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Annual Report 2013-14

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Indian Council of Agricultural Research

NBAIM
रा.कृ.उ.सू.ब्यू.

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो

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Preface

The microbial world is the largest unexplored reservoir of biodiversity on the Earth. It is a frontier area in biology under intensive investigations. The microbial community is a gold mine for genes and pathways that encode novel biocatalysts for biosynthetic or biodegradation processes, including degradation of pollutants, synthesis of biofuels and production of novel drugs. The vast majority of global microbial diversity was inaccessible or largely underestimated. However, Microbial diversity is the largest untapped resource for understanding how biological systems function as well as for new biotechnologies. The uniqueness of microorganisms and their often unpredictable nature and biosynthetic capabilities, given a specific set of environmental and cultural conditions, has made them likely candidates for solving particularly difficult problems in the life. With improved methods for analysis, funding stimulated by recent triumphs in the field, and attraction of diverse scientists to identify new problems and solve old ones, the branch of science will expand and continue to enrich our understanding of microorganisms.

Microbes have remained the oldest unseen life forms on the earth and pioneer colonizers of different habitats with their cosmopolitan occurrence. They are natural inhabitants of normal and sub-normal ecological niches right from the extremes of hot and cold situations, pressure, pH, drought, acidic and saline stresses, etc. They inhabit mountain tops, the prairies and the plains, and thrive well even when exposed to high wind velocities and open sunlight conditions. Their versatility in the biosphere in almost all forms of plant- microbe-animal assemblages that has the capacity to regulate natural biological cycles and nutrient dynamics is essential for the sustenance of the life. The inherent functional properties of microbial communities essentially make them important in agricultural ecosystem and environment. Soils, being the most natural inhabitant of countless microbes are biological entities, not only because of their base presence, but because of multitrophic interactions that the inhabiting microbes create with several other natural flora and fauna. Diversity of microbial communities in the soil and other habitats is the most important asset for the crops as this has many implications from building soil bio-physical network and improving chemical characteristics to strengthening biological functions. This is why, for agriculture, microbial diversity is one of the most fundamental aspects to maintain and sustain natural processes and conserve global microbial genetic resources.

Microbial communities have remained the most vital component of ecosystem function. Diversity of microbial communities encompasses total number of species or overall species richness along with the richness of the functionalities that these communities cover during their existence with the expression of their genome. In agro-ecosystem, microbes exist in the form of diverse communities in the soil, air, water and plants (epiphytic and endophytic conditions). Even with the most modern scientific approaches, we are able to explore less than 1% of the total existing microbial population on the earth and the rest poses challenge for the scientific community. Huge number of microbes are of agricultural importance and their role beneath the soil and with the plants can not be ignored. Therefore, a first hand great task is to conserve and make the native communities flourish well in agro-ecosystem by making the agricultural practices favourable for growth and proliferation which can only be done at the farmer's level who is the major stakeholder. The conservation and management of microbial communities in the soils is the biggest challenge before all the stakeholders, i.e., the scientists, extension workers and farmers. This cannot be achieved without a proper well managed plan regarding the cropping patterns, application of farm inputs and knowledge about the native

population and methods to regenerate native microbial population. A global approach for the assessment of microbial diversity lies in the identification of the culturable life forms, characterization of the metagenomes from the environmental samples and illustration of the functional properties of the microbes that can have major impact on ecology, climate and soil system phenomena. Therefore, the issues of microbial diversity covers all related issues like key concepts and methods defining the nature of microbial species, the use of microscopic, cultural, molecular and phylogenetic systematics leading to the studies on evolutionary diversification, environmental impacts, biogeochemical cycles and species interactions. Since inception, National Bureau of Agriculturally Important Microorganisms (NBAIM) has dedicated itself in deciphering microbial diversity existing all across the country and finding out functions of microbes for the benefit of agriculture. Other emerging research areas under this vital task like plant microbe interaction, development of biocontrol agents, microbial molecular biology and omics science (genomics, proteomics, metabolomics) as well as bioinformatics have also been addressed to uncover the challenges in structural and functional analysis of microbial communities and their conservation for future agriculture.

I would like to extend my sincere thanks to Dr. S. Ayyappan, Secretary (DARE) and Director General (ICAR), Dr. S. K. Dutta, DDG (CS), Dr. P. K. Chakarbarty, ADG (PP) and Dr. T. P. Rajendran, former ADG (PP), for encouragement and valuable guidance in our endeavour of microbial diversity conservation at NBAIM. I extend my appreciation to the scientific and technical staff who have come up with this Annual Report 2013-14 in a very attractive and informative manner.

Arun Kumar Sharma
Director

Executive Summary

The Bureau was established with the major objective of collection, preservation, conservation and utilization of microbial diversity in the country. The rationale to establish an ex-situ collection of agriculturally important microorganisms is due to the recognized role of microorganisms in agriculture and closely related sectors, environment and human welfare. Not only are they global players in the metabolism of nitrogen, phosphate, oxygen and carbon, but many are of immense scientific and economic benefits in the form of resources as biomolecules, drugs and bioenergy. Considering the importance of microorganisms, the Bureau has prioritized the research in the area of :

- Isolation of extremophiles from extreme environments
- Exploration and collection of AIMs from: soil, plants and freshwater from different agro-climatic regions.
- Procurement of AIMs from existing culture collections
- Diagnostic kit development and molecular taxonomy
- Decoding of whole microbial genome & functional genomics/metagenomics/ bioinformatics
- Plant microbe interactions & rhizoengineering
- Augmentation of cultures through exchange from various national Microbial Resource Centres (MRCs)
- Development of state-of-the-art microbial gene bank
- Initiation of research in the area of bioenergy and biofuels
- Nutrient mobilizers/ nanotized bioformulation
- Biotransformation & agrowaste management/ bioremediation and bioleaching/ fermented products/ microbial food supplements
- Human Resource Development
- Issues related to biosafety and intellectual property rights.

In the year 2013-14, the microbial culture accessions deposited in NAIMCC, NBAIM has reached around 5000. To safeguard these cultures from any unforeseen menace situation, a safety storage facility created at NBPGR, New Delhi is being strengthened and equipped with cryopreservation facilities. A set of lyophilized cultures and glycerol stock has been kept as safety duplicate of the collection. Another remarkable achievement is the establishment of high performance computing (HPC) facilty, the infrastructure has been established at NBAIM to cater to the need of high performance computing in the field of agricultural bioinformatics and computational biology under National Agricultural Bioinformatics Grid (NABG) project of NAIP. The supercomputing hub consists of hybrid architecture of high performance computing environment. Bioinformatics resources at NBAIM include 16 node Linux clusters with each of 96 GB, one master node and total storage capacity of 126TB connected with three workstations. The facility is supported by CLC Genomics workbench, Discovery Studio package and more than 100 freely accessible softwares to perform high end bioinformatics research at NBAIM.

At present in the Bureau, ten institute projects, one inter institutional project and five externally funded projects are running which are mainly focused in the area of diversity mapping of specific group of microorganisms, their characterization, biocontrol and plant growth potential and barcoding. NBAIM is the nodal unit for the network project Application of Microorganisms in Agriculture and Allied Sectors, 'AMAAS', which is SFC linked plan scheme of NBAIM continuing since Xth plan period running over 68 centers throughout the country. Under the project, structural and functional diversity of varied

agro-ecological Indian regions, including Indo-gangetic plains, drought affected areas, eastern and western Himalayan regions and other extreme conditions have been studied last year in order to explore microbial communities present over there and their functionalities. Soil metagenome from Leh has been submitted to NCBI genome database.

The hypersaline niches are the prominent source of haloarchaea, 17 haloarchaea morphotypes isolated from Bhayander and Meera Road salt pans, Mumbai were characterized morphologically and through 16S rRNA gene . Promising bacterial antagonistic isolates preliminary screened under laboratory against different soil borne pathogens of vegetable crop were evaluated for the presence of different antibiotic gene important in suppression of plant pathogens. Black rot diseased samples from different host plants, like cauliflower, cabbage and rai from different locations in India were collected and isolation of *Xanthomonas campestris* pv *campestris* (Xcc) and their characterization by polyphasic approaches via morphologically, biochemically, carbon source utilization pattern as well as at molecular level in addition to in planta pathogenicity test and host range was completed. Different cyanobacterial strains of *Oscillatoria acuta*, *Calothrix geitonus*, *Anabaena doliolum* and *Nostoc cornium* were evaluated for NaCl stress (100, 200, 300 and 400 mM NaCl concentration) in relation to growth, metabolic changes and antioxidant properties of the extracts and culture filtrates. Varied accumulation of phenolic acids and flavonoids (gallic, trans-chlorogenic, caeffic, vanilic, ferulic, salicylic and cinnamic acids), along with the flavonoids quercetin hydrate, naringenin and kaempferol was observed in stressed cultures. Change in phenylpropanoid metabolites was correlated with stress tolerance level of cyanobacterial strains.

Computational Mining and Genome Wide Distribution of Microsatellite in *Fusarium oxysporum* f. sp. *lycopersici* was studied for the genome wide distributional pattern of SSRs motifs. The identification and phylogenetic analysis of *Alternaria* spp. on the basis of ITS sequence is not able to differentiate the closely related species of *Alternaria*. Therefore, the work has been initiated on Multilocus sequence typing, using *Alternaria brassicae* (15), *A. brassicicola* (10), *A. porri* (09) and *A. sesame* (09) with ITS, Beta tubulin, Histone-3, EF-alpha gene specific primers. Sequencing of the microbial genome will also provide insights into the ecology of microorganisms that are beneficial to, or threaten, crop production, and that ensure the quality and provision of ecosystem services.

NBAIM provides training on cutting age technologies in the area on microbial identification, diversity analysis through culture dependent and culture independent techniques, and microbial interactions for its exploitation in abiotic and biotic stress management. During 2013-14, four training programmes were organized. NBAIM is consistently enhancing the efficiency and creating awareness regarding application of microorganisms in sustainable agriculture in the country.

कार्यकारी सारांश

आधुनिक शोध अनुमानों के अनुसार इस पृथ्वी पर सूक्ष्म जीवों की जैव विभिन्नता सर्वाधिक है। जिसका अधिकांश भाग अभी तक अन्वेषित नहीं है। वर्तमान में सूक्ष्म जैव विभिन्नता में अन्वेषण के सघन शोध कार्यक्रम चलाये जा रहे हैं। सूक्ष्म जीव, जैव रासायनिक, चक्रण एवं पर्यावरण संरक्षण में महत्वपूर्ण भूमिका निभाते हैं। अतः सूक्ष्म जीव जैव मण्डल में सूचारु रूप से संघटन हेतु अति महत्वपूर्ण हैं। सूक्ष्म जीवों की असीमित उपापचयिक क्षमता, जैव मण्डल एवं मानव आर्थिकी में इनकी भूमिका के कारण इनमें जैव विभिन्नता का अध्ययन, संरक्षण एवं समाज हेतु सन्तुलित दोहन महत्वपूर्ण है। लाभदायक सूक्ष्म जीवों का खेती में प्रयोग पृथ्वी के पारिस्थितिक तन्त्र को बनाये रखने में सहायक होता है। सूक्ष्म जैव विभिन्नता का पौधों की विभिन्नता से सीधा सम्बन्ध है। भिन्न-भिन्न प्रकार के जटिल रासायनिक परिवेश में उगने वाले भिन्न-भिन्न पौधों की जड़ों में पाये जाने वाले सूक्ष्म जीवों का संघटन इनके प्रकार, संख्या एवं समुदाय के आधार पर भिन्न होता है और यह भिन्नता जीवन-विज्ञान के लिये स्वर्ण खदानों की तरह है। इनके अध्ययन से जैव संश्लेषण अथवा विखण्डन हेतु नये प्रकार के जैव उत्प्रेरक, प्रदूषकों के विघटन, जैव ईंधन में संश्लेषण, नये कृषि रासायनों एवं औषधियों के निर्माण हेतु नये जीन एवं संश्लेषण चक्रों का पता लगाया जा सकता है।

राष्ट्रीय कृषि उपयोगी सूक्ष्म जीव ब्यूरो की स्थापना सूक्ष्म जीवों के संग्रहण एवं देश की सूक्ष्म जैव विभिन्नता के उपयोग के लिये की गयी है। इन सूक्ष्म जीवों के संग्रह के पीछे इनका कृषि एवं तत्सम्बन्धी क्षेत्रों तथा पर्यावरण एवं मानव कल्याण में इनका उपयोग है। स्पष्टतया ये नाइट्रोजन, फॉस्फोरस, ऑक्सीजन एवं कार्बन के चक्रण के अतिरिक्त उपयोगी जैव अणु, औषधि एवं जैव ऊर्जा के स्रोत हैं। सूक्ष्मजीवों के महत्व एवं उपयोगिता को देखते हुए ब्यूरों ने निम्न क्षेत्रों में प्राथमिकता निर्धारित की है।

- अतिवादी परिवेश से सूक्ष्म जीवों का पृथक्करण
- मृदा, पौधौं एवं विभिन्न कृषि पर्यावरणीय परिवेशों से सूक्ष्मजीवों का अन्वेषण एवं संग्रहण
- अन्य सूक्ष्मजीव प्रवर्ध केन्द्रों से कृषि उपयोगी सूक्ष्मजीवों की प्राप्ति
- जैव आणविक पहचान-किट का विकास
- सूक्ष्मजीवों का पूर्ण जीन अनुक्रमण, कार्यकारी जीनोमिक्स/मेटाजीनोमिक्स एवं बायोइन्फॉर्मेटिक्स
- पादप सूक्ष्मजीव पारस्परिक सम्बंध एवं राईजोइंजीनियरिंग
- विभिन्न जैव सम्पदा केन्द्रों से विनिमय आधारित सूक्ष्मजीव संवर्धों की वृद्धि
- अत्याधुनिक सूक्ष्मजीव जीन बैंक का विकास
- जैव उर्जा एवं जैव ईंधन हेतु शोध
- नैनो बायोफार्मुलेशन एवं पोषक तत्वों का संचालन
- जैव रूपांतरण एवं कृषि अपशिष्ट प्रबन्धन/बायो रेमिडियेशन एवं बायोलीचिंग / किण्वन उत्पाद एवं सूक्ष्मजीव आधारित भोजन
- मानव संसाधन विकास
- जैव सुरक्षा एवं बौद्धिक सम्पदा अधिकार से जुड़े बिन्दु

गत वर्ष 2013-14 में ब्यूरो स्थित राष्ट्रीय कृषि उपयोगी सूक्ष्म जीव संग्रहण केन्द्र (NAIMCC) में संग्रहीत सूक्ष्मजीव प्रवर्धों की संख्या लगभग 5000 है। इनकी किसी आमसयिक परिस्थिति में सुरक्षा हेतु, इनकी एक द्वितीय प्रतिरूप राष्ट्रीय पादप आनुवंशिक संसाधन ब्यूरो नई दिल्ली में विकसित भण्डारण केन्द्र में रखी गयी है तथा इस भण्डारण केन्द्र को क्रायो संरक्षण क्षमता हेतु विकसित किया जा रहा है। वर्तमान में यहाँ पर लायोफिलाइज्ड एवं ग्लीसराल में संरक्षित प्रवर्धों को रखा गया है। गत वर्ष ब्यूरो में एन.ए.आई.पी. द्वारा पोषित 'राष्ट्रीय कृषि जैव सूचना ग्रिड' परियोजना के अन्तर्गत उच्च क्षमता आधारित कम्प्यूटिंग सुविधा का आरम्भ हुआ। यह सुपर कम्प्युटिंग केन्द्र संकर विन्यास आधारित संगणन आधार पर कार्य करता है। इस सुविधा के अन्तर्गत 16 नोड का 96 गिगा बाईट क्षमता (प्रत्येक) का लाइनेक्स गुच्छ, एक मुख्य नोड तथा कुल 126 टेराबाइट संग्रहण के क्षमता के तीन कार्य स्टेशन लगाये गये हैं। इस सुविधा के संचालन हेतु सी.एल.सी. जिनोमिक्स, डिस्कवरी स्टुडियो पैकेज एवं 100 से अधिक विमुक्त सॉफ्टवेयर जैव सूचना शोधकर्ताओं हेतु उपलब्ध

है। वर्तमान में ब्यूरो में 10 संस्थान परियोजना / 1 अर्न्तसंस्थान परियोजना / 5 वाह्य वित्त पोषित परियोजनायें संचालित हो रही हैं। ये सभी परियोजनायें मुख्यतया सूक्ष्मजैव विभिन्नता निरूपण, इनके गुण चिह्नाकंन, जैव नियंत्रण एवं पादप वृद्धि वर्धन संभावनायें एवं डीएनए बार कोड के क्षेत्र में केंन्द्रित हैं। रा.कृ.उ.सू. ब्यूरो कृषि एवं तत्संबंधी क्षेत्रों में सूक्ष्मजीवों का अनुप्रयोग नामक संजाल परियोजना का नोडल इकाई है यह परियोजना ब्यूरो के एस.एफ.सी से जुड़ी योजनागत परियोजना है जो कि दसवीं पंचवर्षिय योजना से 68 केन्द्रों पर संचालित हो रही हैं। इस परियोजना में भारत के विभिन्न कृषि पारिस्थितिकी क्षेत्रों जैसे - गंगा के मैदानी क्षेत्र, सूखा प्रभावित क्षेत्र, पूर्वी एवं पश्चिमी हिमालय क्षेत्र एवं अन्य अतिवादी परिवेश में पाये जाने वाले सूक्ष्मजीवों के संरचना एवं कार्यिकी विभिन्नत का अध्यन्न गत वर्षों से किया जा रहा है। गत वर्ष लेह के मृदा मेटाजिनोम को एन.सी.बी.आई. जीनोम डाटाबेस में जमा किया गया।

उच्च लवणीय सान्द्रतायुक्त मीरा रोड एवं भएन्दर मुम्बई के नमक क्षेत्रों से 17 हैलोआर्किया विलगित किये गये। जिनका आकारिकी एवं 16 S राइबोसोम जीन आधारित गुण चिह्नांकन किया गया है। कुछ जैव कीटनाशी क्षमतायुक्त जीवाणुओं का प्रयोगशाला में सब्जियों पर लगने वाले रोग कारकों के ऊपर परीक्षण किया गया। पत्तागोभी, गोभी एवं अन्य सब्जियों में होने वाले ब्लैकराट रोग के रोगजनक जीवाणु जैन्थोमोनास कम्प्रेस्टिस पी.वी. कम्प्रेस्टिस के गुण चिह्नांकन हेतु बहुचरणीय पद्धति जो कि आकारिकी, जैव रसायन, कार्बन स्रोत उपयोग, पौधों में रोग उत्पन्न करने की क्षमता इत्यादि पर आधारित को प्रयोग किया गया। आसिलैटोरिया एक्यूटा, कैलोथ्रिक्स गिटोनम, एनाविना डोलिओलस एवं नास्टाक कार्नियम प्रजातियों के विभिन्न नील हरित शैवालों को लवण न्यूनता स्थितियों (100, 200, 300 एवं 400 एम.एम. नमक) में वृद्धि, उपापचयिका का परिवर्तन एवं प्रतिआक्सीकरण क्षमता हेतु आंकलित किया गया। न्यूनता प्रभावित प्रर्वधों में फिनोलिक अम्ल एवं फ्लैवोनायड (गैलिक, ट्रांसक्लोरोजेनिक, कैफीन, वैनिलिक, फेरूलिक, सैलीसिलिक एवं सिनैमिक अम्ल) तथा फ्लैवोनायड (क्वरसीटिन हाइड्रेट, नार्निजेनिन एवं केम्पफराल) पाये गये। इनके फेनिल प्रोपेनायड मेटाबोलाईट में परिर्वतन एवं लवण न्यूनता सहिष्णुता में सीधा सम्बंध पाया गया।

उकठा रोगजनक फ्यूजेरियम आक्सीस्पोरम लाइकोपसिसि के जीनोम में एस.एस.आर. मैटिफ युक्त माइक्रोसेटेलाइट मार्कर की पहचान की गयी। आई.टी.एस. आधारित डी.एन. अनुक्रम एवं विभिन्न बिन्दु (Locus) पर आधारित गुण चिह्नांकन पद्धति केद्वारा अल्टरनेरिया की प्रजातियों, अल्टरनेरिया ब्रैसिकी (15), अल्टरनेरिया, ब्रैसिकाला (10) अल्टरनेरिया पोरी (09) एवं अल्टरनेरिया सिसेमी (09) का अध्ययन एवं चिह्नांकन आई.टी.एस., बीटा ट्युबूलिन, हिस्टोन 3 एव ई.एफ. अल्फा जीनके माध्यम से किया गया।

रा.कृ.उ.सू. जीव ब्यूरो वैज्ञानिक क्षमतावर्धन एवं मानव संसाधन विकास के तहत सूक्ष्म जीवों की पहचान, जैव विभिन्नता, अध्ययय, अजैविक एवं जैविक न्यूनता प्रबन्धन इत्यादि के क्षेत्र में प्रतिवर्ष प्रशिक्षण कार्यक्रम एवं कार्यशालाओं का आयोजन करता है। गत वर्ष 2013-14 में कुल 4 प्रशिक्षण कार्यक्रम आयोजित किये गये।

Infrastructure

Indian Council of Agricultural Research (ICAR) has taken a visionary and thoughtful step for long term conservation of agriculturally important microorganisms (AIMs) by establishing National Bureau of Agriculturally Important Microorganisms (NBAIM). The mandate of the Bureau is to act as a nodal center at national level for acquisition and management of indigenous and exotic microbial genetic resources for food and agriculture. The Bureau has well equipped research laboratories, central instrumentation facility, separate fungal and bacterial labs, molecular biology lab, genomics lab, lyophilization unit, culture collection facility including cyanobacteria, microbial genome resource repository (MGRR), administrative block, library, conference hall and mini conference room equipped with audio-visual equipments and agricultural knowledge management unit (AKMU), etc. The entire campus as well as laboratories is under electronic surveillance system. Tube wells and heavy duty power generators have been installed in campus for assured water and electricity supply around the clock. Furthermore, smooth functioning of laboratories including NAIMCC unit has been ensured by putting high power DG set installed in campus.

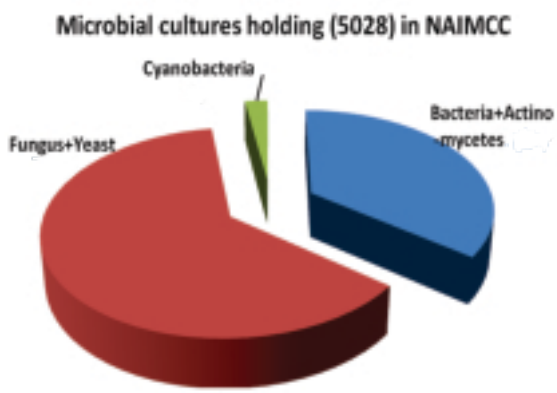
National Agriculturally Important Microbial Culture Collection (NAIMCC)

Microorganisms play a vital role in agriculture such as promotion of plant growth, suppression of plant pathogens, conversion of organic matter and maintenance of ecological balance. They represent the richest diversity in nature and comprise the most diverse forms of life. This microbial biodiversity is a crucial component of the agro-ecosystems. The need to conserve and manage the biodiversity of microorganisms of food and agricultural importance necessitated the development of integrated sustainable management strategies that conserve this resource for the future and enhance the delivery of ecosystem services. With a view to conserve microbial heritage of country, the ICAR developed National Agriculturally Important Microbial Culture Collection (NAIMCC) as one of the units of NBAIM for collection, maintenance, conservation and supply of microorganisms all over the country. Biodiversity Authority of India has recognized NBAIM culture collection (NAIMCC) as the National Repository. It also offers the facility for registration of elite microbial germplasm to facilitate the flow of such germplasm among scientist under MOU for further research. World Federation for Culture Collections (WFCC) developed an international database named as "World Data Center for Microorganisms (WDCM)" and presently this data resource is maintained at National Institute of Genetics (NIG), Japan. This database has records of nearly 660 culture collections from 70 countries and the records contain data on the organization, management services and scientific interests of the collections. The NAIMCC has been added in the database (WDCM 1060; www.wfcc.info/ccinfo/collection/by_id/1060) in the year 2014 and these biological resource centers (BRCs) may be the preferred mechanism for the appropriate exploitation of microbial resources by offering the guarantee of accessibility and transparency of supply, taking into account all relevant regulations and stakeholders rights, as required by the Convention on Biological Diversity (CBD).

Various types of agriculturally important microorganisms (AIMs) including fungi, bacteria, actinomycetes and cyanobacteria are maintained at NAIMCC under short term as well as long term storage conditions. Since cyanobacteria are photosynthetic organism, they are maintained in a dedicated growth chamber by providing the required growth conditions. NAIMCC has state-of-the-art technologies for short term and long term conservation of microorganisms. AIMs are conserved and maintained by at least two methods depending on

type of microorganism, i.e., short term preservation through storage at 4ºC and mineral oil stocks (5-10 years), and long term storage through lyophilization (20-25 years) under vacuum at -60ºC and glycerol stock at -80ºC. At present NAIMCC has around 5000 microbial cultures (Fungus & Yeast, 3137; Bacteria & Actinomycetes, 1763; Cyanobacteria, 125) and they have been published in its first catalogue in 2009, second edition in 2011 and third edition is planned to be published in this year. For cultivation, characterization and preservation of microbes, the facilities available in NAIMCC are bright field microscope, confocal laser microscope, scanning electron microscope (SEM), cold room, deep freezer (-80ºC) growth chamber for cyanobacteria and lyophilizer.

NAIMCC have been digitized for the retrieval of information and for this, software Microbial Culture Collection Database (MCCD) has been developed by the Bureau to list the characteristics of AIMs in terms of origin, ecology, morphology, physiology and biochemical parameters, pathogenic or non-



pathogenic and molecular tools are being used for the characterization of AIMs. Also variety of data related to isolates can be accommodated in fields like passport data information, geographical location of isolation, name of the donor (person or institute) or depositor and the form in which it is preserved, etc. This software is developed for rapid searches and to facilitate communication between database and the user.

