Phytophthora, Pythium, Fusarium, Thielaviopsis paradoxa, Ganoderma* sp, *Macrophomina phaseolina* and *Sclerotium rolfsii* in black pepper, ginger, coconut, cocoa, sorghum, tomato, brinjal, chilli and oil seed crops were screened.
- Promising isolates of entomogenous fungi have



been identified against sucking pests like, *Aphis craccivora, Scirtothrips dorsalis, Bemisia tabaci* and *Myzus persicae* based on laboratory bioassay studies.

Management of Agrowaste, Bioremediation and Microbes in Post Harvest and Processing

- Drastic changes in functional and structural diversity of soil microbial community structure were observed due to long term application of pulp and paper mill effluent onto the agricultural lands.
- A novel species of *Sterptomyces* with unique and uncommon growth and pigmentation pattern was isolated having tremendous potential in reclaiming contaminated soils.
- *Serratia marcescens* is reported for the first time to reclaim soils contaminated with Poly Aromatic Hydrocarbons.
- Potential HCH degrading sphingomonad and non-sphingomonads strains have been isolated which can effectively reclaim the soils contaminated with HCH.
- An economically viable and rapid method for compost production has been developed for Mushroom production using fungi *S. thermophilum*
- Two novel p-nitrophenol degrading *Bacillus* sp. and one o-nitrophenol degrading *Bacillus* sp. were isolated from flooded rice soils retreated with respective isomers of nitrophenol.
- A consortium has been developed for Bio-Processing agricultural wastes and bioremediation of aquaculture effluents.

Microbial Management of Abiotic Stress

- A bacterial isolate identified as *Bacillus pumilus* increased grain yield of wheat by 21% under saline soils.
- Seed bacterization with stress tolerant strains of *Pseudomonas* helped sorghum and pearl millet seedlings to survive at 50 °C up to 21 days. Seed inoculation also induced synthesis of a novel high molecular weight protein.

Microbial Genomics

NBAIM has honour of being a Nodal Center for complete Genome Sequencing of an agriculturally important bacterium *Mesorhizobium ciceri* Ca181. It will be the first microorganism to be sequenced in the country. *Mesorhizobium ciceri* is highly specific and promising bacterial strain for chick pea with multiple plant growth promoting activities.

Genomic DNA library of *Mesorhizobium ciceri* was prepared in pUC 19. A total of 2000 clones were sequenced and Blast searched. Several genes have been identified that can be further used in different studies.

Trainings Organized

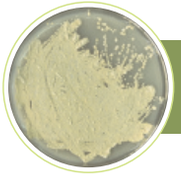
Organized a National Training Programme on "Novel and innovative biochemical and molecular tools for characterization of Agriculturally Important Microorganism" from 5th to 23rd January 2009.

- Identification module for important group of plant pathogenic and beneficial fungi
- Identification module for important bacteria
- Identification of Important actinomycetes
- Molecular biological tools to study microbial diversity
- Biolog Automated Microbial Identification System
- Microbial identification based on gene sequencing
- 16S rDNA-based microbial identification for bacteria and actinomycetes
- ITS rDNA-based microbial identification for fungi.
- Development of molecular probe for detection of microbes
- Bioinformatic tools used in identification of Microbes.

Participants of the National Training Programme on "Novel and innovative biochemical and molecular tools for characterization of Agriculturally Important Microorganism" from 5th to 23rd January 2009 at NBAIM

MEETINGS

- Arranged and attended the QRT meeting of NBAIM, Mau at IISR, Lucknow on 5th January 2009.
- Organized Institute Research Committee Meeting at NBAIM, Mau on 12.01.09.
- Attended the Director's Conference at NASC, New Delhi on 15-16 January 2009.
- Organized the Institute Management Committee Meeting at NBAIM, Mau on 23.01.09



- Organized the meeting of Experts on Cyanobacteria at NBAIM, Mau on 24.01.09
- Organized the AMAAS Review Meeting at CRRI, Cuttack on 28.01.09.
- Attended the Kisan Mela organized by IIVR on 29-30.01.09.
- Attended the International Conference on "Grain Legumes: Quality Improvement, Value Addition and Trade" at IIPR, Kanpur on 14-16th February 2009.
- Organized the Research Advisory Committee Meeting on 14.03.09.
- Organized the National Training Programme on "Novel and Innovative Biochemical and Molecular Tools for Characterization of Agriculturally Important Microorganisms" from 12th January 2009 to 1st February 2009.