A separate storage facility of microbial cultures, an axillury unit of NAIMCC, was established in January, 2013 in the building of NBPGR, New Delhi and is being maintained by scientists of NBAIM. The aim of this unit is to safe guard the microbial gene pool from unforeseen natural calamities. The important equipments and apparatus are housed in the unit to carry out preservation work. To initiate work on cryopreservation of microorganisms, temperature gradient system along with other essential items had been transferred to this unit keeping in view the expertise and availability of liquid nitrogen at NBPGR.

LIBRARY

The NBAIM library is enriched with about 1966 books on various subjects like microbiology, biochemistry, plant pathology, environmental science, genetics and genomics, administration, botany, bioinformatics, IPR, molecular biology, nanotechnology, mycology, proteomics, statistics, soil biology, virology, etc. It has collection of about 43 scientific journals/ periodicals. Publications by NBAIM, like newsletters, annual reports, vision document, laboratory manuals, etc. are available in the library. Besides this, other miscellaneous literature like annual reports and newsletters from various organizations, ICAR news and bulletins are also maintained and can be accessed by users. Library is equipped with internet facility and has an access to many international and national journals *via* CERA (Consortia for e Resources in Agriculture) maintained at AKMU/ARIS cell. Library also has subscription to one weekly and four daily newspapers.

Agricultural Knowledge Management Unit (AKMU)

ARIS cell of NBAIM was renamed as AKMU as per ICAR guidelines (F.No. 3-1/2010-GAS (DIPA) dt 27 January, 2011. A static IP fully dedicated to access many national and international journals through Consortium of e-resources in Agriculture (CERA) is being maintained for users of library facility at NBAIM. To strengthen the ICT infrastructure for learning and capacity building including implementation of Management Information System (MIS)- Financial Management System (FMS) a fund of Rs 25 lakhs was released under non recurring head. In order to strengthen the ICT infrastructure of NBAIM desktop computers (25), laser printers (5), online UPS (5), multimedia projector (1) and extension cum training kit (1) was procured. Besides this, in the whole institute, including guest house, networking was done through LAN as well as wi-fi. Also an ERP solution for ICAR is being implemented under NAIP sub-project "Implementation of Management information System (MIS) including Financial Management System (FMS) in ICAR" in which after ICT infrastructure strengthening, data digitization

will be done in which migration of records from register/service books to digital format in the data templates designed for different solutions will be done. AKMU is currently being renovated in terms of it having sitting capacity while accessing e resources as well as utilizing the space as training centre also.

IPR

IPR cell established at NBAIM for the management of intellectual knowledge and technologies generated at NBAIM is equipped with wealth of information on IPR. The Bureau is making efforts to identify, register and document the novel microorganisms, genes, and microbiological processes for patents as per the ICAR and other GOI guidelines. The manual of patent practice and procedure of Indian patent office is also being applied as guidelines for the Bureau as per ICAR guidelines. ICAR Guidelines for Intellectual Property Management and Technology Transfer/ Commercialization is being followed at the Bureau.

NBAIM WEBSITE

The NBAIM website (*www.nbaim.org.in*) has been designed, based on the ICAR guidelines for uniformity of website, updated information about various activities of the Bureau in different profiles, *viz*., mandate, about the Bureau, culture collection, scientific plan, gene bank, library, future activities, etc. A list is also displayed about available agriculturally important fungi, bacteria and actinomycetes at culture collection with information regarding utility, preservation and conservation. Currently the website is being updated and is under revision to provide the latest information in various heads.

MGR PORTAL

Microbial Genetic Resource Portal (*www.mgrportal.org.in*) developed and maintained by in house team of ARIS/AKMU of NBAIM, contains information pertaining to conservation of microbial genetic resource with special reference to agriculturally important microbes in National Agriculturally Important Microbial Culture Collection (NAIMCC) housed at National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, Uttar Pradesh. The portal is informative in various aspects of microbial conservation, microbial diversity with special reference to India, need for microbial genetic resources conservation and International and National status of conservation of microbial genetic resources, list of different federations, societies and networks of Microbial Resource Centres in the world, some leading culture collections in the world, some important microbial culture collections in India and microbial repositories in India recognized by National Biodiversity Authority (NBA), India. Details on conservation and management of AIMs at NAIMCC, various services offered by NAIMCC, dynamic database search engine, and accessibility to catalogue, microbial registration facility, easily downloadable forms for various purposes and linkages to important sites can also be accessed. The portal was launched by Dr. R.S. Paroda, Chairman, of National Advisory Board for Management of Genetic Resources and Dr. S. Ayyappan, Co- Chairman, of National Advisory Board for Management of Genetic Resources and Secretary, DARE & Director General, ICAR in 4th Meeting of National Advisory Board for Management of Genetic Resources at National Bureau of Agriculturally Important Insects (NBAII), Bengaluru on 10th October, 2013.

Microbial Genomic Resource Repository (MGRR)

Microbial Genomic Resource Repository (MGRR) has been established with the aim for collection and long term conservation of genomic resources, like microbial DNA, clones, novel gene constructs vectors, etc. The different forms of microbial genetic material, e.g., DNA, RNA, cDNA, mRNA, plasmid, cosmid, primer and vector, etc., can be utilized for further research in agriculture in many ways like, for the enhancement of the soil fertility, crop production, crop quality and their resistance to diseases. In XIIth plan, MGRR has the following objectives : Nation wide survey and Collection of information about Microbial Genetic Resources, characterization, validation and molecular typification of reference microbial cultures, and generation of molecular dataset and generation of barcode as reference, exploration for collection of environmental microbial samples from different agroclimatic regions and direct DNA isolation through metagenomic approaches; collection of DNA materials from microorganisms and other relevant organisms which result from the various molecular genetics and genomics research programmes; acquisition of gene constructs from various sources, and production/multiplication and quality control for distribution.

Guest House

NBAIM is having a well furnished Guest House with 10 rooms including two Suites and a well managed Transit House. The charges for accommodation are according to the ICAR norms.

Research Achievements

Project : Characterization of beneficial rhizobacteria and its role in induced systemic resistance (ISR) and horizontal resistance in plants

PI : Alok Kumar Srivastava
Co PIs : Sudheer Kumar and Prem Lal Kashyap

Rationale

The direct effects of the rhizosphere microorganisms on plant growth and development are crucially important for agricultural uses and for understanding the roles of these micoorganisms in natural and managed ecosystems. Root colonization by PGPRs frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients. Plant diseases play a direct role in the destruction of natural resources in agriculture. In particular, soil-borne pathogens cause important losses, fungi being the most aggressive. The distribution of several phytopathogenic fungi has spread during the last few years due to changes introduced in farming, with detrimental effects on crops of economic importance. Chemical compounds have been used to control plant diseases (chemical control), but abuse in their employment has favored the development of pathogens resistant to fungicides. By contrast, the use of microorganisms that antagonize plant pathogens (biological control) is risk-free when it results in enhancement of resident antagonists. The ability of antagonistic strains to protect plants against root pathogens has long been attributed to an antagonistic effect against the invasive pathogen. However, these plant-bacteria associations also stimulate plant defensive mechanisms, which points to the induction of resistance mechanisms similar to the hypersensitive response (HR) and induced systemic resistance (ISR) in plants.

Objectives

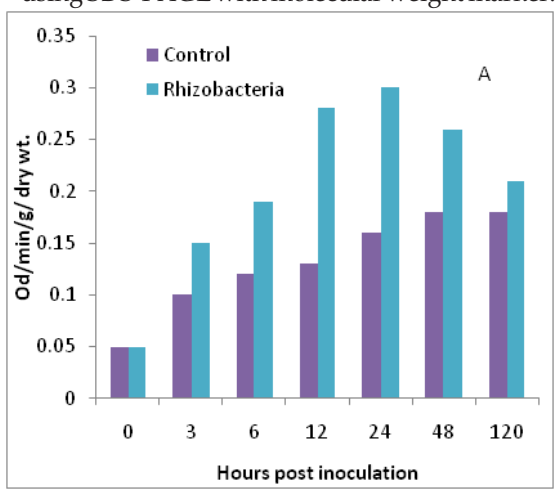
- Induction through rhizobacteria treatment and challenge inoculation
- Histochemical staining of phenolic compounds
- Extraction of glycoside-linked phenolics
- Bioassay and analysis of glycoside-linked phenolic compounds
- Analysis of acidic chitinase

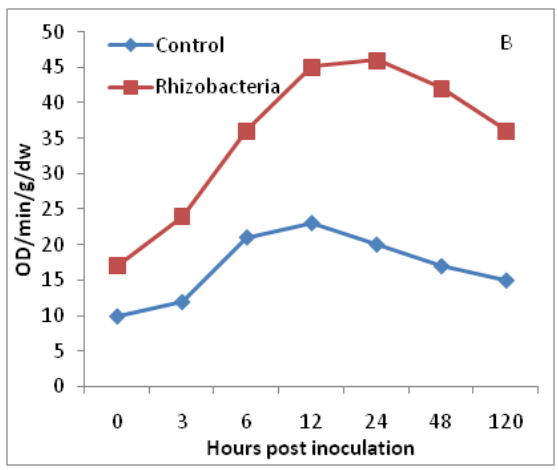
Achievements

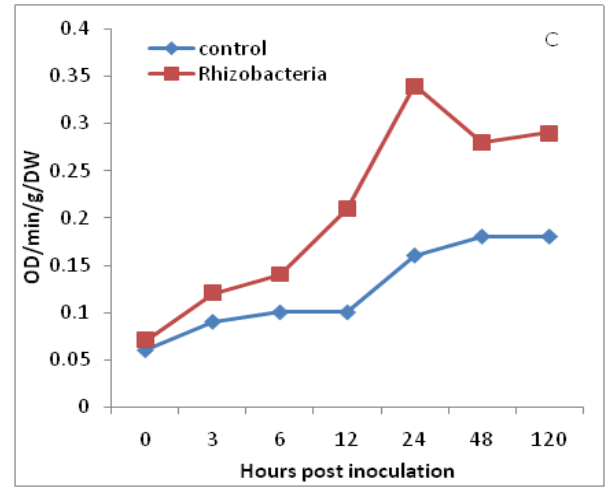
- The level of defensive enzymes *viz*: chitnase and PPO and phenolic compounds in the cucumber plant treated with a potential *Pseudomonas fluorescens* isolate LH 1 obtained earlier in the study, and challenge inoculated with *Rhizoctonia solani* was evaluated.
- Higher peroxidise activity was recorded in the leaves after 3 hrs post inoculation of rhizobacteria which increased significantly till 48 hrs after inoculation. The level of PO ranged higher up to 195-330% with treatment over control. The enzyme activity is expressed as the changes in absorbance min^{-1} mg^{-1} protein.
- The polyphenol oxidase in the leaves was determined up to 5 days post inoculation through colorimetric assay. The reaction mixture contained 0.2 ml leaf extract in 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5), and 0.2 ml of 0.01 M catechol was added to start the reaction. The PPO activity is expressed as the change in absorbance at 495 nm min^{-1} g^{-1} f.wt. A 2.25 to 2.5 fold increase in the level of PPO was recorded after 12-36 hours post inoculation after which a gradual decrease was observed.
- Phenylalanine ammonia lyase (PAL) activity was determined in the leaves as the rate of conversion

of L'Phenylalanine to cinnamic acid at 290 nm. The amount of cinnamic acid synthesized was calculated and enzyme activity is expressed as µg cinnamic acid min^{-1} g^{-1} f.wt. Maximum activity was recorded at 36 h post inoculation which gradually decreased and stabilized afterwards.

- The level of phenolic compounds in cucumber leaves inoculated with rhizobacteria and cross inoculated with *R. solani* was significantly higher in plants treated compared with that in control plants. Throughout the sampling period, the accumulation of phenolic compounds in *P. fluorescens* -treated cucumber plants was always found to be higher than those in control plants.
- Higher chitinase activities were observed in cucumber plants treated with rhizobacteria. The chitinase was purified by $(NH_4)_2SO_4$ precipitation followed by dialysis and column purification. The molecular weight was determined as 39 kDA using SDS-PAGE with molecular weight marker.







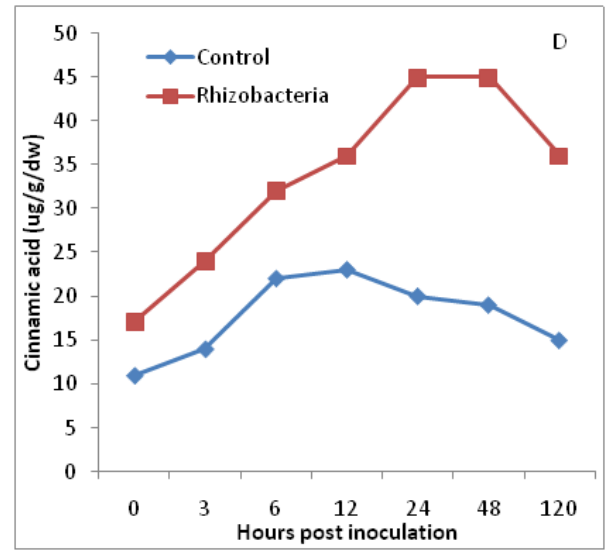


Fig: Estimation of (A) Peroxidase, (B) Polyphenol Oxidase, (C) PAL and (D) Total Phenolics in cucumber leaves inoculated with *P. fluorescens* and challenge inoculated with *R. solani*.

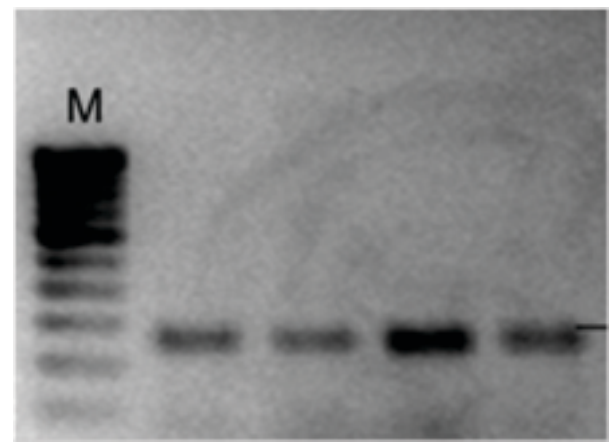


Fig: Purification of Chitinase

Conclusion

Elicited by a local infection or nonpathogenic inoculation, the cucumber plant responded that may lead to the systemic expression of defense related enzymes, changes in cell wall composition, production of pathogenesis related proteins such as chitinases, and synthesis of phytoalexins are associated with resistance. Resistance mechanisms attain their maximum electiveness at three to five days after the application of an inducing agent, but the level of persistence of resistance generally decreases over time. These criteria may determine the number of applications of PGPR needed to maintain the resistance level in the crop plants.

Project : Isolation and Identification of cyanobacteria from saline soil habitats, characterization of their salt-adaptation mechanisms and application for stress-tolerance in rice

PI : Dhananjay Pratap Singh
Co-PI : Anurag Chaurasia

Rationale

Cyanobacteria are the most primitive and widespread group of gram-negative prokaryotic organisms evolved approximately 3.8 billion years ago on the earth. Assessment of biodiversity of these organisms using morphological and physiological variables often provides misleading information and therefore, polyphasic approach using molecular identification and characterization methods is finding a viable and comparatively authentic way of cyanobacterial taxonomic identification. It is proposed to identify and characterize functional traits of cyanobacteria in saline habitats. Since the organisms inhabiting saline systems (soil or water) are regularly facing salt and osmotic stresses and therefore, evolving biochemical, morphological and cellular mechanisms to overcome such stresses, it is also proposed to evaluate their mechanisms of adaptation and survival under salt stress conditions. Cyanobacteria are the major inhabitants of rice ecosystem and in the field, besides fixing nitrogen, solubilising phosphate and releasing phytohormones to support plant growth and development, they are supposed to act as plant growth promoting rhizobacteria (PGPR) for imparting resistance against biotic stresses (pathogens) and tolerance against abiotic stresses (especially salt and osmotic stress) in rice. It is therefore, proposed to assess the role of efficient cyanobacterial strains in the tolerance of rice plants to salt stress conditions.

Objectives

- To isolate, identify and characterize agriculturally important cyanobacterial strains for saline soil habitats
- To evaluate molecular, biochemical, morphological and cellular mechanisms of adaptation and survival of selective cyanobacterial strains under salt stress conditions
- To assess the role of efficient cyanobacterial strains in the tolerance of rice plants to salt stress conditions

Achievements

Explorations were conducted in the local salt affected areas of Eastern Uttar Pradesh (Mau, Azamgarh and Ghazipur districts) for soil sample collection from rice fields and water bodies (pH range 7.8-8.7, EC 4.5-5.6 mmhos/cm, ESP range 11.7 to 14.3). Cyanobacterial strains belonging to the genera *Nostoc*, *Anabaena*, *Oscillatoria, Calothrix, Microcoleus, Scytonema, Plectonema, Hapalosiphon, Cylindrospermum, Microchaete, Tolypothrix* etc. were isolated from 56 samples of salt affected soils and water bodies. in rice grown fields. Overall 21 isolates were found from the rice soils. These isolates were identified on the basis of morphology and microscopic character at genera level. 16S rRNA gene amplification of these isolates has been carried out and sequencing is under process. All isolates are being maintained in the cyanobacterial culture collection at NBAIM, Mau. These isolates were again characterized for different plant growth promoting traits including quantification of the phytohomone indole acetic acid (IAA) production and phenylpropanoids (phenolics, flavonoids, carotenoids and various enzymes like

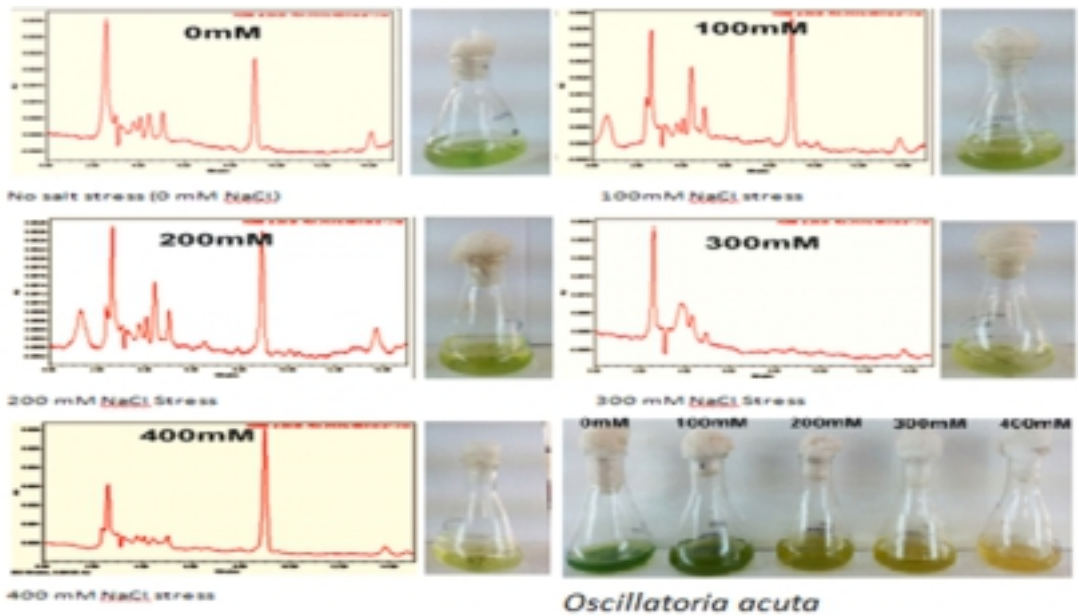


Fig. Accumulation of phenolic acids and flavonoids in *Oscillatoria acuta* (cyanobacteria) treated with different salt (NaCl) concentrations

phenylalanineammonialyase (PAL), SOD and POD. Secondary metabolic responses in cyanobacteria has been worked out with special reference to the phenylpropanoid pathway metabolites, especially phenolics, flavonoids and phytohormones production and accumulation in cell-free extract and culture filtrate. Different cyanobacterial *Oscillatoria acuta, Calothrix geitonus, Anabaena doliolum* and *Nostoc cornium* were grown under NaCl stress (100, 200, 300 and 400 mM NaCl concentration) and growth, metabolic changes and antioxidant properties of the extracts and culture filtrates were observed and correlated. Cyanobacterial strains grown under different salt concentrations showed varied accumulation of phenolic acids and flavonoids (gallic, trans-chlorogenic, caeffic, vanilic, ferulic, salicylic and cinnamic acids along with the flavonoids quercetin hydrate, naringenin and kaempferol. Change in phenylpropanoid metabolites was correlated with stress tolerance level of cyanobacterial strains.

Project: Isolation, characterization and conservation of bacteriophages associated with some important phytopathogenic bacteria and their evaluation for use in agriculture

PI : Renu
CO-PI : Dipak T. Nagrale and Udai Bhan Singh

Rationale

Diseases caused by bacteria in agricultural crops bring down the economy drastically as they affect agriculture production. Due to variability in pathogen population, high probability for mutation or gene transfer in the pathogen when confronted with resistance genes or bactericides, high pathogen multiplication rate during optimal conditions for disease development, and lack of adequate chemical-based approaches for control the management of disease caused by bacteria is very difficult. Integrated management approach utilize combination of proper cultural practices, chemicals such as bactericides or plant activators where applicable, introgression of plant resistance genes, and biological control strategies. In all cases disease control has been variable. Recently, there has been resurgence in interest in use of bacteriophages for control of bacterial plant diseases.

Use of bacteriophages for controlling plant diseases is an emerging field with great potential. The concern about environment-friendly sustainable agriculture and the rise of organic production necessitates improvements in biological disease control methods, including the use of bacteriophages against bacterial plant pathogens. *Xanthomonas campestris* pv. *Campestris* (*Xcc*), the causal agent of black rot also known as blight, black stem, black vein, stem rot, and stump rot, infects a large number of cruciferous plants, including agriculturally important crops such as cabbage, broccoli, and cauliflower. Management of disease is generally by proper cultural practices, usage of chemical control methods and disease free planting material and usage of resistant varieties. However, the bacteria are rapidly becoming resistant to copper sprays and copper residues are poisoning our environment. Alternative control chemicals are few and toxic. Bacteriophages provide highly specific control opportunities for bacterial diseases by specifically infecting and destroying the disease-causing bacteria. Hence it is necessary to investigate potentials of bacteriophages in controlling black rot disease as one of the options for environmentally safe disease control method. Keeping the above facts in mind, the project was formulated to collect and characterize phages of phtyopathogenic bacteria and to look out for their possible role in disease management programme.

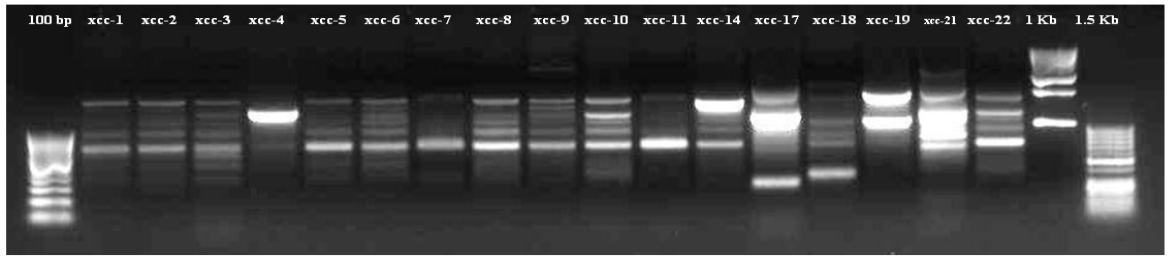
Objectives

- Isolation and characterization of pathogenic bacteria of important crops
- Collection and isolation of bacteriophage from bacterial infected fields.
- Characterization of bacteriophages.
- Screening for evaluation of selected phages for disease control potentiality.

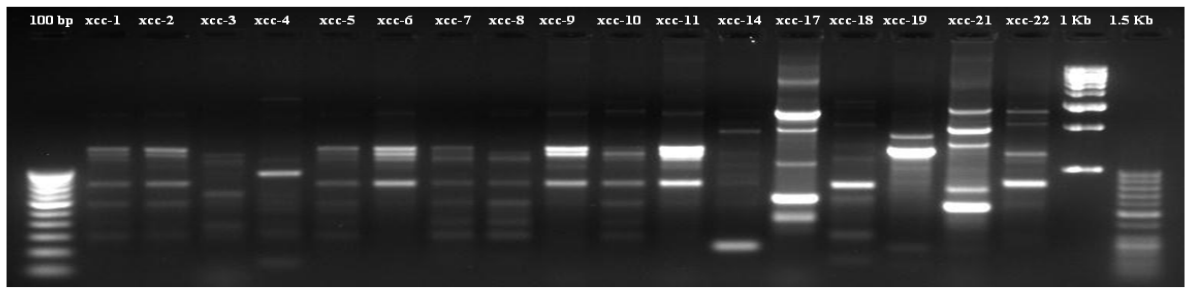
Achievement

During year 2013-14 various studies and experiments related to project were carried out:

- **Symptom expression, disease incidence and intensity:** The *in planta* pathogenicity test of 17 *Xcc* strains was carried out by the leaf-clipping inoculation method on susceptible Cauliflower (*Brassica oleracea* var. *botrytis*) cv. Kataki 1. Data on symptom expression, black rot incidence and intensity revealed that the pathogen could successfully cause infection of the susceptible cauliflower host by with varied incidence and intensity and some variation in symptom expression when inoculation was conducted by clip inoculation method. All the inoculated plants were infected (100% incidence) with all the isolates. Lesion progression was recorded at 10, 15 and 20 days after inoculation and percent (%) leaf area infected was calculated. Highest % leaf area infected and lesion progression was found to be by Xcc-6 isolate from Ranichauri followed by Xcc-5 isolate from Himachal Pradesh.
- **Molecular fingerprinting:** Molecular fingerprinting of different *Xcc* strains was done using 10 bacterial RAPD primers *viz*. RBa 1-10 (Banglore GeNei™). The binary matrix (1= presence of band, 0= absence of band) was constructed based on gel patterns. Using the software NTSYS version 2.02e, the similarity triangular matrixes were constructed using the

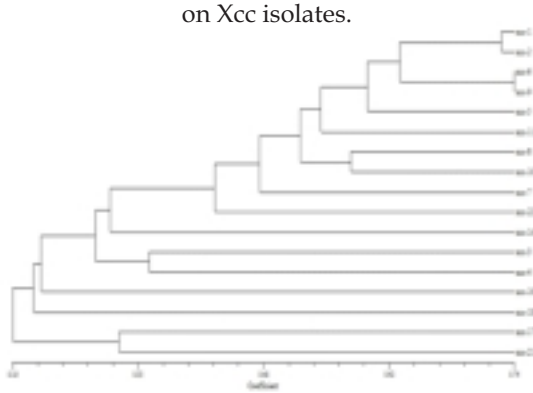


Gel electrophoresis of Bacterial RAPD primer RBa 1products from 17 *Xanthomonas campestris* pv. *campestris* strains alongwith molecuilar weight markers 100bp, 1kb and 1.5 kb (Banglore GeNei)



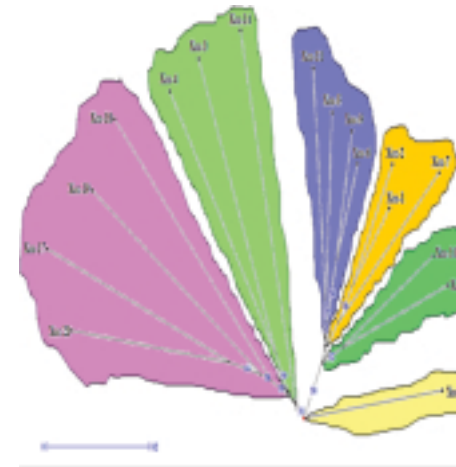
Gel electrophoresis of Bacterial RAPD primer RBa 5 products from 17 *Xanthomonas campestris* pv. *campestris* strains alongwith molecuilar weight markers 100bp, 1kb and 1.5 kb (Banglore GeNei)

Dendogram based on RAPD data obtained on Xcc isolates.



band-based Jacard's similarity coefficient (J). From each similarity matrix, the unweighted pair group method with arithmetic mean (UPGMA) was used to cluster the patterns and dendogram was obtained.

- A high genetic diversity was obtained. Total number of bands obtained were 94 out of with 93 were polymorphic. Band size ranged from 200bp – 5 kb and number. of bands ranged from 6-16 with respective primer. Isolate Xcc6 from Ranichauri was closest to xcc 9 from Faizabad (76% similarty) where as isolate Xcc 1 from Varanasi was most dissimilar to isolate Xcc 17 from H.P. exhibiting only 11 % similarity based RAPD pattern.



Unrooted tree based on RAPD data obtained on Xcc isolates. The tree was created by the neighbour-joining method. The numbers on the tree indicates the percentages of bootstrap sampling derived from 1,000 replications. Bar inferred nucleotide substitutions per nucleotides. (DARwin5)

- All Xcc strains were submitted to NAIMCC at NBAIM (Accession no. NBAIMCCB-01238-01248 ; NBAIMCCB-01358-63). Partial 16SrRNA gene sequences of 17 isolates were submitted to NCBI GenBank and were assigned GenBank accession no. JQ698512-JQ69822 , KF498594-KF498599.
- **Collection and isolation of bacteriophage**

  Collection of soil and plant samples from black

Survey and collection of soil and plant samples from infected fields

Collection of soil and plant samples from black rot infected field at Lucknow in Jan. 2012

Exploratory survey of vegetable field at Shillong, Meghalaya and collection of soil and plant samples from black rot diseased field

rot infected cabbage fields was done and standardization of technique for isolation of bacteriophages with refinement in the technique of soft agar overlay method for isolation of phage from infected plant tissues and soil by overnight enrichment with the indicator bacterial hosts was done. Bacteriophages of different strains of Xcc were isolated and maintained as lysate and kept in SM buffer at 4 ^{0}C in dark.

- **Characterization of bacteriophages**

  The bacteriophages collected from different sources like Lucknow, Shillong, Jaunpur, Mau, Ganga river, etc and on different indicator hosts (different strains of *Xcc*) were further characterized for the number of plaques formed, their type, shape and appearance.

- **Phage titre** of the selected phages was determined which was observed to be in the range of 10^9 to 8 X 10^{10} pfu/ml of lysate for the selected phages.

- **Organic solvent sensitivity test :**Phages of Xcc were subjected to chloroform sensitivity test. Most of the phages were found to be insensitive when subjected to chloroform.

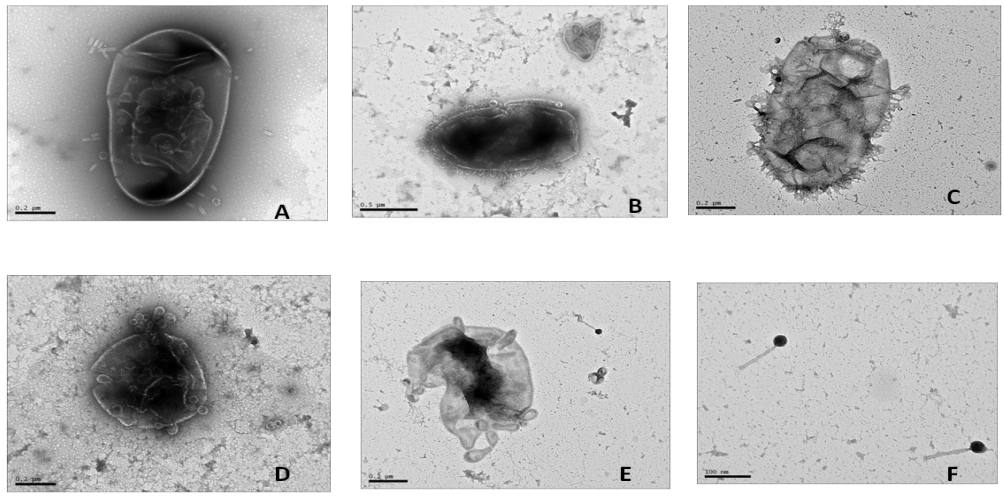
- **Effect of temperature:** Optimum temperature for lysis and plaque formation was found to be 28 ^{0}C ± 2 ^{0}C. Thermal inactivation point was found to be in range of 60-75 ^{0}C for 60 min.

- **Host range:** The host range of each phage isolate was tested against 17 Xcc strains using a plaque assay as well as spot test. A virulent phage Xc9SH3 was found to lyse all tested strains of Xcc. The plaque type produced by this phage is lytic, clear and transparent. This phage was further taken up for detailed characterization studies.

- **Morphological examination:** Selected Phages were observed under a transmission electron microscope (TEM; JEM-2010; JEOL, Tokyo, Japan) at 80 kV.

- *In vitro* evaluation of phage Xc9SH3 was done and its effect on various strains of *Xanthomonas campestris pv campestris*, a plant pathogenic bacteria causing black rot disease in various cruciferous hosts was studied. Infection of Xcc strain collection in NBAIM laboratory with the phage Xc9SH3 caused lysis of all the strains of Xcc under the study. Detailed electron microscopic studies were carried out which established the lytic nature of this phage. Images of different stages of phage cycle were captured starting from attachment of phage to the host cells to its absorption, release of nucleic acid to bursting stage.

Transmission electron microscopy of Xcc9sh3 phage showing different stages of phage cycle (A to F)



Conclusions

The present project enriched NAIMCC with strains of phytopathogenic bacteria , *Xanthomonas campestris pv campestris* (Xcc), causing black rot in crucifers. The identification and diversity of Xcc strains collected from different cruciferous hosts and geographical area based on pathogenic, metabolic and different molecular techniques like *Hrp* gene based region, 16s rDNA region and with random primers was deciphered. It was concluded that different collected strains of Xcc exhibited considerable level of diversity and were able to form group based on geographical origin. Study generated number of lytic phages of Xcc and their characterization was done. Upon comparison with other phages of Xcc isolated in the laboratory phage Xc9SH3 is highly virulent and is able to lyse all the available strains of Xcc in *in vitro* experiments. The bacteriophage is chloroform insensitive and possibly belonging to family Siphoviridae of bacteriophages possessing double stranded DNA. Since the focus of the project besides collecting and characterizing pathogenic bacteria *Xanthomonas campestris pv campestris* causing black rot in crucifer and enriching NAIMCC with the strains of Xcc , also was to isolate and characterize lytic bacteriophages of various strains of Xcc. This was achieved in the project and upon *in vitro* evaluation it was found that some lytic phages especially phage Xc9SH3 possess the ability to be further characterized and evaluated in field conditions for possible use as biocontrol agent against black rot disease.

Project: Exploration, preservation and evaluation of endophytic actinomycetes from Indo-Gangetic plain.

PI : Anurag chaurasia
Co-PI : Dhananjaya Pratap Singh

Rationale

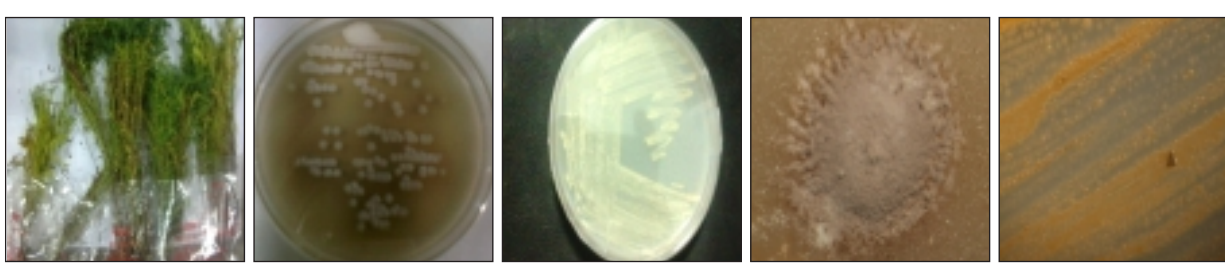
According to Hallmann *et al.* endophytes are defined as microorganisms which make no visible harm to the plant and can be isolated from surface disinfected plant tissues or extracted from inside the plant. While this definition does not include non-extractable endophytic microorganism, it is a practical definition based on experimental limitations and is inclusive of bacterial symbionts, as well as internal plant-colonizing nonpathogenic bacteria with no known beneficial or detrimental effects on colonized plants. Historically, endophytes have been thought to be weakly virulent plant pathogens but have recently been discovered to have several beneficial effects on host plants, such as plant growth promotion and increased resistance against plant pathogens and parasites. Endophytes are ubiquitous and have been found in all the species of plants studied to date; however, most of these endophyte/plant relationships are not well understood. Actinomycetes also known as pigmented microorganisms have been frequently reported to produce antibiotics and other different kinds of bio-active metabolites. Hypothesis and concept behind this formulated project is that certain plants remain healthy in the pathogen infected crop field, one of the possible reason behind this may be the presence of specific endophytic actinomycetes in such plants/crops which produce bio-active compounds and act as antibiotic factory which help the plant in surviving and remain unaffected in the pathogen infected field. With this hypothesis this project was formulated to isolate, preserve and evaluate endophytic actinomycetes with PGPR and biocontrol potential from various crops growing in the Indo-Gangetic plain which will be further used for enhancing agricultural productivity.

Objectives

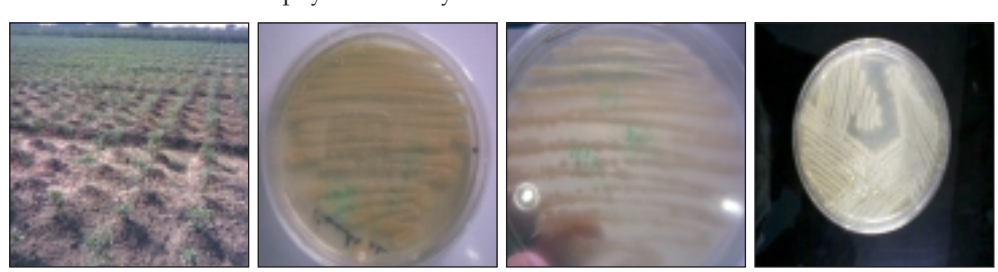
- Isolation of endophytic actinomycetes from the crops growing in the Indo-Gangetic plain.
- Preservation and identification of the isolates.
- Evaluation of the endophytic actinomycetes isolates for enhancing agricultural productivity and human welfare.

Achievements

Endophytic actinomycetes were isolated from the root, stem and leaves of the healthy and infected Chickpea (*Cicer arietinum*) and Tomato (*Lycopersicon esculentum*) plants using sodium hypochlorite or mercuric chloride as surface disinfectant, concentration and time of the treatment were



Endophytic actinomycetes from *Cicer arietinum* root.



Endophytic actinomycetes from *Lycopersicon esculentum* root.

determined using tissue sensitivity test. Sodium hypochloride was found to be suitable for soft tissues like leaves while mercuric chloride was effective for hard tissue like stem and root. Last distilled water wash from surface sterilization was used for the sterility check. No microorganisms were found to be grown on the surface of the explants too. These two steps confirmed that actinomycetes isolated were of endophytic in nature. Actinomycetes isolation agar and the starch casein agar media with nystatin or cyclihexamide as antifungal agents were used for the endophytic actinomycetes isolation experiments. Actinomycetes colony morphology usually start with bacterial shape and end up as a fungal mycelia. Hence microbial plates were observed at regular interval to pick the rightly identified actinomycetes colony. Roots of the plants were reported to have more actinomycetes diversity compared to stem and the leaves. Rare endophytic actinomycetes were isolated using different media reported in the literature. Actinomycetes diversity were different in healthy and the diseased plants. Disease causative fungal strains were isolated from the infected plant parts using PDA media. Endophytic actinomycetes were evaluated for their biocontrol potential against isolated fungal strains using dual plate techniques. Various physiological (temperature and salt) and biochemical (Ammonia, HCN, IAA, Siderophore, Phosphate solubilization) parameters of the isolates were studied. Isolates were preserved in the slant & 20% glycerol.

Project : Deciphering microbe-mediated mechanisms of Induced Systemic Resistance (ISR) and plant growth promotion in rice and tomato

PI : Udai B. Singh
Co-PI : Renu, Dhananjaya P. Singh

Rationale

The ability of plants to defend themselves against pathogen is important not just for plants in their natural environment but also for plants under cultivation. Induced resistance, where the plant can be primed for an intense defense response on pest or pathogen attack, offers the prospect of a more durable approach to disease control in crop plants. Chemical control of phytopathogens is very expensive and also not desirable because synthetic pesticides may affect the agro-ecosystem, they may have detrimental effects on numerous beneficial parasites, predators, phytotoxicity to plants, soil and water pollution and unavoidable natural imbalance in the soil and other useful microbes prevailing in agricultural soils. Growing ecological concerns have led to intensive research on alternative methods for control of plant diseases among which biological control of phytopathogenic microorganisms has emerged as one of the most powerful approaches. The potential benefits of using microbial inoculants for the purpose of plant growth promotion, biological control and induced resistance responses against the disease can be achieved through modification of the rhizospheric microflora. Recognizing the importance of bio-agents, the present study was undertaken with the objectives:

Objectives

- Screening and characterization of existing and new strains against important phytopathogens of rice and tomato.
- Development of eco-and farmer friendly bioformulation of selected biocontrol agents
- Deciphering mechanisms of induced systemic resistance and plant growth under pathogenic stress conditions.
- Development of suitable delivery system for judicious application in agriculture.

Achievements

- The promising bacterial and fungal antagonistic strains preliminary screened under laboratory against soil and seed-borne pathogens of rice and tomato were selected for root colonization and production of antimicrobial compounds/ metabolites.
- These selected strains were characterized on the molecular level for variability, genetic relatedness

and identified on the basis of ITS and 16S rDNA andsequencing.

- These strains were evaluated for their competitive root colonization in rice and tomato.
- Selected isolates of *Pseudomonas fluorescens* and *Trichoderma harzianum* and *T. viride* against *Rhizoctonia solani* were tested *in vitro* for their antimicrobial potential. It was depicted from result that all strains tested were showing antagonistic against *R. solani* and other soil and seed-borne pathogens. Based on percent inhibition of fungal mycelium in petriplates grouping was done and found that *P. fluorescens* PF-08, PF-10, *Trichoderma harzianum* UBSTH-501, NAIMCC-F-3043, NAIMCC-F-3009, NAIMCC-F-2050, NAIMCC-F-3034 and *T. viride* UBSTV-10 were recorded as most potential strains. Among these strains *P. fluorescens* PF-08, PF-10, *T. harzianum* UBSTH-501, *T. viride* UBSTV-10 was used in the development of bioformulations.

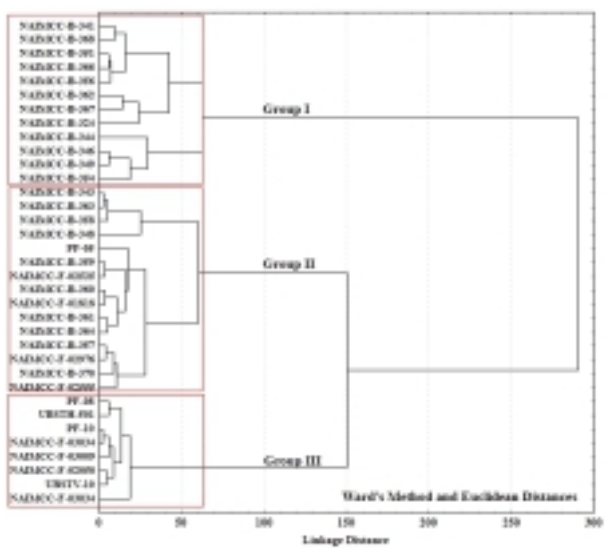


Fig. *In vitro* screening of potential strains of bioagents for greenhouse/field application

Three bioformulations of *P. fluorescens, T. harzianum and T. viride* namely **Eco-Pesticide**: Talc based bioformulation of *Pseudomonas fluorescens;* **Eco-Green Fungicide:** Vermi-based bioformulation of *T. viride* and **Green Fungicide:** Talc based bioformulation of *Trichoderma harzianum,* respectively, were developed successfully and found effective against a number of soil and seed borne pathogens like *Rhizoctonia, Sclerotium, Sclerotinia, Fusarium, Pythium, Ralstonia, Macrophomina, Bipolaris, Phoma,* etc.

Fig. Talc and vermi-based bioformulation of *P. fluorescens, T. harzianum and T. viride*

- These above mentioned bioformulations were tested under nethouse conditions in different crop plants like tomato, brinjal, cabbage, cauliflower and rice. One talk based commercial bioformulation of *T. harzianum* and one sand based formulation of PGPRs developed by SSRI, Karnal were taken as standard.
- The bioformulations tested were found to be potential in enhancing the accumulation of defence related biomolecules, enzymes and exhibited biocontrol potential against *R. solani.* It was depicted from result that application of *T.*

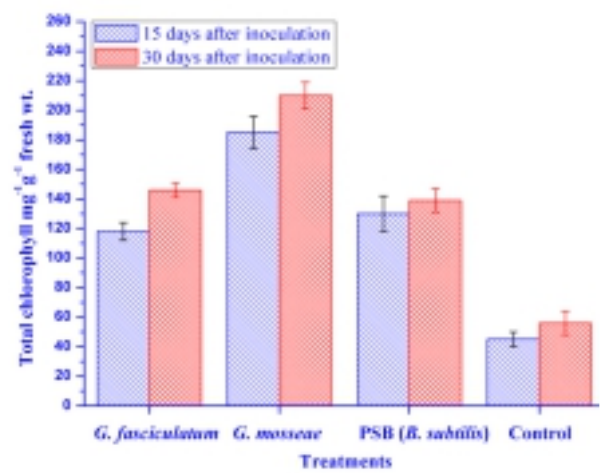


Fig. Effect of AMF and PSB on accumulation of total chlorophyll content in tomato leaves under nethouse condition after 15 and 30 days of inoculation. Data are mean (n=5) and vertical bar represent standard error of mean.

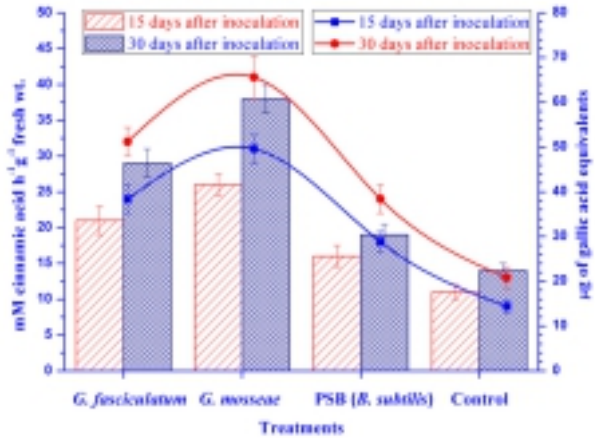


Fig. Effects of AMF and PSB on PAL activities (bar graph) and accumulation of total phenolics (line graphs) in tomato leaves under nethouse condition after 15 and 30 days of inoculation. Data are mean (n=5) and vertical bar represent standard error of mean.

viride UBSTV-10 and *P. fluorescens* PH-08 and PF-10, particularly in combination, not only help in the control of *R. solani* but also increase plant growth as well as enhances the nutritional uptake and translocation in tomato and other vegetable crops. When compared with talk based commercial bioformulation of *T. harzianum* and one sand based formulation of PGPRs developed by SSRI, Karnal, our formulations (in combination) exhibited better result than the other formulation tested.

- The level of defence related enzymatic activities in tomato leaves inoculated with selected bioagents and cross inoculated with *R. solani* was studied. Result showed that many for increase in the enzymatic activities were recorded. Plants treated with PF-08 and *Trichoderma harzianum* in combination exhibited significantly higher enzymatic activities in tomato leave prechallanged with *R. solani* .
- Tomato plants colonized by AMF fungi *Glomus mosseae and G. fasciculatum* (obtained from Dr. D. J. Bagyaraj) accumulate phenolic compounds significantly higher than the plants treated with PSB and their respective control. Similar pattern

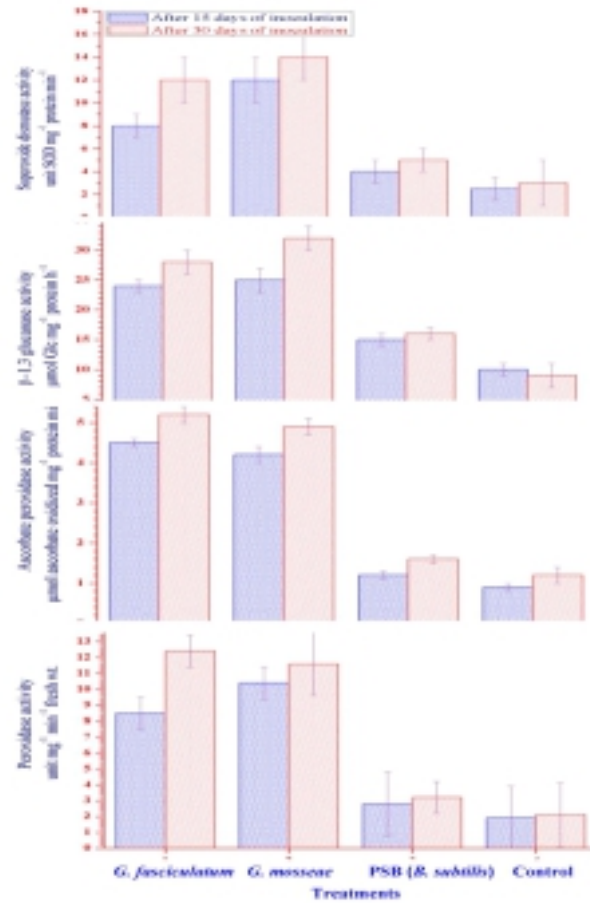


Fig. Effect of AMF and PSB on accumulation of defence related enzymatic activities *viz.* Superoxide dismutase; β 1, 3- glucanase, ascorbate peroxidase and peroxidase in tomato leaves under nethouse condition after 15 and 30 days of inoculation. Data are mean (n=5) and vertical bar represent standard error of mean.

was also recorded for phenylalanine ammonia lyase (PAL) activities.The superoxide dismutase (SOD) activity proved highest in plants colonized by AMF in comparison to plants treated with PSB and their respective control. Similar pattern was recorded in case of β 1, 3- glucanase, ascorbate peroxidase and peroxidase in tomato leaves under nethouse condition after 15 and 30 days of inoculation.

Project: Development of DNA barcode for the identification of *Colletotrichum* species complex

PI : Prem Lal Kashyap
Co-PI : Alok Kumar Srivastava and Hillol Chakdar

Rationale

Colletotrichum is the causal agent of anthracnose and other diseases on leaves, stems and fruits of several horticultural and agriculturally important crops. Accurate species identification is critical to understand the epidemiology and to develop effective management of these diseases. However, there has been considerable difficulty in fast and accurate identification of *Colletotrichum* species complex, due to the lack of reliable morphological features, making species boundaries ambiguous and confusing. Traditionally, several *Colletotrichum* species have been named after their host, which suggests host specificity amongst species. von Arx (1957) reduced the number of *Colletotrichum* species from several hundred to eleven based on morphological characters, with many taxa treated as synonyms of *C. gloeosporioides* and *C. dematium*. Since then, several additional species have been reported and accepted on the basis of morphological criteria. At present, the difficulty in authentic identification of *Colletotrichum* species has resulted from: i) few and variable morphological characters; ii) an extensive host range and variability in pathogenicity; iii) missing and poor conditions of type specimens and; iv) erroneously naming of *Colletotrichum* strains deposited in NCBI database on the basis of rDNA ITS (ITS) and other sequences. Further, a well-documented decline in taxonomic expertise, along with the need to develop rapid and sensitive diagnostic methods has provided an impetus to develop technologies that are both generic and able to complement traditional skills and techniques. Thus, there is an urgent need to develop a barcode by utilizing several characters *viz.*, nucleic acid sequence data, physiology, secondary metabolites and pathogenicity, as part of a polyphasic approach. However, our knowledge on exploration of DNA barcoding for *Colletotrichum* species is still limited and clearly much more work is required. Therefore, the aim of the present project is to develop a standard DNA barcode for rapid detection and identification of *Colletotrichum* species complex associated with agriculturally important crops.