NBAIM was dedicated to the Nation on 24.01.09 by Dr. Mangala Rai, Secretary DARE and Director General, ICAR.

Abroad Visit

Prof. Dilip K Arora, Director NBAIM visited Philippines from 20.02.2009 to 02.03.2009 under short term INSA International collaboration/Bilateral exchange and delivered lectures at National Institute of Molecular Biology and Biotechnology, College of Sciences, University of Philippines.

Institute Management Committee Meeting

The Fifth Institute Management Committee (IMC) Meeting of the National Bureau of Agriculturally Important Microorganisms (NBAIM) was held on 22nd January 2009. The following members attended the meeting:

1. Prof. Dilip K. Arora, Director, NBAIM
2. Dr. R. P. Tewari, Member
3. Dr. D. L. N. Rao, Member
4. Dr. Alok Srivastava, Member Secretary

Research Advisory Committee Meeting

The Fifth Research Advisory Committee (RAC) Meeting of the National Bureau of Agriculturally Important Microorganisms (NBAIM) was held under the Chairmanship of Dr. A. N. Mukhopadhyay, former Vice Chancellor of Assam Agricultural University, on 14th March, 2009. The following members attended the meeting:

1. Dr. A. N. Mukhopadhyay, Chairman
2. Dr. K.V.B.R. Tilak, Member
3. Dr. A.N. Rai, Member
4. Dr. O.P. Rupela, Member
5. Dr. T. P. Rajendran, ADG (PP), ICAR
6. Prof. Dilip K. Arora, Director, NBAIM
7. Dr. A.K. Saxena, Member Secretary

Fifth Research Advisory Committee Meeting held at NBAIM

Kisan Mela

A Kisan Mela was organised on 24.01.2009 jointly with DSR, Mau. The farmers were provided first hand information about the beneficial microorganisms being used in agriculture such as biofertilizer, biopesticides, decomposers etc. Special emphasis was given on the use of *Rhizobium*, Pulse crop and use of *Trichoderma* in vegetables.

Kisan Mela jointly organised by NBAIM & DSR, Mau

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Forthcoming Events

1. National Training program on "The evolutionary diversification of cyanobacteria : Biochemical, Molecular and Phylogenetic Approaches" from July 14 to 19, 2009.

   Theme areas to be covered in the National Training are :

   Morphological, physiological and biological characteristics & growth measurements; Chemotaxonomic approach - Metabolic profiling and bioactivity evaluation; Molecular evolution, diversification and DNA profiling : Isolation of genomic DNA and quantification; Primer design and amplification procedures for phycocyanin IGS and 16S-23S rDNA ITS; Restriction endonuclease digestion of phycocyanin IGS and 16S-23S rDNA ITS; Taxonomic discrimination and Phylogenetic analysis of RFLP; Development of molecular probes for detection of cyanobacteria in different habitats; and Bioinformatic used in the identification of cyanobacterial strains.

   Both lectures from renowned cyanobacteriologists and microbial biotechnologists followed by practical exposure on the above theme areas will be included. Several resource experts from different parts of the country are likely to address the participants. The training will strengthen human resource for identification and microbial diversity analysis.

2. "Farmer Interaction Program" for skill development among farmers on *Trichoderma* based biopestisides, microbial inoculants and Blue-Green Algae based biofertilizers on 14 August, 2009.

3. Summer school on "Recent Advances in Molecular Identification and Characterization of Agriculturally Important Microorganisms" from Sept. 01 to 21, 2009.

   The summer school on Recent Advances in Molecular Identification and Characterization of Agriculturally Important Microorganisms aims at equipping the trainees with the latest development in characterization and identification of microorganisms and infusing competence to set up the bio-agent utilization them effectively. The course content includes lectures and laboratory modules. The trainees will also be exposed to traditional and modern tools for identification of bio-control agents and their molecular characterization.

4. National Training on "Molecular approaches for Identification and Characterization of Actinomycetes" from December 1-10, 2009. This training program will provide critical insights into the psychological, biochemical and molecular approaches for the greater understanding of Morpho-, chemo- and molecular taxonomy and diversity of Actinymycetes.

Staff Joined

Sh. Alok Upadhyay
T-I

Sh. Rajkumar Meena
T-I (Driver)

Staff Transferred

Dr. A. K. Saxena
Principal Scientist
Joined as Head, Division of Microbiology
IARI, New Delhi

Sh. Devendra Fuloria
Junior Clerk
Transferred to ICAR, Krishi Bhavan
New Delhi

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