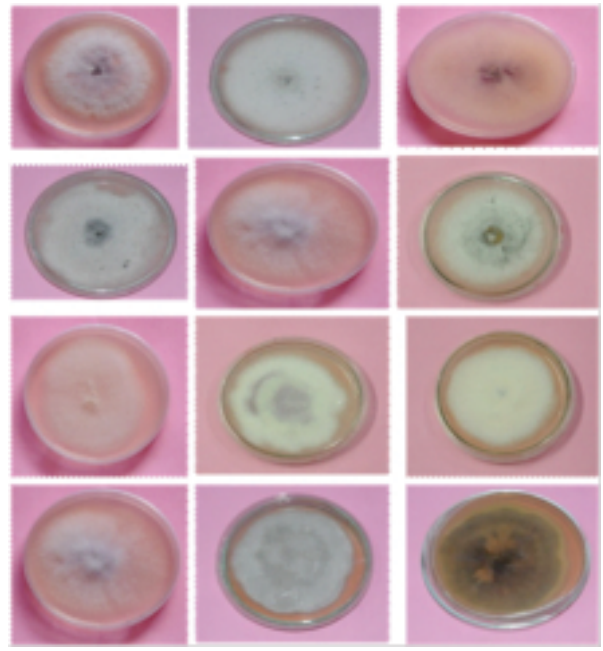


Fig 1: Culture morphology of the representative *Colletotrichum* species.

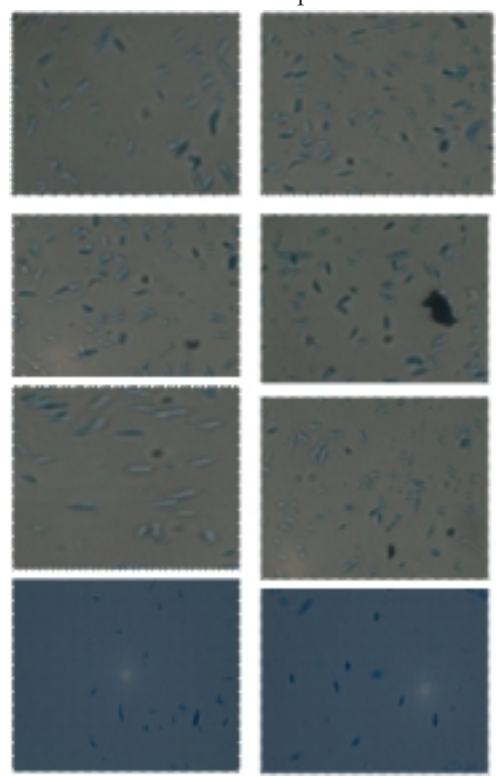


Fig 2: Microscopic view of slightly curved or dumbbell shaped conidia of *Colletotrichum* species.

Objectives

- Isolation, purification and maintenance of

Colletotrichum isolates

- Morphological and molecular characterization of *Colletotrichum* species complex

Achievements

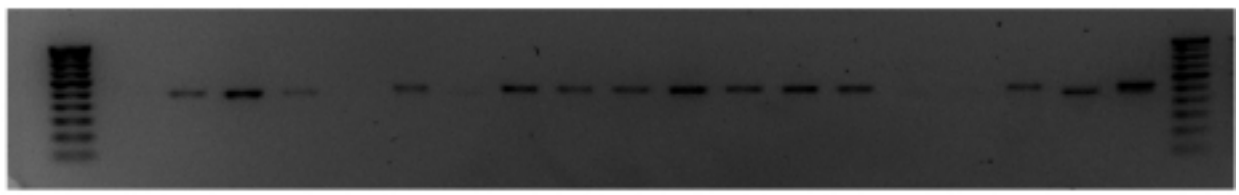
- The survey was performed to collect the isolates of *Collectotrichum* isolates from agriculturally important crops. A total 52 different isolates of *Colletotrichum* species associated with chilli (2), grapes (3), sugarcane (8), mango (37), coffee (1), Tea (1) were isolated and purified on potato carrot agar (PCA) media and sub-cultured periodically. Fig 1 showed the colour morphology of the representative isolates of *Colletotrichum* species.
- Morphological and physiological characterization of 52 *Colletotrichum* species associated with mango, chilli and sugarcane crops were done. For the physiological characterisation, the isolates analysed under different pH (6, 7, 8 and 9) and temperature (15, 20, 25, 30 & 35°C). All the isolates showed luxuriant growth at pH 7 and at 25°C temperature. Under microscopes, all the isolates were analysed and one- celled, ovoid to oblong, slightly curved or dumbbell shaped conidia were observed. Fig 2 showed the micrographs of some of the representative isolates.
- For the molecular characterization of the *Colletotrichum* species, extraction of total genomic DNA of 52 isolates was isolated.
- Amplification genomic DNA with species specific primers (CagInt & ITS4) was performed to identify the *Colletotrichum* species.
- Primers representing different loci *Viz. ITS,* histone, b-tubulin, actin, transcription elongation factor, & pectate lyase (*PelB*) etc. were designed and synthesized (Table 2).

Table 2: Primers /locus used for the development of barcode for *Collettrichum* Species

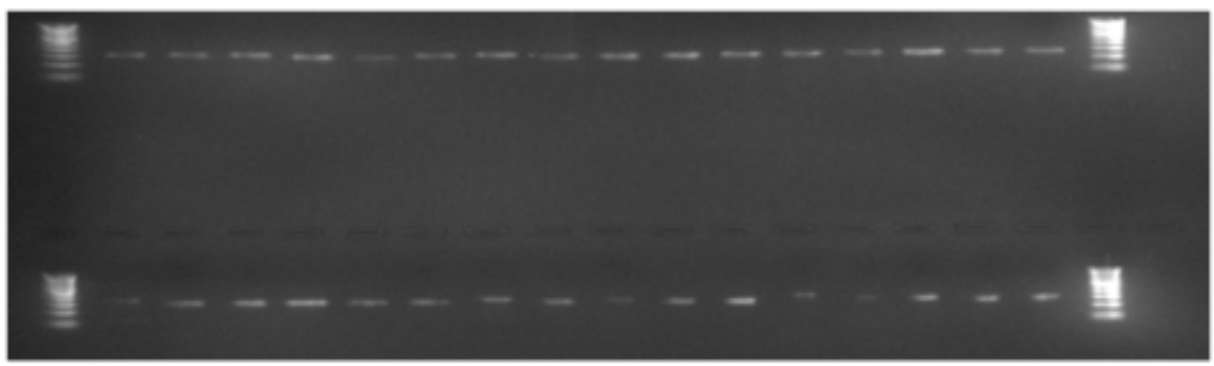
Primer	Primer sequence
TEF1F	ATG GGT AAG GAG GAC AAG AC
TEF1R	GCC ATC CTT GGA GAT ACC AGC
β-tubulinF	GGTAACCAAATCGGTGCTGCTTTC
β-tubulinR	ACCCTCAGTGTAGTGACCCTTGGC
ITS1	TCCGTAGGTGAACCTGCGG
ITS1	TCCTCCGCTTATTGATATGC
pelB F	CAC CAA GCC CGA CTA CAG CT
pelB R	AGC CTT ACC TTG GAG GAG CC
areA F	ACA GAC CAC AGG CAT TGC AA
areA R	TGT GGA GAC GAA ACC CTG AAG
Scd1-s	TGG GAG GCG ATG CCA GCG GAT
Scd1-a	GCT CGC GGC CCT CTG CAA ACA
Brn1-s	TGG GTG GCC GCA TTA TCC TCA
Brn1-a	GAA GCA GAC GAC GCG GGC AAT
BRN2 F	CAT TGC CGC TGG TCT TCT CGG
BRN2 R	AAG CCA CAA CCC TCG CAA CAT
cap20 F	GCA ACA TCT CGT CCG CTC T
cap20R	TGA AGT GGG GAG AAG GGA A
cCap F	GTA GGC GTC CCC TAA AAA GG
cCap R	CCC AAT GCG AGA CGA AAT
CaInt2 F	GGG GAA GCC TCT CGC GG
CgInt 2R	GGC CTC CCG CCT CCG GGC GG
col 1	GCC GTC CCC TGA AAA G
CO1F	AAC CCT TTG TGA ACR TAC CTA
CO1R	TTA CTA CGC AAA GGA GGC T
ITS 4 (col R)	TCC TCC GCT TAT TGA TAT GC

- PCR was optimised for the amplification of ITS, β-tubulin and Elongation factor and results were shown in Fig 3.

B) ITS amplification in different isolates of *Colletotrichum* species.



B) β-tubluin amplification in different isolates of *Colletotrichum* species.



C) EF factor amplification in different isolates of *Colletotrichum* species.

Fig 2: PCR for the amplification of various loci viz. Internal transcribed spacer region (ITS), β-tubulin and elongation 1-alfa factor etc. in different *Colletotrichum* isolates.

Project: Isolation and characterization of bacterial communities and their metabolites in rhizospheric rice ecosystem.

PI : Lalan Sharma
Co-PI : Dipak T. Nagrale

Introduction

Rhizosphere is considered the soil volume surrounding the root-tissue. It is well established that microbial life only occupies a minor volume of soil being localized in hot spots such as the rhizosphere soil (Nannipieri *et al.,* 2003), where micro flora has a continuous access to a flow of low and high molecular weight organic substrates derived from roots. This flow, together with specific physical, chemical and biological factors, can markedly affect microbial activity and community structure of the rhizosphere soil (Sorensen, 1997; & Brimecombe *et al.,* 2001). Both beneficial and detrimental interactions occur between microorganisms of rhizosphere soil and plants. Root exudation is generally confined to apical root zones. However, root architecture, and thus exudation can change depending on the nutritional status of plants. It is also well established that low molecular weight exudates are immediately available to microorganisms inhabiting rhizosphere soil and rhizoplane whereas high-molecular weight compounds are generally hydrolysed by hydrolases in smaller compounds which can be taken up by microbial cells. Therefore, research is to be proposed that there are some metabolic interaction in rhizosphere that may influence plat growth and productivity.

Objectives

- Survey and collection rhizospheric soil samples from rice crop in Indo-gangetic plain of Uttar Pradesh.
- To determine the compounds profile in root tissue and present in rhizospheric soil by using Mass Spectrometry.
- To determine the secondary metabolites produced by the bacterial isolates in broth culture medium by Mass Spectrometry.
- Characterization of HCN/siderophore producing bacterial isolates and develop consortia of beneficial isolates for their nutrient utilization.
- To study plant-microbe interaction by using Gnotobiotic system.

Achievement

A study of consortium effect of potential rhizobacterial isolates on rice plant growth was done. Consortium of MAU 143 + MRT 84 was recorded to improve radical length (3.8cm) followed by MAU 143 + MRT 84 +MRT 92. In another oxidative stress assay experiment, Superoxide dismutase assay (SOD), Peroxidase assay (POD), Polyphenoloxidase assay (POP), and Total Polyphenol Content assay (TPC), it was recorded that maximum oxidative stress was mitigated by treatments like MAU 143 (OD 0.045), MRT 84 (OD 0.223), MAU 143 (OD 0.064), and MRT 84 (OD 3.810), respectively as compared to control. It was recorded that consortium of MAU 143 + MRT 84 have increased POD assay (OD 0.217) and TPC assay (OD 4.010) and also consortium of MAU 143 + MRT 84 + MRT 92 have increased activity of SOD (OD-0.039 and POP (OD-0.099)as compared to control. Rhizobacterial isolates either individually/combinations are potential to increase rice plant growth.

Project: Diversity analysis of archaea from various ecological niches and their characterization

PI : Dipak T. Nagrale
Co-PI : Renu

Rationale

The domain archaea was recognized as a major domain of life quite recently (Olsen and Woese, 1993). It is a general perception that the archaea evolved very early from the universal common common ancestor (UCA) and regarded as the most primitive group of organisms. Earlier, only the methanogens groups were placed in the new domain. However, the archaea were considered as extremophiles that exist only in extreme habitats such as hot springs and salt lakes. Most of the archaea has ability to adapt extreme chemical and/or physical environments such as temperature, pressure, pH, salinity, etc. and the group has broadly divided into hyperthermophiles, halophiles and methanogens. Hypersaline niches are found throughout the world, but extremely hypersaline habitats are rare. Most of the habitats are in hot, dry areas of the world which harbours unique diversity of haloarchaea with potential applications in industry, agriculture and allied sectors. Commercial applications of haloarchaea include acceleration of soy and fish sauce fermentation, β-carotene and extracellular stable hydrolytic enzyme production. Recently, they have reported use in bioremediation of contaminated hypersaline sites. Novel biomolecules like bacteriorhodopsin has application in biocomputing, as food colouring agents and compatible solutes as stress protectants. Thus, there is tremendous diversity of haloarchaea in hypersaline niches which is still not being fully explored. The aim of this study was to analyse the diversity and community analysis of archaea from different ecological niches and their characterization. The hypersaline niches like marine solar saltern pond (Mumabi, India) and natural salt lake (Sambhar salt lake, Rajasthan) were selected for the isolation and characterization of haloarchaeon.

Objectives

- Diversity analysis of archaea from different ecological niches using culturable approach
- Community analysis of archaea from different ecological niches
- Development of molecular diagnostic tools of some agriculturally important archaea

Achievements

- Eleven dark brown to red coloured isolates from niches of Bhayander road marine solar saltern pond, Mumbai, six red to blood red isolates from Mira road east marine solar saltern pond, Mumbai and three from Sambhar lake, Rajasthan were obtained using haloarchaea agar medium containing 25% (w/v) NaCl. All the isolates were Gram negative in nature and variable motile.
- The genomic DNA from all the haloarchaea isolates were amplified with archaea specific primer with amplicon size ~ 1500 bp.
- Isolates B3(6) and M3(1) exhibited potential zone of clearance on skimmed milk agar and showed significant protease production after 72 hrs.
- The protease production estimation by these isolates was made in soybean flour based medium at 37^{0}C. The maximum protease production at 30% (18 ±0.5 U/ml) was observed at 72 hr by B3(6) and at 30% (17.5±0.3 U/ml) by M3(1).
- The enzyme exhibited highest activity for B3(6) in the pH range 7.0-8.0 with maximum activity at pH 7.0 (100 ± 1.5%). Similarly, the enzyme activity for M3(1) was also maximum at pH 7.0 (100± 2.0%). These results indicated that the extracellular protease for B3(6) and M3(1) isolates is a neutral to near alkaline protease.
- The Mira road isolates were grouped in two groups (only from single genus) as strains of *Haloarcula marismortui* M1(1), *Haloarcula marismortui* M3(1) and *Haloarcula marismortui* M2(2). However, others were categorized as *Haloarcula salaria* M2(1), *Haloarcula argetinensis* M4(1) and *Haloarcula quadrata* M4(2).
- All these isolates are unavailable in NAIMCC and first time work has successfully implemented in the bureau.

Conclusion

- On the basis of polyphasic characterization, these isolates were identified as haloarchaeon members of family *Halobacteriaceae*. Strains M1(1) and M3(1) may be novel strains which should be verified by DNA-DNA hybridization with type strains. The marine solar saltern pond were dominant in both

monovalaent and divalent cations like Na^{+}, K^{+}, Mg^{++} etc. however, Sambhar lake brine samples were lacking divalent cations thus, affecting the diversity and community of haloarchaea.

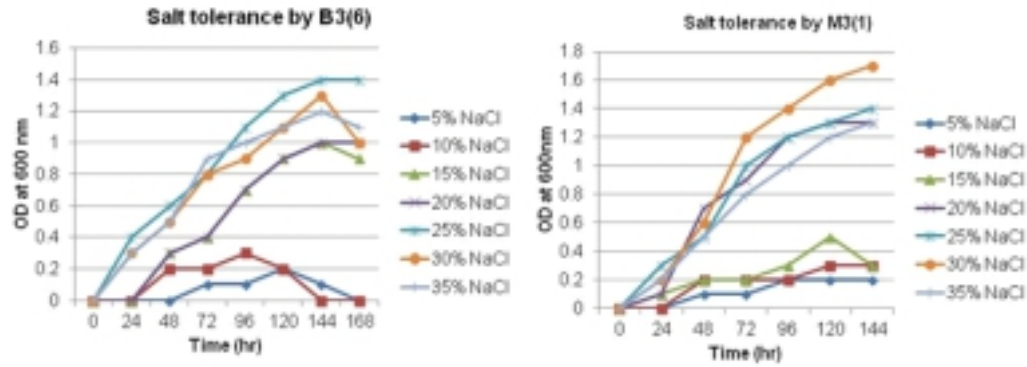


Fig 1. Salt tolerance by haloarchaeal isolates B3(6) andM3(1)

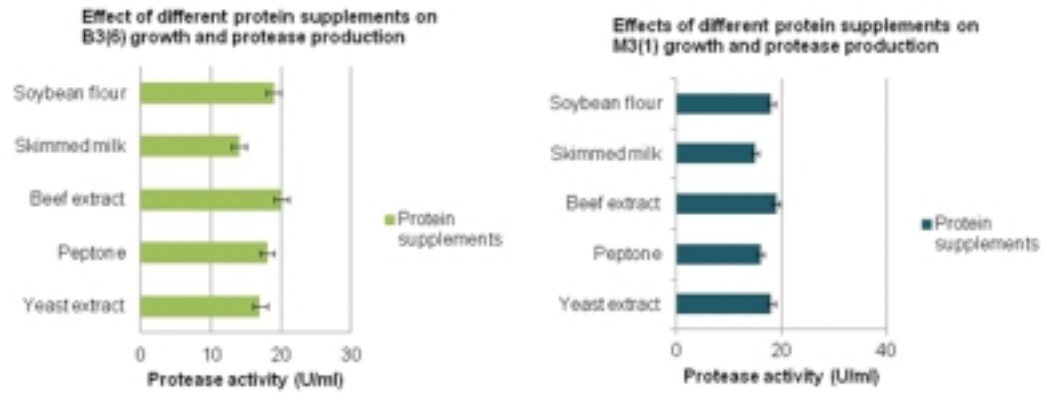


Fig 2. Effect of different protein supplements on B3(6) and M3(1) growth and their protease production

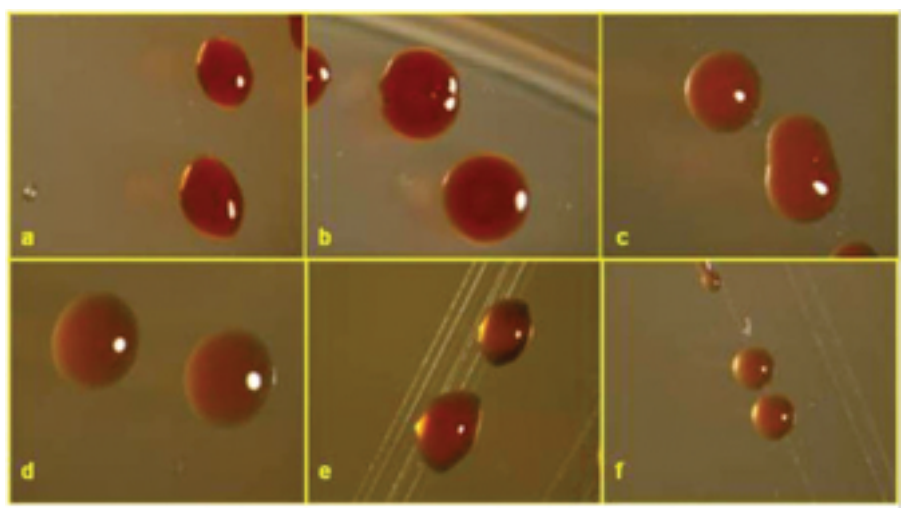


Fig 3. a: *Haloarcula marisportui* M1(1), **b:** *Haloarcula salaria* M2(1), **c:** *Haloarcula argentinensis* M4(1), **d:** *Haloarcula quadrata* M4(2), **e:** *Haloarcula marismortui* M3(1), **f:** *Haloarcula marismortui* M2(2)

Title: Bioprospecting for phosphorus solubilizing bacteria with high phosphatase activity and its application to enhance plant productivity

PI : Hillol Chakdar
Co-PI : Prem Lal Kashyap

Rationale

In India, about 46% of soil is classified as P-deficient soil where available P concentration is not higher than 10 mM and on the other hand India harbors world's largest rock phosphate deposit. But utilization of this huge mineral phosphate resource for application in crop production is a big challenge as it becomes immobilized rapidly and become unavailable to the plants. Microorganisms with the ability to solubilize mineral phosphates hold a great potential to utilize the rock phosphates for agricultural purpose. PSB in combination with rock phosphates which usually contains some form of the mineral apatite, can be applied directly to soil with varying agronomic efficiencies depending on the type of soil and crop.

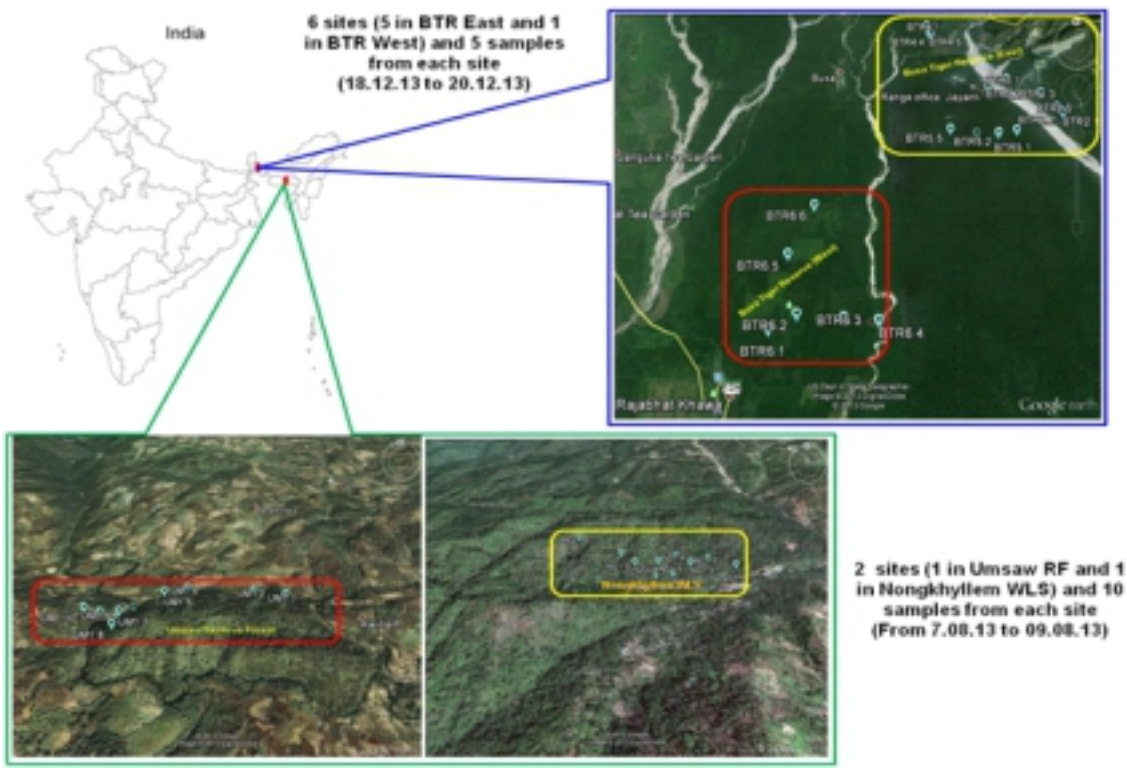
In addition to the P solubilization from mineral phosphates, bacteria have diverse enzymatic machineries for releasing organically bound P. Suitable bacterial isolates having both mineral phosphate solubilization ability and high phosphatase activity may be a good option to make fixed phosphorus available to the plants.

Objectives

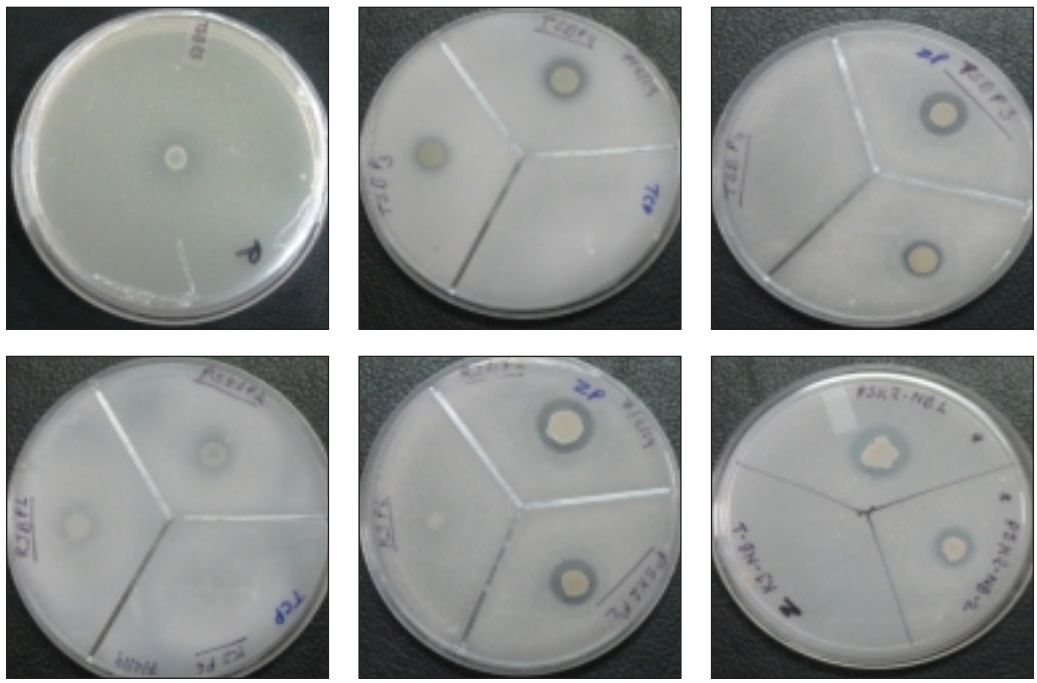
- Exploration of forest soils to isolate bacterial cultures followed by their identification.
- Screening and selection of P solublizing bacteria for high Phosphatase activity.
- Optimization of Phosphatase production and studying MPS activity under different physico-chemical conditions.
- Development of bioformulation followed by their application to sustainably enhance plant productivity

Achievements

Two surveys were conducted for collection of forest soils from Meghalaya and West Bengal. In Meghalaya, samples were collected from one site each from Umsaw Reserve Forest and Nongkhyllem Wild Life Sanctuary while in West Bengal, samples were collected from six sites in Buxa Tiger Reserve.



Bacteria were isolated by spreading appropriate dilutions of the samples on Pikovskya (PKV) and NBRIP agar plates. A total 32 candidate P solubilizing bacterial (PSB) isolates were obtained from soils of Umsaw Reserve Forest while 26 candidate PSB were isolated from Nongkhyllem Wild Life Sanctuary. Total 25 bacterial candidate PSB were isolated from the samples collected from Buxa Tiger Reserve, West Bengal using Pikovskaya Agar medium. Among the isolates obtained from West Bengal, one isolate TSBP3 could solubilize Rock Phosphate, Zinc phosphate as well as Tri-calcium phosphate and four more isolates were also obtained which could solubilize both Zinc and Tri-calcium phosphate



Screening of bacterial strains isolated from West Bengal for solubilization of Rock Phosphate, Zinc Phosphate and Tri-Calcium phosphate

Twenty candidate PSB isolated from Meghalaya were subjected to a screening for their ability to solubilize phosphate (tri-calcium phosphate & iron phosphate) in NBRIP-BPB broth in 50 ml Falcon's tubes. Out of twenty isolates, eight rapid P solubilizers were found. Seven isolates seemed promising as solubilizer of tri-calcium phosphate as substrate and five isolates could solubilize iron phosphate as well. Isolate NG21 showed highest solubilization of tri-calcium phosphate (119 mg/lt.) and isolate UP15 showed maximum solubilization of iron phosphate (21 mg/lt.).

Research Achievements

Developing technique for acceleration of decomposition process using thermophilic organisms

PI : Asha Sahu [1]
Co-PI : Udai B. Singh [2], Dr. J. K. Thakur [1], H.L. Kuswaha [3], M.C. Manna [1], A. Subba Rao [1]
[1] Division of soil Biology, Indian Institute of Soil Science, Nabibagh, Berasia Road, Bhopal 462 038
[2] National Bureau of Agriculturally Important Microorganisms, Kusmaur, Maunath Bhanjan 275 101
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Rationale

Decomposition is the natural process of dead animal or plant tissue being rotted or broken down. This process is carried out by invertebrates, fungi and bacteria. The result of decomposition is that the building blocks required for life can be recycled. During the process of decomposition, the decomposers provide food for themselves by extracting chemicals from the dead bodies or organic wastes; using these to produce energy. During decomposition the organisms vary in the pile due to temperature conditions, but the goal in composting is to create the most favorable environment possible for the desired organisms. Furthermore, stocking and bagging of a wet and immature product can induce compost anaerobic decomposition, with the result of toxic substances such as alcohol, methane and acetic acid during storing up. In addition the concentration of soluble carbon of non-stabilized composts can support pathogen growth. Composting is a microbial process which is influenced by a number of factors like air supply, moisture content, temperature, waste particle size, acidity / alkalinity and some other chemical characteristics. Traditionally in composting process takes longer time duration. Today requirement is that reduces the time and produce good quality compost. Keeping these factors in the mind, the project was formulated with the following objectives.

Objectives

- Isolation and identification of thermophilic bacteria, fungi and actinomycetes.
- Evaluation of selected thermophilic decomposers in municipal solid waste and agricultural waste compost at different stages of decomposition.
- Develop appropriate machinery/protocol for scaled up decomposition process mediated by microbes and its possible application in agriculture.
- Evaluation of physico-chemical properties of compost and economics of composting.

Achievements

- Identification of 7 bacterial isolates by 16S rDNA sequencing. 16S rDNA amplification of bacterial stains was done successfully. Whereas, sequencing of remaining 5 actinomycetes strains (16S rRNA genes) is under process.
- Among 21 strains (9 bacterial, 6 actinomycetes and 6 fungal strains), 18 strains (7 bacterial, 5

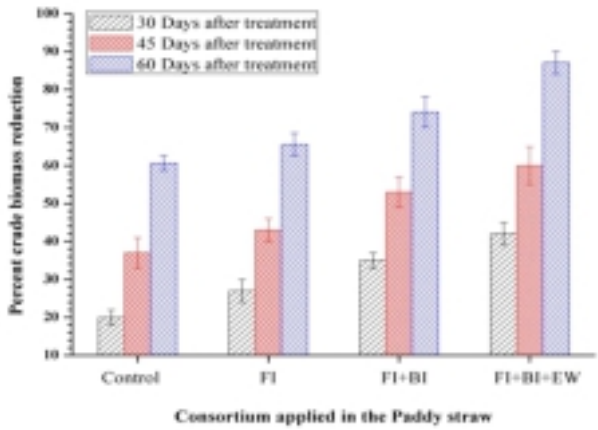

Fig. Effect of different consortium on *ex situ* biomass reduction (%)

actinomycetes and 6 fungal strains) were tested for their cellulolytic and lingo-cellulolytic ability in controlled laboratory condition and found very effective in the decomposition of lingo-cellulosic agro-waste in comparison to control.

- A model was designed and developed by CIAE, Bhopal in collaboration of IISS, Bhopal and NBAIM, Mau to accelerate the decomposition in the presence of suitable microbial strains. Different combinations of 18 selected streains was used to develop three consortia, which is being evaluated.
- These three consortia were tested for faster decomposition of composite agro-waste under normal field condition and consortia 3 was found most effective that can decompose agro-waste within shortest period of time (Fig. 1).
- Compost prepared from rice straw alone and composite agro-waste was tested under net house condition in tomato. It was depicted from result that compost prepared from consortium 3, working well and giving significantly better result than other consortium tested (combination detail was not given here due to IP related issue).

Diversity analysis of *Bacillus* and other predominant genera in extreme environments and its utilization in Agriculture

Consortium Leader : Arun Kumar Sharma
Consortium PI : Alok Kumar Srivastava

Rationale

Species of *Bacillus* and *Bacillus* derived genera are employed in industry as a source of enzymes, in agriculture as inoculants and biocontrol agents. They are also implicated in bioremediation and the insecticidal property of *Bacillus thuringiensis* has been exploited largely. In India there is no baseline information available on the species richness and thus its utilization is not understood. *Bacillus* species predominate in most of the environments and are used as plant growth promoting rhizobacteria as well as potent biocontrol agents. The nutritional versatility of *Bacillus* allows them to use a range of carbon sources including such things as uric acid, herbicides and nicotine. For these reasons they are extremely competitive organisms with remarkable resistance to desiccation and starvation. The optimum growth temperature of most species is 25-30°C. However, species isolated from extreme conditions have developed acclimation proteins allowing them to sustain life under extreme conditions of salinity, drought, high or low temperatures and acidity. Identification of new osmolytes and the relevant genes can be a boon to Indian agriculture as these genes could be utilized to develop transgenics tolerant to abiotic stress. Advances in biotechnology have produced improved prospects for developing new Bt insecticides and an ability to place Bt toxins within crop plants in a variety of ways. . In view of diversity of agroclimatic conditions in the country, genetic diversity of Bt strains could be exploited to develop strains with high and wide range of toxicity to different insect pests, better persistence and nontoxicity to the non-target organisms

Objectives

- Diversity analysis and identification of *Bacillus* and other predominant genera from extreme conditions of salinity.
- 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.

Achievements

- The cellular fatty acid profiles of thirty seven ARDRA representative bacterial strains was analysed by MIDI (USA). Different strains differed in their whole-cell fatty acids composition. Each bacterial species has a unique fatty acid composition, making it a 'microbial fingerprint'. Fatty acids observed in all the *Bacillus* strains were C14:0, C14:0 iso, C15:0 iso, C15:0 anteiso, C16:0 iso, C16:0, C17:0 iso, C17:0 anteiso, C18:0, C18:0 iso, C16:1 w7c alcohol and C17:1 w11c. However, C15:0 iso, C15:0 anteiso, C17:0 iso, C17:0 anteiso, C16:0 iso and C16:0 were the dominant fatty acids detected in *Bacillus* strains.
- Since based on the 16S rRNA gene marker, the similarity ascertained between different orthologues was close to 98-100%, it is evident that more sensitive and discriminating parameters would be required to ascertain the significance of small differences observed in the phylogenetic comparisons. Hence, the FAME analysis of salt tolerant strains was carried out. Both the FAME analysis and 16S rRNA provided almost similar nomenclature for most of the strains. Surprisingly,

fatty acid profile identified strains (MB10, NB22, 203 VB4, VB25 and VB27) up to species level, which could not be differentiated exclusively on the basis of 16S RNA gene sequencing.

- On the basis of germination assay five potent PGPR isolates *viz*., BC39, RC13, RC25, KC30 and KC31 different combination with *Rhizobium* specific to chickpea were further evaluate under the field condition in salt susceptible and tolerant cultivars. Based on the sequencing of 16S rRNA gene and fatty acid methyl ester analysis the isolates were identified as: *Bacillus subtilis* BC39, *Pseudomonas putida* RC13, *Bacillus subtilis* RC25, *Pseudomonas fluorescence* KC30 and *Pseudomonas* sp. KC31
- Results of field experiment revealed that the consortium (T30) *B. subtilis* BC39 + *B. subtilis* RC25 + *P. fluorescence* KC30 + *P.* sp. KC31 + *M. ciceri* was observed more effective in both salt tolerant (CSG8962) and salt susceptible (PG186) chickpea cultivars in terms of growth parameters (like shoot length, root length, shoot/root dry weight & biomass.

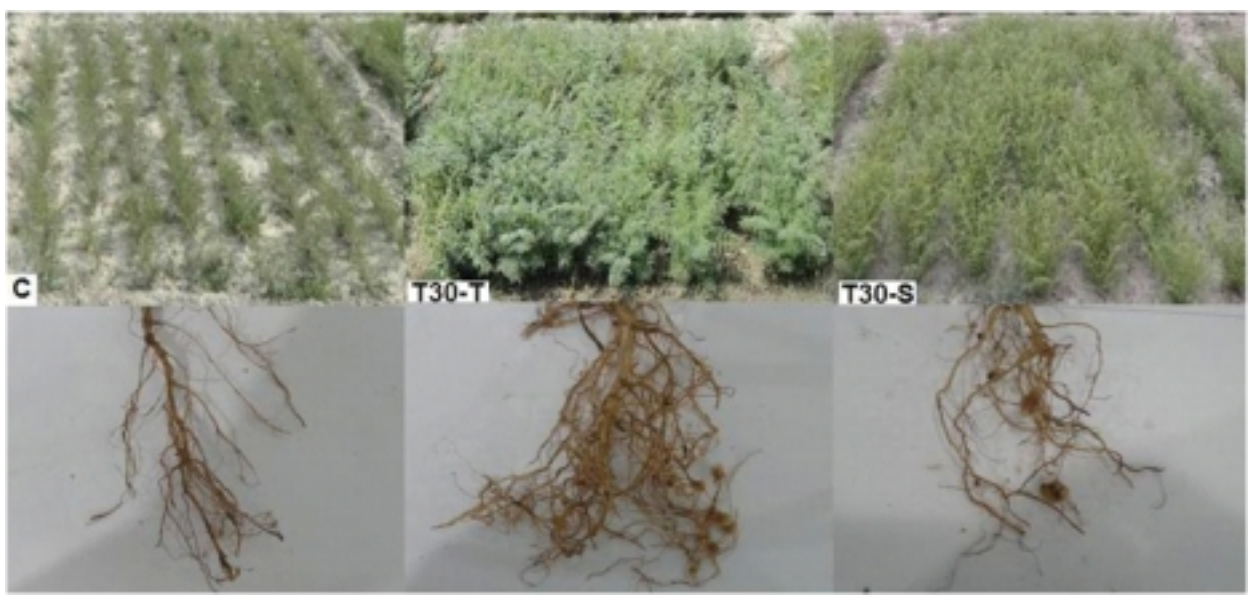

Fig. Effective photograph of field experiment of treatments of C (un-inoculated control), and treatment T30 -T and T30-S (*B. subtilis* BC39 + *B. subtilis* RC25 + *P. fluorescence* KC30 + *P.* sp. KC31 + *M. ciceri)* on Tolerant (T) and susceptible chickpea cultivars growth.

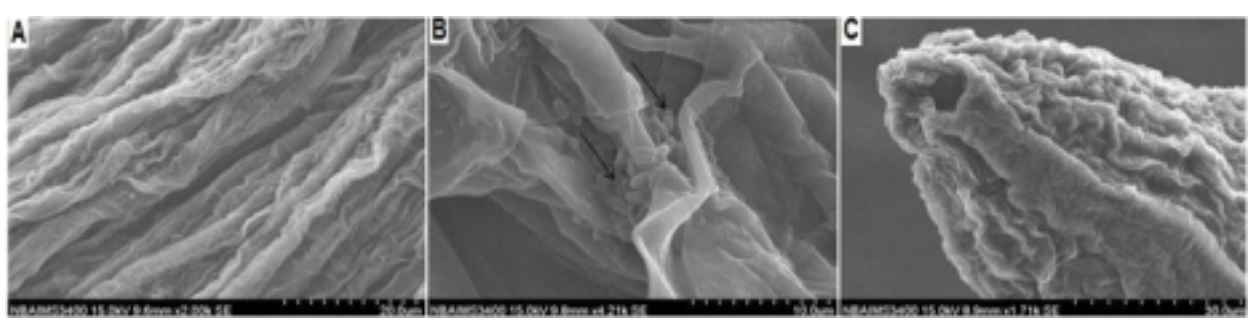

Fig. Scanning electron micrograph of chickpea seedlings (A) Root surface of untreated seedlings free of bacteria; (B) Seedlings treated with PGPR formations of microcolonies are denoted by arrowheads; (C) Formation of Nodule

Project: Georeferenced soil information system for land use planning and monitoring soil and land quality for agriculture

CCPI : Alok Kumar Srivastava

Rationale

Soil contains a large variety of microbial taxa with a wide diversity of metabolic activities. Soil microbial biomass compared with that of superior organisms are a more sensitive indicator and is influenced by different ecological factors like plant diversity, soil organic matter content, moisture, and climate changes. Microbial indicators have been defined as 'properties of the environment or impacts that can be interpreted beyond the information that the measured or observed represents it. Specific indicators are dependent on the geographic zone, climate, soil type and land use history. The diverse microbial pool maintains soil homeostasis. The larger the microbial diversity and functional redundancy, the quicker the ecosystem can return to stable initial conditions after exposure to stress or disturbance. In particular, the size and diversity of specific functional microbial groups such as arbuscular mycorrhiza (AM) fungi and nitrifying bacterial communities have the potential to influence the effects of management on the sustainability of soil. In particular, the search for indicator organisms associated with healthy or deteriorated soil requires a unified concept of soil quality. In this quest of finding out microbes as soil quality indicators we studied a few representative soils of the Indogangetic Plains (IGP).

Objectives

- Determination of CFU for the different micro-organism of samples.
- Determination of the soil dehydrogenase activity.
- Determination of soil urease activity.
- Quantitative analysis of P -solubilization microbes and *Azotobacter*

Achievements

- In the study area of the IGP, *Rhizobium*, mycorrhizae and nitrifying bacteria are found as specific indicators because of their high sensitivity to agrochemicals or management regimes, and

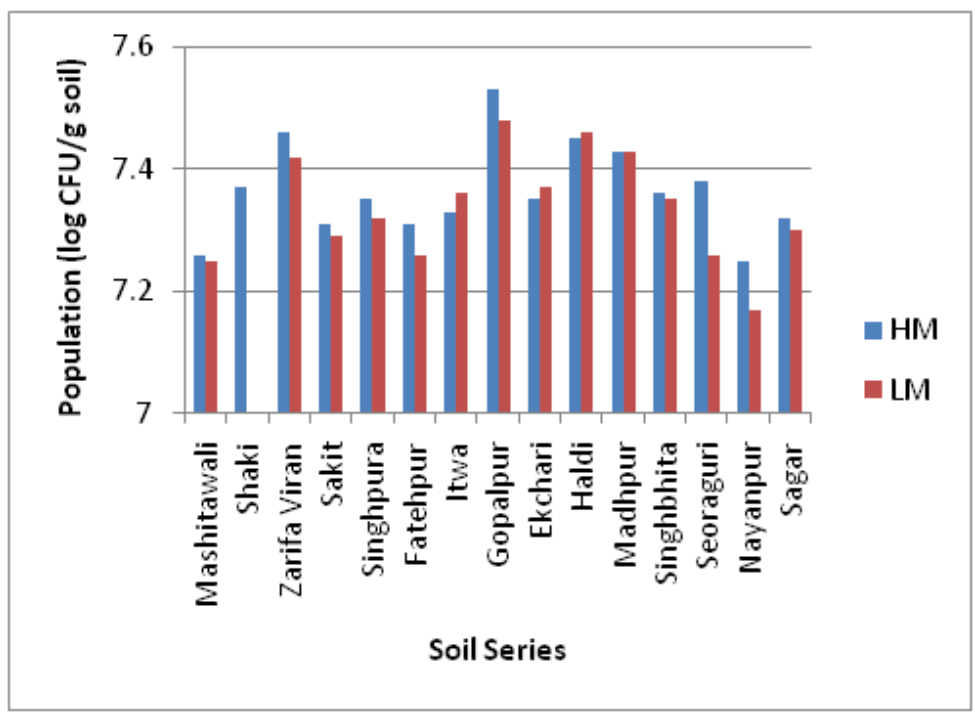

Fig. Population of Bacteria at different benchmark spots of IGP at a depth of 0-50 cms. (HM: high management, usually soils receiving fertilizers and manures and other management practices; LM: low managememt , usually soils receiving very little management in terms of fertilizer and manures)

clearly defined roles among soil functions. The total mass of micro-organisms in organic systems is 20-40% higher than that in the conventional system with manuring and 60-85% than that in the conventional system without manuring. The management practices also influence the population of microorganisms in the soil. In some of the benchmark spots from IGP, it was observed that population of bacteria remains higher in the field under high management practices.

- The major microbial functional indicators in soil include the activity of extracellular enzymes involved in the transformations of carbon (amylase, cellulase, invertase), nitrogen (protease) and phosphorus (phosphatase); the activity of intracellular enzymes such as dehydrogenase; as well as microbial biomass carbon (MBC) and basal respiration (CO_2 evolution).
- Dehydrogenase enzyme is known to oxidize soil organic matter by transferring protons and electrons from substrates to acceptors. These processes are the part of respiration pathways of soil microorganisms and are closely related to the type of soil and soil air-water conditions. Since these processes are the part of respiration pathways of soil microorganisms, assessment of activities of dehydrogenase enzyme in the soil is very important as it provide indications of the potential of the soil to support biochemical processes which are essential for maintaining soil fertility as well as soil health.
- In the IGP soils higher dehydrogenase activity was observed in top soil (0-30 cms) to indicate the intense microbial load compared to the subsurface horizons from 100 cms and 150 cms. The activity was significantly, higher in high management (HM) soil compared to low management (LM) soils. Additionally, dehydrogenase enzyme is often used as a measure of any disruption caused by pesticides, trace elements or management practices to the soil, as well as a direct measure of soil microbial activity.
- Generally, urease activity increases with increasing temperature. Therefore, it is recommended to farmers in IGP regions that urea be applied at times of the day when temperatures are low. Since urease plays a vital role in the hydrolysis of urea fertilizer, it is important to uncover other unknown factors that may reduce the efficiency of this enzyme in the ecosystem. However, a marked decrease in the activity of urease along the depth was obtained in the semi arid. sub humid and humid bioclimatic systems. The activity was higher in high management soil which corresponds with more bacterial population.

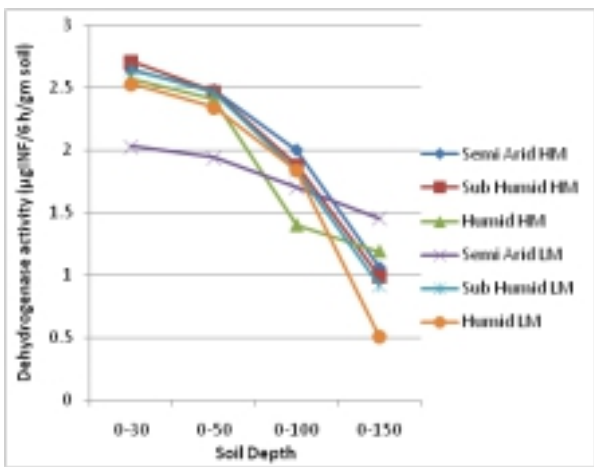

Fig. Soil Dehydrogenase activity in the soils represented by different bioclimatic systems (HM: high management, usually soils receiving fertilizers and manures and other management practices; LM: low managememt , usually soils receiving very little management in terms of fertilizer and manures)

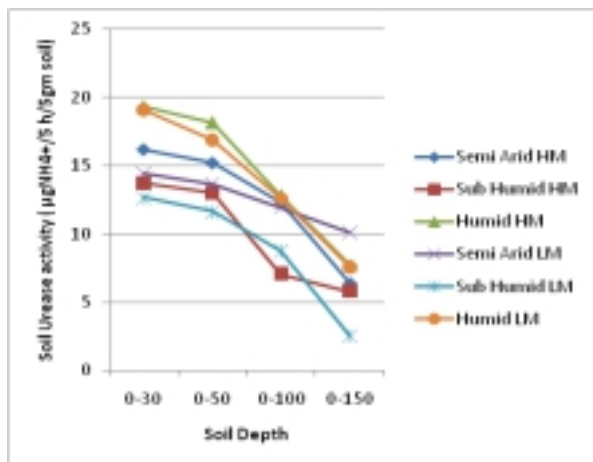

Fig. Soil Ureasein the soils represented by different bioclimatic systems (HM: high management, usually soils receiving fertilizers and manures and other management practices; LM: low managememt , usually soils receiving very little management in terms of fertilizer and manures)

Establishment of National Agricultural Bioinformatics Grid

CCPI : Dhananjay Pratap Singh
Co-PIs : Alok Kumar Srivastava, Anurag Chaurasia, Dipak T. Nagrale and Lalan Sharma

Rationale

Omics research in biological research, especially microbial research has opened new vistas for increasing the productivity and quality attributes of agricultural systems. During last several decades genomics has witnessed an information explosion. Genomic databases contain huge amounts of information that are not amenable to traditional analytical approaches. Therefore, bioinformatics has emerged as an inter-disciplinary programme which links computational and mathematical sciences with life sciences. The proposed centre of bioinformatics aims to bring the biologists, statisticians and computer scientists together from the point of view of system biology approach and effective problem solving. It is proposed to establish a National Agricultural Bioinformatics Grid (NABG) not only for the Indian Council of Agricultural Research (ICAR) institutions but also for the country as a whole. NBAIM, being a domain partner of this project is looking into various programs assigned by the nodal centre i.e. IASRI, New Delhi. The specific objectives of the centre are as under .

Objectives

- Development of agricultural bioinformatics grid for the country
- Creation of local databases and Bioinformatics Data Warehouse (BinDW) for genomic resources across species
- Human resource development in agricultural bioinformatics
- Create and promote inter-disciplinary research groups with focus on agricultural bioinformatics

Achievements

HPC data centre developed

Research work carried out at microbial domain of the project "National Agricultural Bioinformatics Grid" (NABG) at NBAIM, Mau included six major themes namely 1. Evolutionary genomics: whole genome phylogeny; 2. Comparative genomics analysis; 3. Genome annotation; 4. Protein structure prediction and modeling; 5. Combinatorial library designing for important small molecules; 6. Identification of novel antimicrobial targets in phytopathogens.

Evolutionary Genomics: Use of genome sequences for phylogenetic analysis

Genomic sequences of 12 strains of high- and low light inhabiting *Prochlorococcus marinus* (marine cyanobacteria) was downloaded from NCBI and whole genome phylogeny was prepared based on genome alignment (Fig. 1. a) and overlapping gene-content and overlaping gene-order (Fig. 1 b). The whole genome phylogeny supported morphological differentiation of *P. marinus* strains and differed with that based on 16S rDNA gene.

Fig. 1 Whole genome phylogeny of *Prochlorococcus marinus* based on (a) genome alignment and (b) overlapping gene-content and overlaping gene-orderWhole genome sequences of 41 cyanobacterial genomes available with NCBI were downloaded and whole genome phylogeny was constructed and finally compared with the traditional taxonomy (five groups of cyanobacteria) and molecular taxonomy based on 16S rDNA.

Comparative genomic analysis was carried out on genome size, gene content, GC composition, codon selection pattern, nucleotide distribution pattern, mono- and di- nucleotide frequency and oligonucleotide (tri-, tetra- and hexa- nucleotide) frequency in all the 41 cyanobacterial genomes. Genome atlas for distribution of oligonucleotides in one of the 41 cyanobacteria i.e. *Prochlorococcus marinus* MIT9313 has been developed (Fig. 2). Genome alignment of all the twelve genomes of *P. marinus* was carried out with Mauve software having Progressive approach using default parameters (Fib. 3). Results suggested evolutionary trends in cyanobacterial genomes and reflected the adaptation patterns adapted by cyanobacteria in different ecological niches.

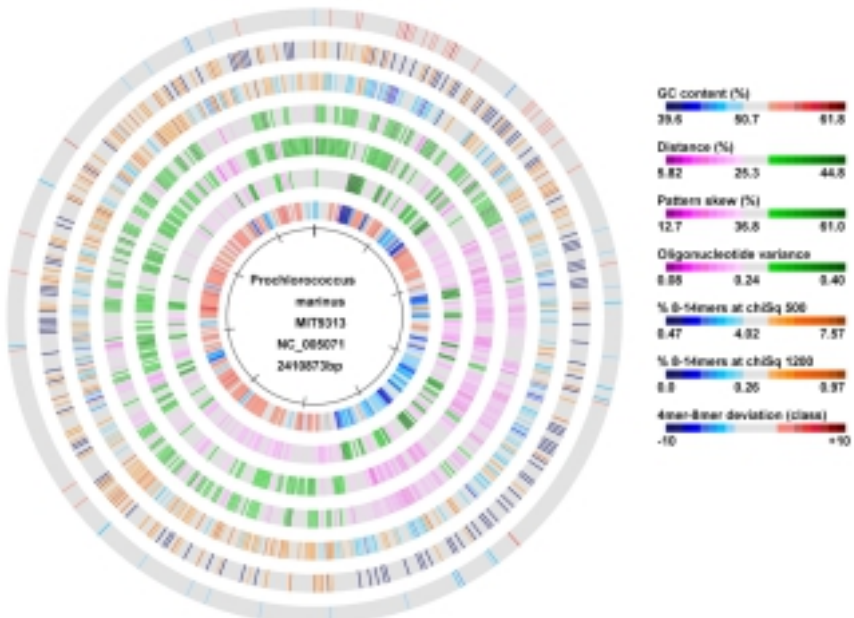

Fig 2. Genome atlas for distribution of oligonucleotides in *Prochlorococcus marinus* MIT9313

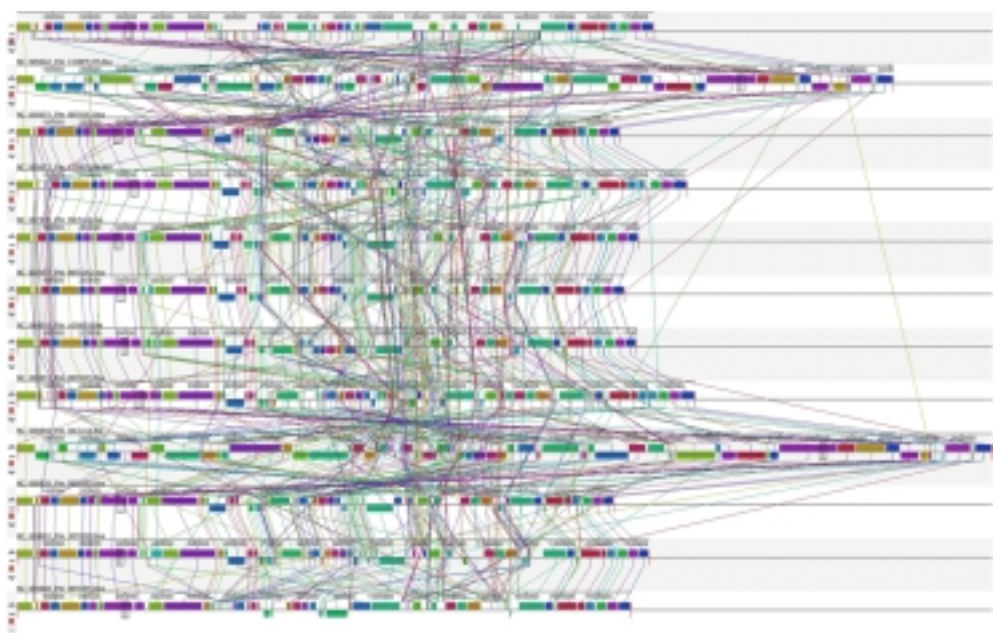

Fig 3. Genome alignment of all the twelve genomes of *Prochlorococcus marinus* (cyanobacteria) with Progressive Mauve using default parameters

Outreach Programmes

Outreach programme on *Phytophthora, Fusarium* and *Ralstonia* diseases of horticultural and field crops

Sub Project : Conservation, characterization and documentation of different species of Fusarium

PI : Prem Lal Kashyap
Co-PI : Alok Kumar Srivastava

Rationale

A detailed understanding of pathogen's phenotypic and genetic diversity is imperative to interpret their contribution to disease epidemiology and management. In this context, a polyphasic approach was undertaken with the aim of characterizing the *Fusarium udum* (*Fu*) and *F. oxysporum* f. sp. *ciceri* (FOC) species responsible for causing wilt disease in piegonpea and chickpea, respectively in India. To ascertain the variability in *Fu* and *Foc* isolates, the robustness of different molecular marker systems *viz.*, random amplified polymorphic DNA (RAPD), enterobacterial repetitive intergenic consensus (ERIC), BOX elements and mating type locus were employed. All techniques yielded intra-specific polymorphism, but different levels of discrimination were obtained.

Objectives

- Characterization of *Fusarium* species using molecular markers.

Achievements

Genetic diversity analysis by mating type sequences

PCR assay was performed to assign mating types (MAT1 and MAT2) for 20 different isolates of *Fu* and *Foc*. A single product was generated by PCR from each *Fu* and *Foc* isolates using primer pairs complementary to the alpha domain and HMG domain genes, respectively. A 320 bp portion of the alpha domain (MAT1) was obtained from isolates Fu1, Fu2, Fu3, Fu4, Fu5, Fu8, Fu11, Fu12, Fu15, Fu18, Fu19 and Fu20. Similarly, a 650bp portion of HMG domain (MAT2) was detected in isolate Fu6, Fu7, Fu9, Fu10, Fu13, Fu14, Fu16 and Fu17 (Fig 1). Similar trend was obtained with the isolates of FOC (Fig 2). The presence of MAT-1 was detected in 12 isolates of FOC, while rest of the isolates showed the presence of MAT-2 gene.

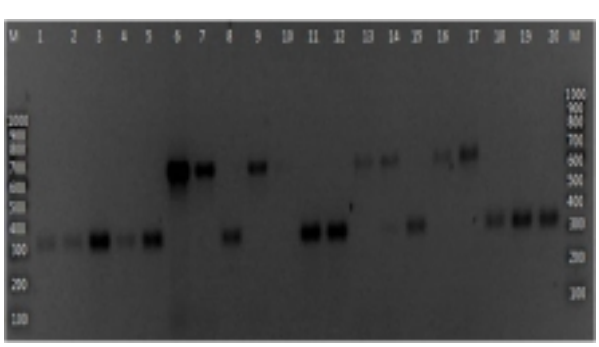

Fig 1: PCR amplification of a MAT1 (320-bp) and MAT2 (650 bp) gene in *Fu* isolates representing distinct geographical lineages. Lanes 1–20 are different *Fu* isolates. M is a 100-bp DNA marker.

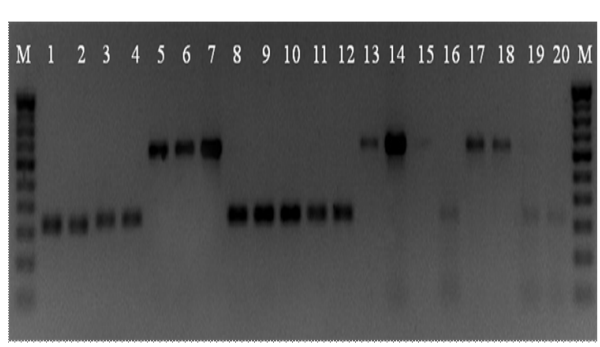

Fig 2: PCR amplification of a MAT1 (320-bp) and MAT2 (650 bp) gene in FOC isolates representing distinct geographical lineages. Lanes 1–20 are different Foc isolates. M is a 100-bp DNA marker.

Genetic diversity analysis by RAPD-PCR analysis

Among the ten RAPD primers, three primers *viz.*, OPA-2, OPA-3 and OPA-11 were chosen based on their capacity to reveal polymorphisms among isolates (Fig. 3). These primers produced a total of 83 fragments among all the 20 isolates, giving a ratio of three polymporphic bands/primer. The size of RAPD fragments ranged from 300 to 1600 bp. RAPD analysis of genomic DNA from the pathogenic isolates revealed the presence of thirteen clusters at the arbitrary level of 50% similarity (Fig 3). Maximum isolates were clustered in group I (Fu1, Fu2, Fu3 and Fu4) followed by group II (Fu19 and Fu 20), III (Fu 15 and Fu 18), IV (Fu6 and Fu7) and IX (Fu 13 and Fu16). In case of FOC isolates, ten clusters at the arbitrary level of 50% similarity were obtained (Fig 4). Maximum eight isolates were grouped in cluster III.

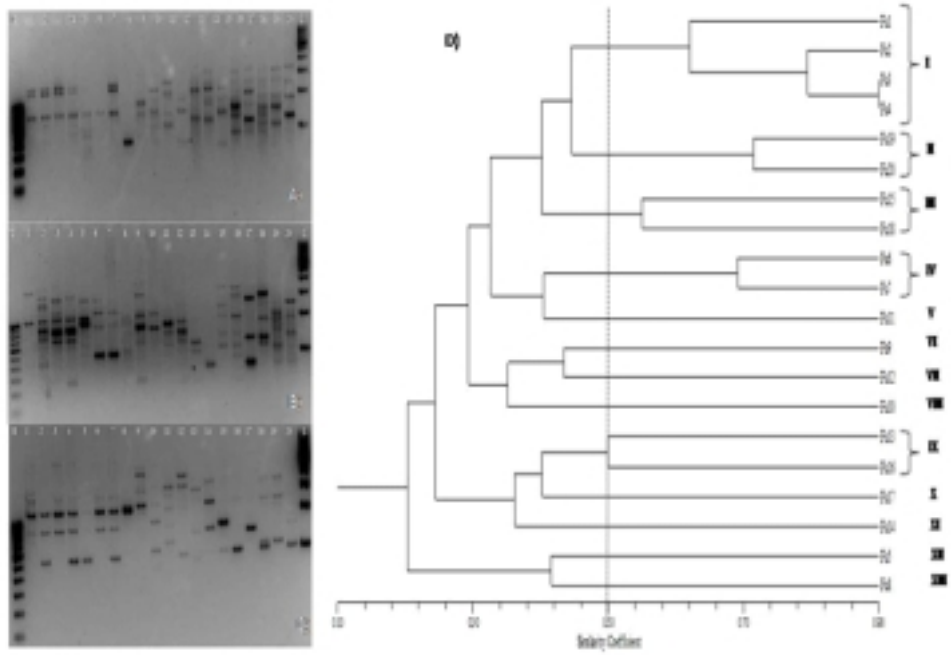

Fig 3: Banding pattern of Fu isolates obtained using OPA2 (A), OPA3 (B) and OPA11 (C). M and L is a 100bp and 500 bp DNA ladder, respectively. D) Dendrogram based on banding pattern obtained from RAPD markers.

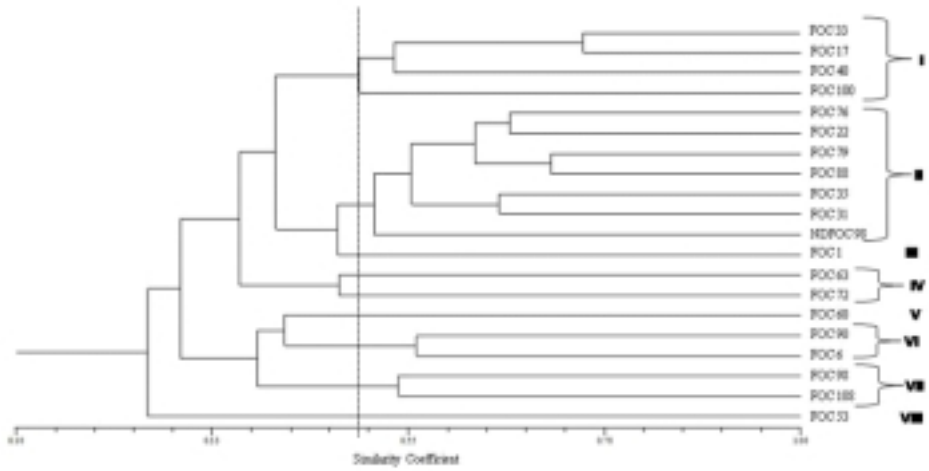

Fig 4: Dendrogram based on banding pattern of FOC isolates obtained from RAPD markers.

Genetic diversity analysis by ERIC-PCR analysis

The genetic variability among the 20 Fu isolates was assessed using ERIC-PCR and a high level of polymorphism in the banding pattern was obtained (Fig. 5). The number of bands in the amplification profile was 188, and their size was found to vary from 150-3000 bp among these isolates. One band of approximately 500bp amplicon size was present in all Fu isolates (Fig 5). Cluster analysis based on the Jaccard's similarity coefficient (50%) showed that the isolates were divided into eight groups and giving a ratio of eight bands/isolate. Similarly, in case of *Foc* isolates, cluster analysis based on the the Jaccard's similarity coefficient (50%) showed that the isolates were divided into fourteen clusters (Fig 6).

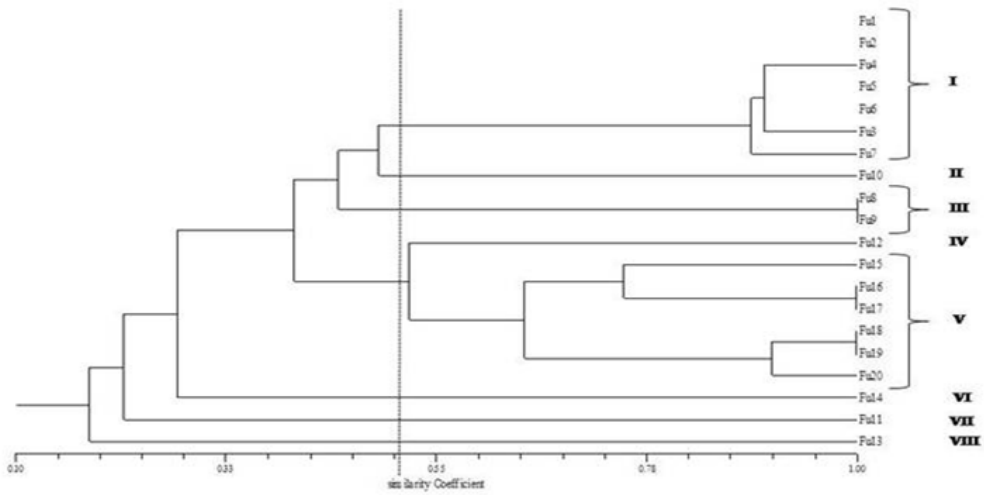

Fig 5: UPGMA dendrogram showing genotypic diversity among the 20 Fu isolates from ERIC-PCR fingerprinting

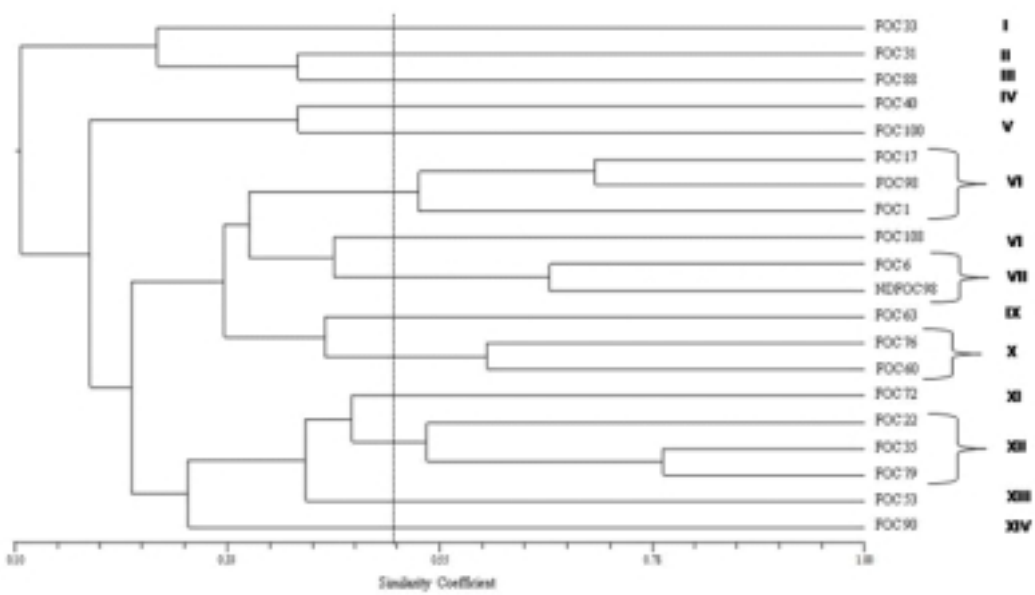

Fig 6: UPGMA dendrogram showing genotypic diversity among the 20 FOC isolates from ERIC-PCR fingerprinting

Genetic diversity analysis by BOX-PCR analysis

Analysis of BOX-PCR banding pattern (Fig 7) showed that the Fu isolates were clustered into five clusters, sharing 50-100% similarity. The banding pattern showed a total of 246 fragments in the range of 200-4000 bp, giving a ratio of five polymporphic bands/isolate. A perusal of the dendrogram revealed that the thirteen isolates (Fu1, Fu2, Fu3, Fu4, Fu5, Fu7, Fu9, Fu10, Fu13, Fu14, Fu15, Fu16 and Fu17) were formed a major cluster (Cluster I), while only three (Fu18, Fu19 and Fu20) and two (Fu11 and Fu12) isolates were grouped in third (cluster III) and fourth cluster (cluster IV), respectively. In case of FOC isolates, genotypic diversity analysis by BOX-PCR resulted in the formation of nine distinct clusters (Fig 8). The banding pattern showed a total of 170 fragments in the range of 200-4000bp, giving a ratio of eight polymporphic bands/isolate. A perusal of the dendrogram revealed that the eight isolates (FOC33, FOC63, FOC72, FOC22, FOC60, FOC90, FOC35 and FOC53) were formed a major cluster (Cluster I), while only three isolates were grouped in cluster VII (FOC40, FOC79, FOC17, FOC100) and cluster IX (FOC1, FOC31 and FOC88), respectively.

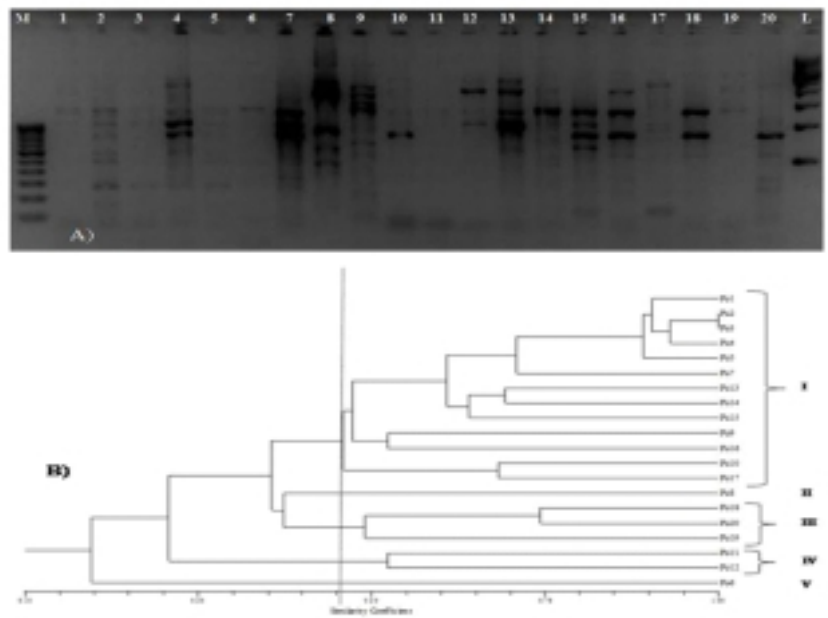

Fig 7: Genotypic diversity analysis by BOX-PCR between 20 *Fu* isolates having distinct geographical lineages

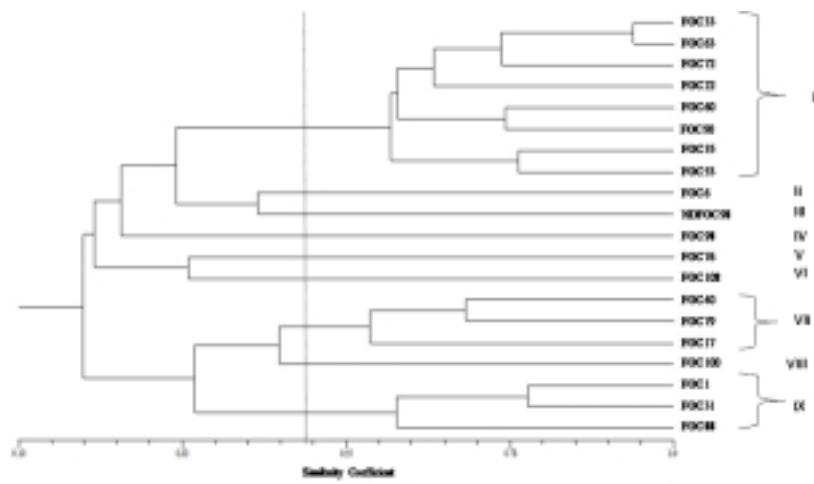

Fig 8: Genotypic diversity analysis by BOX-PCR between 20 *FOC* isolates having distinct geographical lineages

Outreach Programme on Diagnosis and Management of Leaf Spot Diseases in Horticultural and Field Crops

Sub project: Conservation, characterization and documentation of different species of *Alternaria*, *Colletotrichum* and *Cercospora*

PI : Prem Lal Kashyap
Co-PI : Alok Kumar Srivastava

Rationale

Alternaria blight caused by *Alternaria brassicicola* and *A. brassicae* is one of the most important disease of rapeseed-mustard and characterized by the formation of spots on leaves, stem and siliquae with premature defoliation and stunting of growth. The amount and distribution of genetic variation within and between populations in these fungi is of interest, as it gives an indication of the potential for development of pathogenic specialization, host-pathogen co-evolution, and resistance management. In this context, microsatellite based approach was undertaken to characterize characterize transferable microsatellite loci from *A. brassicicola* to *A. brassicae* for the development of diagnostic marker.

Objectives

- Characterization of transferable micro satellite loci from *A.bramicola* to *A.brasicae.*
- Assessment of micro satellite marker as diagnostic marker.

Achievements

Virulent isolates of forty fungi including twenty of *A. brassicae,* ten of *A. brassicicola*, five of *A. porri,* five of *A. sesame,* four of *A. solani* and one each of *A. polenderi, A. gossypina, A. arborescence, A. poneasis, A. alternata, A. zinnae, A. tennuisima, Fusarium udum, Fusarium oxysporum* f. sp. *lycospersci, F. ciceri, Colletotrichum falcatum* and *C. gleosporoides* obtained from from different participating Institute under network project and National Agriculturally Important Microorganisms Culture collection (NAIMCC), Mau, Uttar Pradesh, India were used in present study. A total of thirty one primer sets were tested on ten isolates of *A. brassiscola*. Fourteen (45.1%) of them successfully produced at least one bright and distinct amplicon in *A. brassiscola* isolates ranged from 117-400 bp, while seventeen microsatellite markers showed no amplification (Table 1).The number of detected alleles at these loci ranged from one to two. Locus ABS1generated two alleles for all the tested isolates of *A. brassisicola* and rest of them were found monomorphic. Microsatellite transferability was assessed with the same conditions of DNA extraction and PCR amplification on twenty isolates of *A. brassicae* (Table 1). Five of these microsatellite loci (ABS1, ABS7, ABS9, ABS14 and ABS24) showed amplification signal for all the tested isolates.

Table 2: Amplification patterns of designed EST-microsatellite primer sets in *A. brassisicola* and *A. brassicae* isolates

Locus	Primer sequence (5′-3′)	Motifs	T_m (°C)	No. of alleles	Expected size (bp)	Amplicon size (bp)	
						A. brassicicola	*A. brassicae*
ABS1	CTTTGGTGGTTGTGTTCCTGT GCTCCGGTCTGAGTAGTAGCAC	$(TGCTGG)_3$	61.0	2	300-400	(300, 400)	369
ABS7	ACACTATGGCTACCCTCAGCAT GACGGACTAACACAGACGATCA	$(GAGC)_3$	60.3	1	256	256	256
ABS9	CCCCAACTCACACGCATAC GTCTCTCAGGAAGTCCATCCAC	$(GAGC)_3$	61.1	1	166	166	166
ABS12	GTCAAAGATTTCCGGTTAGTGC CTCCCCTTTCCCTTCTTCTC	$(GGGAAA)_3$	58.9	1	238	238	-
ABS14	ACAGTCTTCTCTACCTCCCACG AGTGGTCTTTGCTTTGAGCTTC	$(CAT)_4$	60.3	1	322	322	322
ABS15	AGATTGAGGTTTGAGGGTGAAG CGCCTTGTTCTCTTCTAGCAAT	$(CGG)_5$	58.4	1	312	350	-
ABS16	TATGGAGTTGGATCACCTTTGG GTGAAATCAGTCTCGTTCGTCA	$(GCCA)_3$	58.4	1	277	277	-

ABS17	ATGATCTCTACTATCCTACCACTGC CACGTATTCATTTCCGACAAC	$(CGCT)_3$	58.6	1	117	117	-
ABS18	ACCCAGCATTATCTTCATGGAC TCGTAAAAGAGCGGGATCTAAA	$(TCGAG)_3$	57.5	1	196	196	-
ABS24	TATCCCAGCTCAACGAAGCTAT TCTGTGGGTCCAGTTGTGTTAG	$(ACAAC)_3$	59.2	1	295	350	350
ABS25	CAGAGTAGTGGTCGTAAGCACG GATTTGACGCTCTCGAAGAACT	$(TCTGG)_3$	60.3	1	287	287	-
ABS28	TATGAAACTCGGTGACAACCAC TACACTCAGCGGAATATGCAAC	$(CATCA)_3$	58.4	1	359	380	-

To evaluate the effectiveness and specificities of ABS28F/ABS28R primer set, PCR was performed using purified DNA from a panel of ten isolates of *Alternaria brassicicola* resulted in desired amplification of 380 bp amplicon (Fig 1). No amplification was observed in *Alternaria* related and unrelated fungal species (*A. polenderi*, *A. gossypina*, *A. arborescence*, *A. poneasis*, *A. alternata*, *A. zinnae*, *A. tennuisima*, *A. porri*, *sesame*, *A. solani*, *A. brassicae*, *Fusarium udum*, *Fusarium lycospersci*, *Fusarium cicri*, *Colletotrichum falcatum* and *Colletotrichum gleosporoides*).

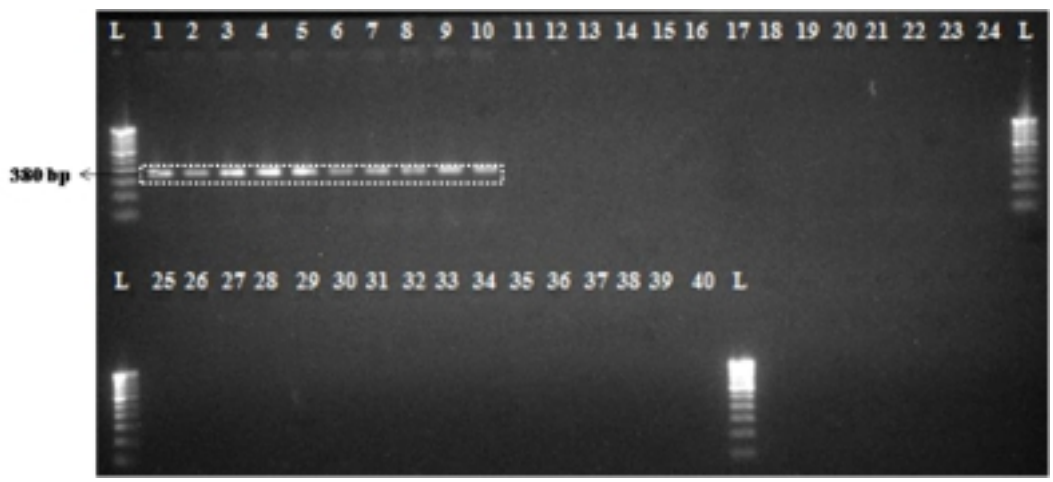

Fig 1: PCR amplification using primer pair ABS28F and ABS28R showing amplification product of 380 bp. Lane L is a 100 bp DNA marker. Lanes 1–10 represent *Alternaria brassicicola*; while lanes 11–39 are *A. polenderi*, *A. gossypina*, *A. arborescence*, *A. poneasis*, *A. alternata*, *A. zinnae*, *A. tennuisima*, *A. porri* OOA3, *A. porri* OOA6, *A. porri* OOA13, *A. porri* OOA14, *A. porri* OOA5, *A. sesame* OSA15, *A. sesame* OSA22, *A. sesame* OSA23, *A. sesame* OSA42 , *A. sesame* OSA53, *A. solani* JT4 , *A. solani* HPT6, *A. solani* JT1, *A. solani* JT2, *A. brassicae* BAB50, *A. brassicae* BAB6, *A. brassicae* BAB42, *Fusarium udum*, *Fusarium lycospersci*, *Fusarium cicri*, *Colletotrichum falcatum* and *Colletotrichum gleosporoides*, respectively. Lane 40 is a no template control (NTC).

Specificity tests with target DNA of *A. brassicicola*, a precise increase of fluorescence was obtained. Fluorescence remained below threshold values for the no template water controls (Fig 2a). Characterization of the amplicons was achieved by melting point analysis 82.3°C. PCR reactions performed on 100ng of *A. brassicicola* DNA with primers ABS28F/ABS28R led to Ct values (23.95 to 34.85) for the *A. brassicicola* and dissociation curves compared to control devoid of DNA matrix, showing the specificity of fungal quantification (Fig 2a). In the 10-fold dilutions series of A. brassicicola DNA, the sensitivity of ABS28F and ABS28R primer was 0.01 ng µl–1 (Fig 2). The correlation between the Ct-value and the target DNA concentration was linear with a high coefficient of determination (R^2> 0.992, y= -2.858x + 37.00) (Fig. 2b). All products were subjected to analytical gel 185 electrophoresis to confirm the 380 bp amplicon size and results were shown in Fig. 2c.

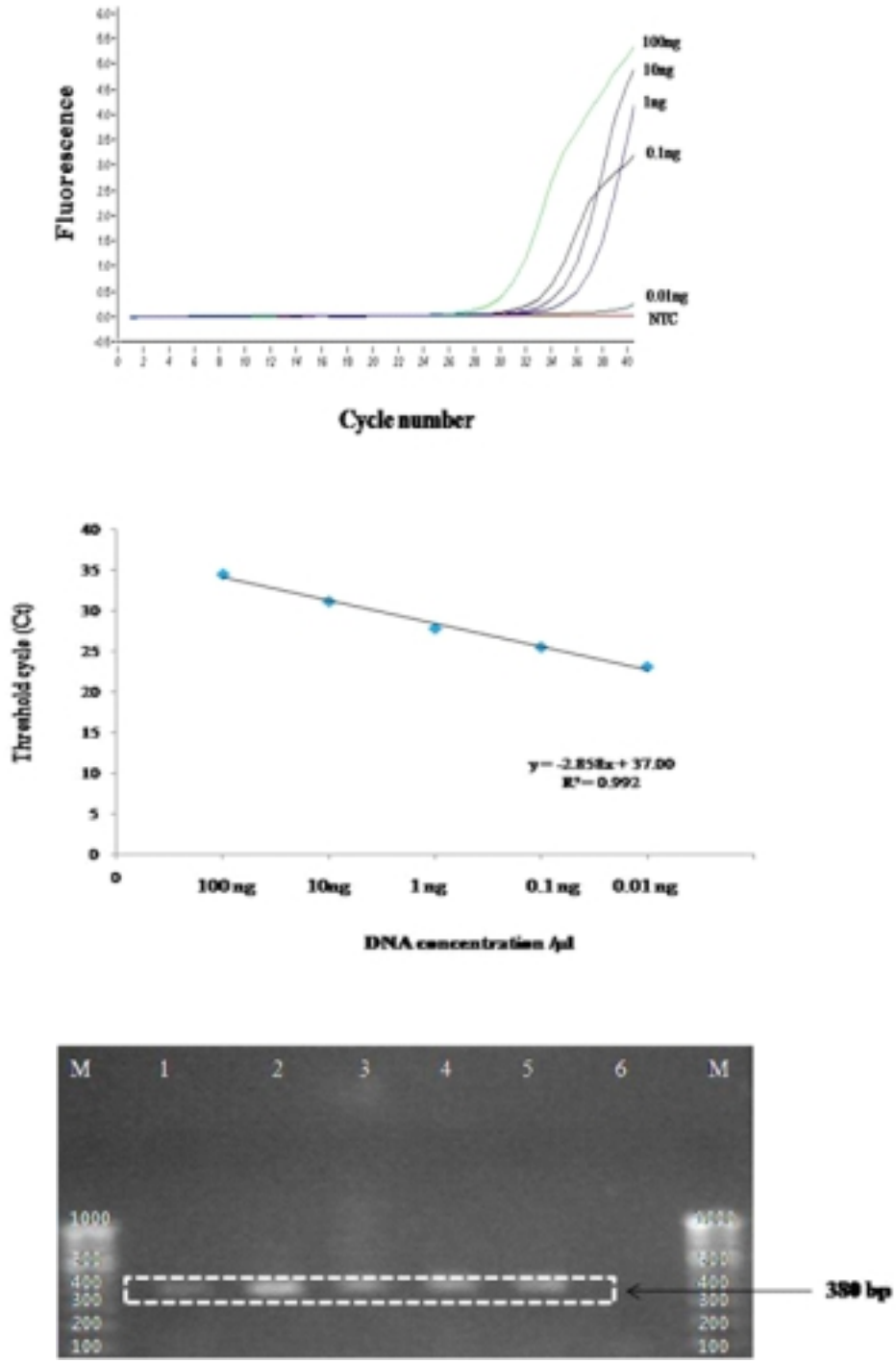

Fig. 2. Standard curve for absolute quantification of genomic DNA generated with 10-fold serial dilutions of genomic DNA isolated from pure cultures of *A. brassicicola* OCA1 using ABS28 primer sets. (a) The curves show the relative fluorescence intensity with respect to the number of PCR cycles. (B) Linear relation between the cycle number and DNA template concentration. The detection limit for genomic DNA is in the range from 100 ngµl^{-1} to 0.01 ngµl^{-1}. c) Gel photographs for absolute quantification of genomic DNA generated with 10-fold serial dilutions of genomic DNA (100, 10, 1.0, 0.1 and 0.01 ng µl^{-1})isolated from pure cultures of *A. brassicicola* OCA1 (Lane 1-5) using ABS28 primer sets; Lane 6, no template control; lane M, 100 bp DNA marker.

Conclusions

In summary, fourteen monomorphic and one polymorphic microsatellite markers were identified from the necrotrophic phytopathogenic fungus *A. brassicicola*. Although, the identified polymorphic marker (ABS1) needs to be developed to enable a more study of the genetic structure of natural *A. brassicicola* populations and to compare the diversity of isolates originating from distinct host and geographical location. Moreover, primer set, ABS28F and ABS28R amplified a specific amplicon of 380 bp and would be useful as an internal diagnostic marker to discriminate and diagnose *A. brassicicola* from other fungi associated with leaves, stem and siliquae of rapeseed mustard

Externally funded project: DST Funded Project

Development of Eco-friendly, Sustainable and cost effective on-farm mass production technology of nematode trapping fungi for resource poor marginal farmers

PI : Udai B. Singh

Rationale

Annual loss of major crops by nematodes is estimated to be 12.3%, which may accounts for billions of rupees annually and root knot of tomato caused by *Meloidogyne* spp. is a serious problem in U.P. and other states of India, which can be controlled by few nematicides which are known for their pollution hazards. Judicious use of chemicals like pesticides and nematicides has shifted approaches towards the use of biological means. Among different bio agents, nematode predacious fungi an important role for parasitizing nematodes eggs, juveniles, and adults. Considering the immediate need to address the problems of nematode in India, biological control of root-knot of rice and tomato deserves priority. There are some potential predaceous fungi, viz., *Arthrobotrys dactyloides*, *A. oligospora* and *D. brochopaga*, available, which have been mass cultured on suitable substrates after screening a large number of substrates such as grains, brans and straws. However, there is need to modify and improve the formulations, and then test efficacy in pots and at farmers fields.

Objectives

- To develop bioformulations of selected nematode trapping fungi, viz., *Arthrobotrys dactyloides*, *A. oligospora* and *Dactylaria brochopaga*
- To evaluate the bioformulations for their biocontrol potential against root knot of rice and tomato caused by *Meloidogyne* species
- To determine the shelf life of bioformulation and standardize the technique to maintain the quality of bio product
- To develop schedule and delivery system of bioformulation application according to crop and season to achieve maximum biocontrol potential of selected BCA.

Achievements

- Grain and vermin-based bioformulations of nematode trapping fungi were developed. Promising nematode capturing isolates *D. brochopaga* and *M. eudermatum* were grown on sorghum (*Sorghum bicolor* L.) seeds and vermicompost for mass culture. Very good colonization were recorded for the all the isolates tested and were found very effective in controlling plant parasitic nematode (PPN) in *in vitro* condition (Fig. 1).

Fig. 1. Mass multiplication of *Dactylaria brochopaga* and *Monacrosporium eudermatum* on sorghum grains.

- Tomato seedlings (cv. HS 101) were transplanted into pots each containing 2500 second stage juveniles kg^{-1} of soil. The nematode infested soil was thoroughly mixed with 10 g sorghum grain formulations containing 4.50 x 10^6 cfu g^{-1} of *D. brochopaga* and *M. eudermatum* alone or in combination. Nematode free soil without fungal

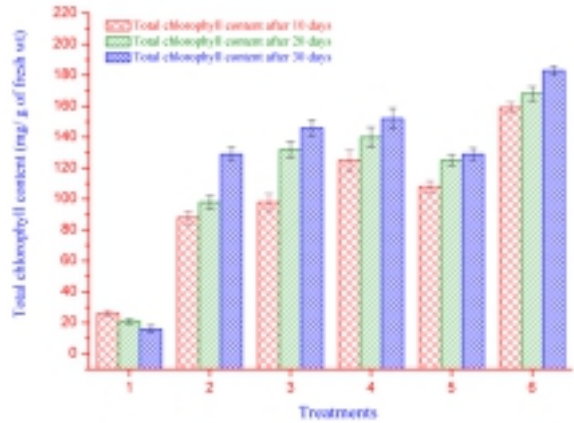

Fig. 2. Total chlorophyll content (mg g^{-1} fresh wt.) in tomato leaves after 10, 20 and 30 days of inoculation; treatment- 1. *Meloidogyne incognita,* 2. *Meloidogyne incognita* + *Dactylaria brochopaga,* 3. *Meloidogyne incognita* + *Monacrosporium eudermatum*, 4. *Meloidogyne incognita* + *Monacrosporium eudermatum* + *Dactylaria brochopaga*, 5. *Meloidogyne incognita* + carbofuran, 6. Control; Control- untreated plants. Data are mean and vertical bar represent standard error of mean.

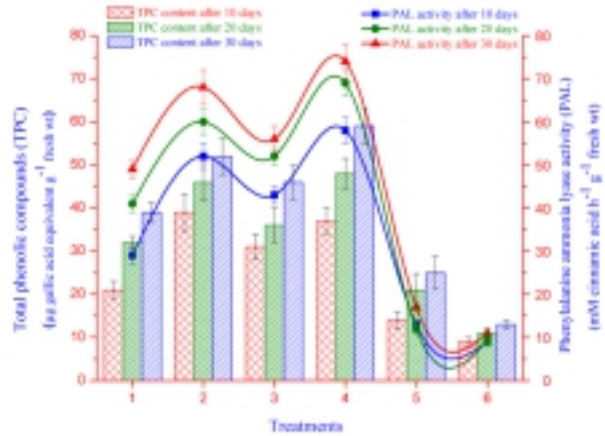

Fig. 3. Total phenolic compounds (µg gallic acid equivalent g^{-1} fresh wt.) and Phenylalanine ammonia lyase (PAL) activity (mM cinnamic acid h^{-1} g^{-1} fresh wt.) in tomato plants after 10, 20 and 30 days of inoculation; treatment- 1. *Meloidogyne incognita,* 2. *Meloidogyne incognita* + *Dactylaria brochopaga,* 3. *Meloidogyne incognita* + *Monacrosporium eudermatum*, 4. *Meloidogyne incognita* + *Monacrosporium eudermatum* + *Dactylaria brochopaga,* 5. *Meloidogyne incognita* + carbofuran, 6. Control; Control- untreated plants. Data are mean and vertical bar represent standard error of mean.

inoculum served as a control and carbofuran @ 2 kg a.i. ha^{-1} (Walia and Bajaj, 2003) was taken as chemical control.

- Plants subjected to biotic stress (*M. incognita*) tends to overproduce defence related biomolecules and enzymes in plant tissues. The activation and accumulation of total chlorophyll ((Fig. 2)), defence related biomolecules and enzymes (Fig. 3) were studied in the *D. brochopaga* and *M. eudermatum* (individually as well as in combination) treated tomato plants pre-challenged with *M. incognita*.

Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)

Project: Complete Genome Sequencing of *Mesorhizobium ciceri* Ca181

PI : Alok Kumar Srivastava,
Co-PIs : Sudheer Kumar and Prem Lal Kashyap

Rationale

The interaction between rhizobia and the host plants of Leguminosae family is very important in view of the nitrogen supplementation to plants. These bacteria induce nodules into the roots of such plants and convert dinitrogen to ammonia. *Mesorhizobium ciceri*, a chickpea rhizobium is one such diazotroph, which forms symbiotic nodules on the roots and provides nitrogen to chickpea (*Cicer arietinum*) plants. Chickpea is the predominant pulse crop in India, accounting for 37% of the production of all pulses grown. It covers the largest area under legume cultivation in our country. More than 70% of the nitrogen economy of the crop is obtained through its symbiotic association with M. *ciceri*. Therefore, improvement of chickpea-rhizobia nitrogen fixation is an important goal to bring a quantum leap in Chickpea productivity. *Mesorhizobium ciceri* strain Ca181 was selected for whole genome sequencing because of its tested qualities like, efficient nitrogen fixation, shows good nodulation, competitiveness and performed well at different locations in different agro-climatic regions, different soil types in All India Coordinated trials. The whole genome sequencing of this bacterium unveils the specific properties of which is encoded by genes that works in the coordinated form of specific metabolic pathways. After the completion of gene prediction and annotation, the reason of uniqueness of bacterium can be unrevealed

Fig. 1 : Comparative mapping of *Mesorhizobium ciceri* Ca181 on *Mesorhizobium loti*

and explained which in turn will help in selection and development of superior strains.

Objective

Complete Genome Sequencing of *Mesorhizobium ciceri* Ca 181

Achievements

- Gap filling of the Contigs: Hybrid Assembly method was used to complete the circular genome by Sequence data generated by different sequencing flat forms. Backbone was generated by large contigs generated by 454-pyrosequencing reads. Illumina contigs (generated with high Phred values) were aligned on backbone of 454 contigs by using MIRA software tool. Multiple iterations were allowed to obtained least number of contigs and large scaffolds were obtained.
- Comparative Genome Analysis (structural and Genetic Variations) against *Mesorhizobium ciceri* Ca181 with other related genomes sequenced.
- The Whole Genome Sequence of Mesorhizobium ciceri Ca 181 was submitted to NCBI genome database; Bio project ID- PRJNA40923, Bio sample ID SAMN02470606 and is available online.

Conclusion: The hybrid assembly and comparision with *Mesorhizobium loti* revealed that it has only about 40% similarity with that genome and indicates the because of absence of plasmid in *M. ciceria* ca. 181, position of genes are at different loci in both the genomes.

Project: Diversity analysis of microbes in extreme conditions

PI : Prem Lal Kashyap
Co-PI : Alok Kumar Srivastava and Hillol Chakdar

Microbes, being the pioneer colonizers on this planet, have come to stay as a cosmopolitan conglomerate of highly compatible organisms. They abound in habits with extremes of temperature, pH and salt stresses. The recognition of 'deep hot biosphere' with unique microbial-animal assemblages and nutrient dynamics, speaks of versatility and importance of microbes in sustaining the life. Bestowed with remarkable inherent physiological and functional diversity, microbes have found application in agriculture and allied sectors. With the existing knowledge regarding microbes and microbial processes, we are still at the base of microbial diversity, which needs to be explored, investigated and exploited. In this project, an attempt has been made to investigate microbial community structure and phylogenetic diversity from the extreme regions of India.

Objectives

- Microbial diversity analysis from cold environment of Arunachal Pradesh
- Diversity analysis of thermo tolerant fungi from Jharia coal mines.

Achievements

I. Microbial diversity analysis from cold environment of Arunachal Pradesh

- A total 205 bacteria and 52 fungi were isolated from cold environment of Sella Pass and Nuranang sites of Arunachal Pradesh (Fig. 1).
- The isolated strains were able to grow at a temperature range of 4-28°C and showed considerable variation with respect to their morphological characteristics on PDA, SDA, MYA and RBA media. The morphotypes of all

Fig 1: Satellite view of the sampling sites (Sella Pass and Nuranang) in Arunachal Pradesh

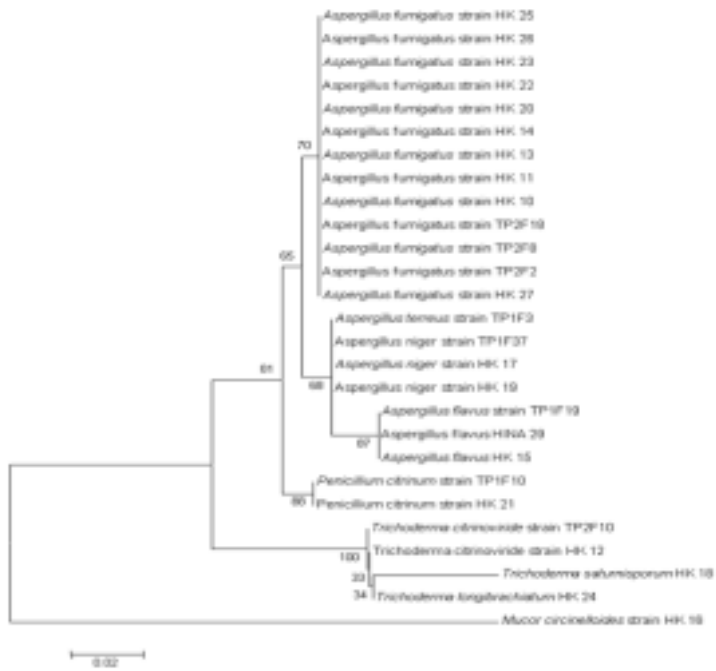

Fig 4: Phylogenetic tree of the fungal isolates obtained from cold environment of Arunachal Pradesh**.**

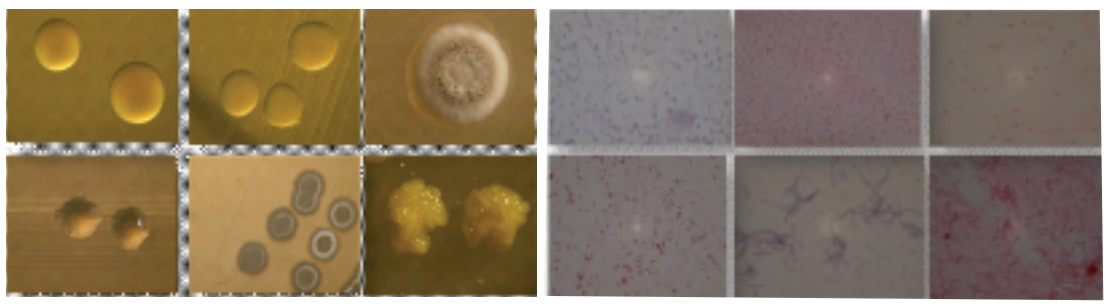

Fig 2: Stereomicroscopic (A) and light microscopic view of the bacterial colonies

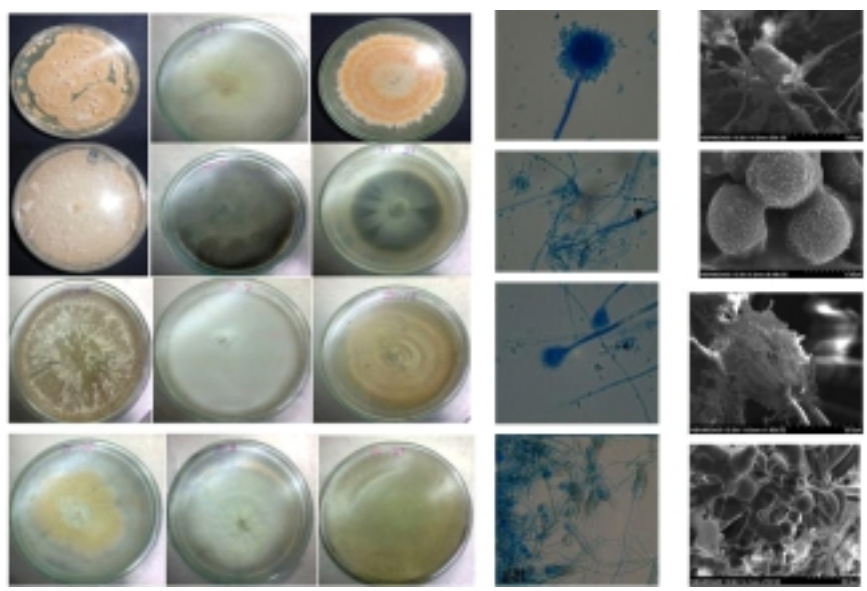

Fig 3: Morphological and microscopic view of the representative fungal isolates.

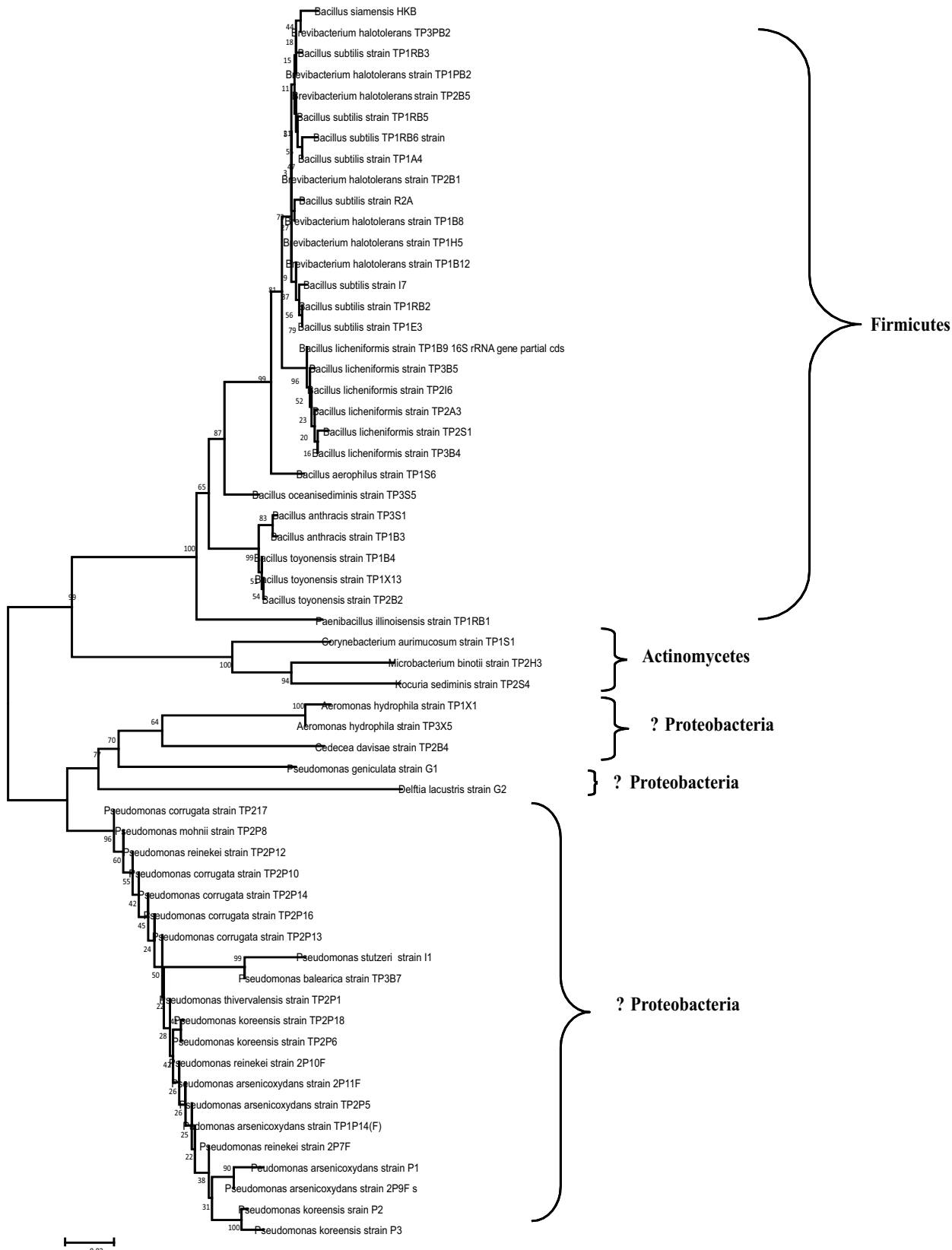

Fig 5: Phylogenetic analysis of the bacterial isolates based on 16S rDNA homology

representative isolates are shown in Fig 2.

- The microscopic examination of all the isolated bacteria and fungi was performed (Fig 3).
- All the isolates were subjected to *in vitro* plant growth promotion activities such as IAA production; siderophore production; HCN production; phosphate, potassium and zinc solubilization. Results showed that out of 52 fungal isolates, 7 isolates were positive for IAA production, 17 for siderophore, 6 for HCN, 5 for phosphate solubilization, 10 for K-solubilization and 2 were positive for zinc solubilization.
- Among 205 bacteria, 35 strains were found positive for P solubilization, 10 for K solubilization and 8 for zinc solubilization.
- Two antagonistic strains *viz.* HKB and R2A gave antagonistic activity against *Alternaria sessame*, *Macrophomina phaeseolina*, *Rhizoctonia solani*, and *Fusarium oxysporum* f.sp. *ciceri*.
- Molecular identification of the potent strains was performed and phylogenetic tress based on 16S rRNA gene and ITS regions for bacteria and fungi was constructed. The cultured bacterial strains were belonged to *Pseudomonas stutzeri*, *Pseudomonas geniculata*, *P. koreensis*, *P. arsenicoxydans*, *Pseudomonas reinekei*, *P. corrugate*, *P. balaerica*, *P. mohnii*, *P. thivervalensis*, *Bacillus subtilis*, *B. erophilus*, *B. anthracis* ,*B. siamensis*, *B. Licheniformis*, *B. toyonensis*, *B. oceanisediminis*, *Paenibacillus dendritiformis*, *P. illinoisensis*, *Delftia lacustris*, *Brevibacterium halotolerans*, *Microbacterium binotii*, *Stenotrophomonas nitritireducens*, *Corynebacterium aurimucosum*, *Kocuria sediminis*, *Aeromonas hydrophila*, *Cedecea davisae*, etc. Among cultivated fungal isolates, *Aspergillus fumigates*, *A. niger*, *A. terreus*, *A. flavus*, *Penicillium citrinum*, *Trichoderma citrinoviride*, *T. saturnisporum*, *T. longibrachiatum* and *Mucor circinelloides* were the most prominent species dwelling in the cold environment of surveyed site.

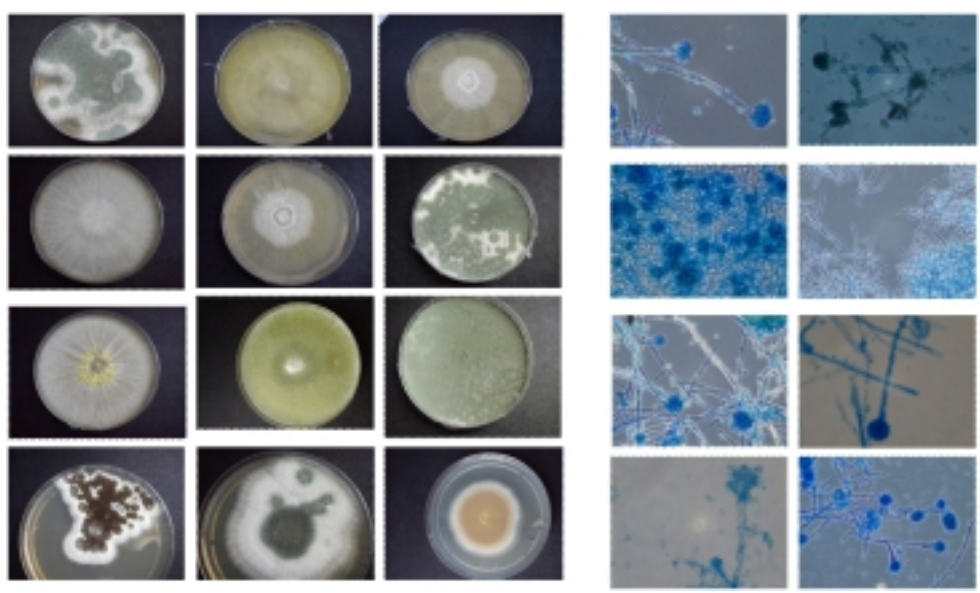

Fig 6: Morphological and microscopic view of the representative fungal isolates from Jharia coal mines

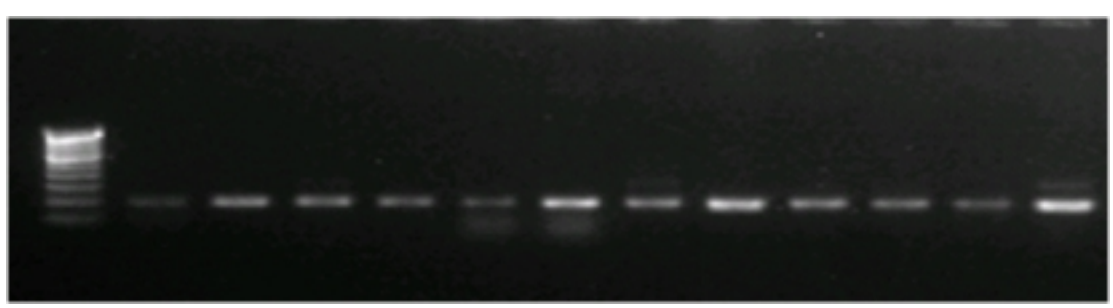

Fig 7: Amplification of heat shock protein (*hsp* 60) in thermotolerant isolates

II. Diversity analysis thermotolerant fungi from Jharia coal mines

- A total of 89 fungal morphotypes were isolated from Rajapur Bengali kothi, Kushtour, Kendua deh sites of Jharia.
- The isolates were able to grow at a temperature range of 25-55ºC and showed considerable variation with respect to their morphological characteristics on YPSS, MEA, GS, PDA media etc. The morphological and microscopic features of the representative isolates were shown in Fig 6.
- Fifteen fungi were able to grow at a 55^{0}C temperature.
- Among all the isolates, 18 isolates were found positive for siderophore production, 24 for ammonia and 33 isolates for HCN production. None of the isolate was able to solubilize phosphorous.
- Molecular identification using ITS region of the 15 thermotolerant fungi was performed. The identified species were: *Neosartorya glabra*, *Aspergillus fumigates*, *Cladophialophora modesta*, Penicillium *solitum*, *Aspergillus terreus*, *Thielavia terricola* etc.
- Genomic DNA of the thermotolenat fungi was amplified by using heat shock primers (hsp60). Only twelve isolates showed the amplification of *hsp* 60 gene with an amplicon of 200 bp (Fig 7).

Project: Exploration, Collection and characterization of some agriculturally important bioagents suitable for disease management (PI)

PI : Prem Lal Kashyap
Co-PI : Alok Kumar Srivastava

Rationale

Salinity is an important abiotic factor limiting crop productivity throughout the globe. It is an intricate phenomenon involving osmotic stress, specific ion effect, nutrient deficiency, etc. thereby affecting different physiological and biochemical mechanisms related to plant growth and development. Among several strategies used to improve crop production under salinity stress, use of NaCl tolerant *Trichoderma/Hypocrea* spp. could be an effective and easily adaptive strategy against plant pathogen. Therefore, the present study was attempted to test the efficacy of salinity tolerant antagonistic *Trichoderma/Hypocrea* strains under saline soil for crop disease management.

Objectives

- Characterization of salt tolerant antagonistic *Trichoderma/Hypocrea* strains.
- To test the efficacy of salinity tolerant antagonistic *Trichoderma/Hypocrea* strains under saline soil.

Achievements

- To investigate the growth response of three *Trichoderma/Hypocrea* strains under saline stress, all strains showed significant cultivation in a PDA/PDB medium containing NaCl (0-50, 100, 150, 200, 250mM). They showed elevated osmotic strengths up to 250mM NaCl and increased mycelia growth diameter and dry weight, and a significant ($P<0.05$) interaction resulted between *Trichoderma/Hypocrea* and NaCl (Table 1).
- Maximum growth resulted *in H. lixii* and followed by *T. asperellum*, while *H. virens* showed minimum tolerance up to 74 to 75% (Table 1).
- The *in vitro* antagonism assays showed that antifungal and volatile compounds produced by *Trichoderma/Hypocrea* spp. had an inhibitory effect on growth of *R. solani.*
- In dual culture assay, comparison of percent inhibition by *H. lixii, H. virens* and *T. asperellum* illustrated that *H. lixii* was more effective as compared to *H. virens* and *T. asperellum* against *R. solani* in normally as well as in the presence of NaCl. Among three, only *H. lixii* showed the maximum antifungal activity by volatile metabolite in the presence of NaCl, and a non-significant interaction resulted between *Trichoderma/Hypocrea* and NaCl with dual culture

Table 1 Growth response of antagonistic *Trichoderma/Hypocrea* isolates at different concentrations of NaCl.

Treatments	NaCl (mM)	Mycelia growth (cm)	% growth over control	Mycelia dry weight (g)	% growth over control
H. lixii	0*	8.97±0.03^{a}		1.47±0.01^{a}	
	50	8.83±0.07^{b}	(98.51±0.38)	1.44±0.00^{abc}	(97.53±0.89)
	100	8.54±0.03^{c}	(95.22±0.31)	1.39±0.02^{cde}	(94.15±1.90)
	150	8.46±0.03^{cd}	(94.35±0.07)	1.33±0.04^{fg}	(90.31±3.29)
	200	8.20±0.06^{e}	(91.45±0.73)	1.29±0.03^{ghi}	(87.59±2.69)
	250	7.87±0.04^{g}	(87.81±0.10)	1.21±0.00^{k}	(81.98±0.71)
H. virens	0	8.97±0.03^{a}		1.45±0.00^{ab}	
	50	8.53±0.03^{c}	(95.17±0.73)	1.42±0.00^{a-d}	(97.51±1.17)
	100	8.35±0.03^{d}	(93.09±0.21)	1.36±0.01^{ef}	(93.63±1.70)
	150	7.79±0.06^{g}	(86.88±0.97)	1.27±0.01^{hij}	(87.43±1.47)
	200	7.31±0.06^{h}	(81.57±0.91)	1.22±0.01^{jk}	(83.98±1.39)
	250	6.79±0.02^{i}	(75.73±0.41)	1.08±0.01^{m}	(74.13±1.83)
T. asperellum	0	8.97±0.03^{a}		1.44±0.00^{ab}	
	50	8.73±0.07^{b}	(97.39±0.38)	1.41±0.00^{b-e}	(96.88±0.84)
	100	8.43±0.04^{cd}	(94.02±0.40)	1.37±0.01^{def}	(93.92±1.12)
	150	8.07±0.03^{f}	(89.96±0.04)	1.32±0.01^{fgh}	(90.63±0.93)
	200	7.77±0.03^{g}	(86.62±0.05)	1.26±0.03^{ijk}	(86.46±1.81)
	250	7.27±0.03^{h}	(81.04±0.07)	1.16±0.00^{l}	(79.40±0.87)

Mean ± SE value of three independent replicates. Means followed by same letter (in superscript) within a column are not significantly different ($p<0.05$) according to Duncan's multiple range test and growth percentage over control (*without NaCl) shows in the parentheses.

Table 2: Growth parameter of antagonistic *Trichoderma/Hypocrea* isolates at different salt concentrations

Treatments	NaCl (mM)	Dual culture assay	Volatile metabolite assay	Chitinase (µmol of GlcNAc min^{-1}mg^{-1} protein)	Glucanase (nmol of glucose min^{-1}mg^{-1} protein)	Protease (µmol min^{-1}mg^{-1}protein)
H. lixii	0	76.83±2.31^{a}	65.17±3.55^{a}	212.79±7.37^{a}	35.71±1.24^{a}	66.20±2.29^{a}
	50	75.10±2.13^{b}	63.67±2.21^{ab}	208.75±7.23^{a}	34.87±1.21^{a}	64.70±2.24^{ab}
	100	70.03±2.01^{bc}	60.73±2.10^{abc}	182.89±6.34^{b}	31.33±1.09^{b}	60.70±2.10^{abc}
	150	63.77±1.94^{cd}	57.17±1.98^{bcd}	159.57±5.53^{cd}	30.37±1.05^{bc}	55.19±1.91^{cde}
	200	61.53±1.86^{cd}	55.73±1.93^{cd}	134.19±4.65^{efg}	29.16±1.01^{b-e}	53.27±1.85^{efg}
	250	59.37±1.79^{d}	51.33±1.78^{de}	127.77±4.43^{fg}	27.40±0.95^{c-f}	48.21±1.67^{ghi}
H. virens	0	51.90±1.57^{efg}	45.63±2.48^{efg}	148.28±5.14^{de}	36.68±1.27^{a}	63.83±2.21^{ab}
	50	48.77±1.48^{e-h}	44.43±2.43^{e-h}	143.96±4.99^{def}	35.48±1.23^{a}	60.53±2.10^{abc}
	100	44.47±1.33^{hij}	38.43±2.08^{g-j}	130.43±4.52^{fg}	30.07±1.04^{bcd}	51.17±1.77^{e-h}
	150	42.93±1.30^{ij}	36.93±2.02^{ij}	123.93±4.29^{g}	26.00±0.90^{efg}	45.43±1.57^{hi}
	200	41.40±1.27^{j}	36.40±1.99^{ij}	107.14±3.71^{h}	23.97±0.83^{gh}	39.23±1.36^{jk}
	250	39.97±1.23^{j}	33.20±1.82^{j}	86.63±3.00^{i}	20.17±0.70^{i}	35.25±1.22^{k}
T. asperellum	0	53.93±1.60^{e}	47.53±2.60^{ef}	220.42±7.64^{a}	30.38±1.05^{bc}	61.73±2.14^{ab}
	50	52.30±1.57^{ef}	46.00±2.51^{ef}	214.82±7.44^{a}	29.67±1.03^{bcd}	59.60±2.06^{bcd}
	100	51.90±1.57^{efg}	44.70±2.45^{e-h}	172.41±5.97^{bc}	27.25±0.94^{c-f}	54.43±1.89^{def}
	150	50.10±1.52^{efg}	42.93±2.37^{f-i}	151.89±5.26^{d}	26.88±0.93^{d-g}	49.20±1.70^{f-i}
	200	48.33±1.45^{fgh}	37.60±2.05^{hij}	126.25±4.37^{g}	24.89±0.86^{fg}	43.47±1.51^{ij}
	250	46.63±1.40^{ghi}	32.30±1.76^{j}	108.13±3.75^{h}	21.67±0.75^{hi}	37.13±1.29^{k}

Mean ± SE value of three independent replicates. Means followed by same letter (in superscript) within a column are not significantly different ($p<0.05$) according to Duncan's multiple range test.

and volatile metabolite. These results showed that NaCl caused negative effect on the antagonism of all three strains. Moreover, in the case of chitinase and glucanase enzymes, a significant interaction resulted between *Trichoderma/Hypocrea* and NaCl. *H. lixii, H. virens* and *T. asperellum* produced these enzymes in the presence of NaCl (Table 2). However, NaCl caused a negative effect on the protease accumulation. Among three strains, *H. lixii* showed maximum enzyme accumulation and also showed *h*igher stability of chitinase up to 60%, β-1, 3-glucanase 77% and protease 73% up to 250mM NaCl concentration (Table 2).

- SDS–PAGE analysis of soluble proteins from mycelia revealed that an enhanced intensity of bands at ~43 and ~65 KDa in NaCl stressed *Trichoderma/Hypocrea* strains as compared to non-stressed (Fig. 1). Among three strains, *H. lixii* showed less intensity of bands under NaCl stress as compared to other two strains *viz. H. virens* and *T. asperellum* (Fig 1).

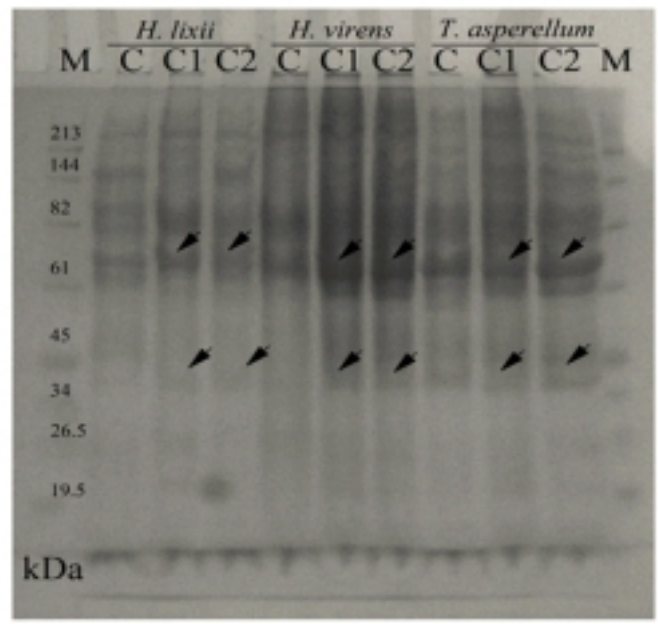

Fig 1: Effect of NaCl stress on the expression of proteins of three antagonistic *Trichoderma/Hypocrea* strains (M-marker, C-without NaCl, C1-100mM NaCl and C2-250mM NaCl) were fractionated by 12% SDS-PAGE.

Project: Development of microbial consortium for alleviation of salt and drought stress

PI : Alok Kumar Srivastava

Rationale

Plant growth-promoting rhizobacteria (PGPR) are known to participate in many important ecosystem processes, such as the biological control of plant pathogens, nutrient cycling, seedling growth and abiotic stress tolerance. PGPR strains possess the enzyme ACC deaminase and this enzyme can cleave the plant ethylene precursor ACC, and thereby lower the level of ethylene in stressed plants. Plants that are treated with ACC deaminase-containing PGPR are comparately more resistant to the deleterious effects of stress ethylene that is synthesized as a consequence of stressful conditions such as, heavy metals, presence of phytopathogens, drought and high salt. In each of these cases, the ACC deaminase-containing PGPR markedly lowered the level of ACC in the stressed plants, thereby limiting the amount of stress ethylene synthesis and hence the damage to the plant. These bacteria are beneficial to plant growth, as in the natural environment, plants are often subjected to ethylene producing stresses. More specifically, the *Pseudomonas* spp. and *Bacillus* spp have received particular attention throughout the world because of their catabolic versatility, excellent root colonizing ability and their capacity to produce a wide range of enzymes and metabolites that favour the plants to withstand both under biotic and abiotic stress conditions.

Osmolytes are zwitterionic, non-charged, or anionic and act without inhibiting normal metabolism of the cell and at the same time providing osmotic balance under osmotic conditions. Cytoplasmic accumulation of osmolytes by uptake or by synthesis is a general phenomenon of osmoadaptation in halophiles and halotolerant microbes. However, uptake is more energetically favorable than synthesis because biosynthesis of osmolytes was found to be more expensive and needs more ATP. This initial response of *B. subtilis* to the increase in the external osmolarity is followed by the intracellular accumulation of organic osmolytes by either synthesis or uptake from the environment. Important example of such compatable solutes are trimethyammonium compounds glycine betaine and the amino acid proline. Both substances have been adopted by a wide variety of prokaryotic and eukaryotic organisms as effective osmoprotectants.

Objectives

- Survey of drought affected area of India.
- Isolation of microorganisms from rhizotic zones of cereal crop grown under drought stress.
- Screening of salt & drought tolerant bacteria at different NaCl and PEG concentration.
- Biochemical & molecular characterization of selected microorganisms.

Achievements

- Twenty six soil samples were collected from rhizosphere and non-rhizosphereic soil of wheat grown under semi-arid fields of Hamirpur, Uttar Pradesh and 106 bacteria and 48 fungi were isolated. Biochemical screening of bacteria was performed at different moisture stress conditions using PEG. The isolates were further characterized for their capability to produce IAA. In the absence of an indole derivative (tryptophan) as a precursor in the culture medium, the produced low levels of IAA. Substantial production of IAA (63.77-508.91 µg/ml) was observed by few isolates when they were grown in tryptophan supplemented nutrient agar in comparison to control (53.96-478.38 µg/ml). Isolate H11303 was able to produce maximum amount of IAA (508.91µg/ml) in comparison to isolates from wheat (H130112, 304.67µg/ml) and potato (H13025, 285.99µg/ml) rhizosphere.
- On PGP activity based selection, 30 bacteria and 15 fungal isolates were characterized for IAA (Indole acetic acid) and phosphate solubilisation.
- About 70% of the bacterial isolates screened from wheat rhizosphere were able to produce ammonia. Out of thirty distinct bacterial isolates screened from different crop rhizospheres, only 3 isolates were able to produce hydrogen cyanide.
- *In vitro* siderophore production assay revealed that 35% of the potent rhizobacterial strains were able to produce siderophores
- All the bacterial strains screened from rhizospheres of agricultural crops from different locations of Hamirpur were able to produce

significant amount of cell wall degrading enzymes *viz*. chitinase, β, 1-3, glucanse and protease.

- The activity of β-1, 3 glucanase was varied in the range of 0.13-0.48mg/l, among various bacterial isolates screened from crop rhizospheres. The highest amount of β-1, 3 glucanase production was shown by H13025 in comparison to other bacterial spp.
- Five potential isolates (H1303, H1305, H13025, H13085, H130112) were further characterized for multiple PGP attributes. The results are given in table 1.

Fig. Sampling site

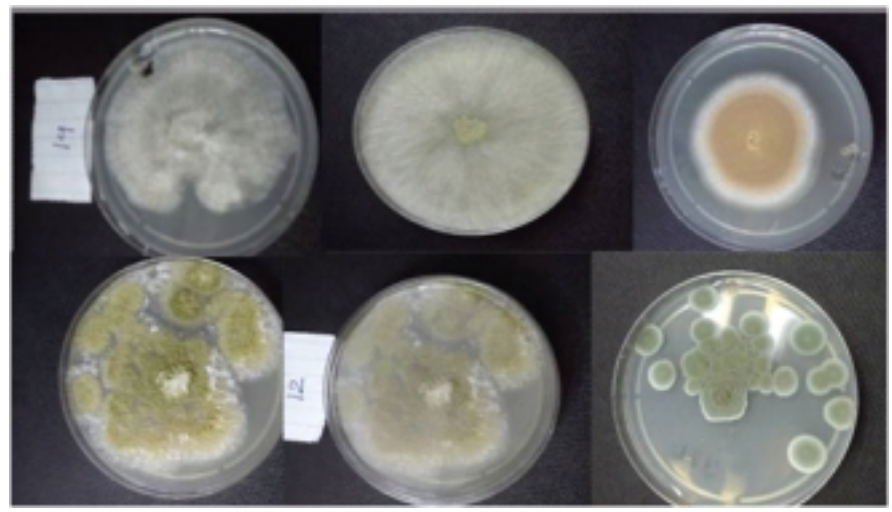

Fig. Fungal morphotypes isolated from Hamirpur Soil

Table 1: Characterization of potential isolates from Hamirpur Soil

Traits	Bacterial Isolates				
	H1303	H1305	H13025	H13085	H130112
Siderophore	+	+	_	+	+
Catalase	+	+	+	+	+
HCN	_	+	+	+	+
P-solublisation	+	+	+	+	+
IAA	+	+	+	_	+
Starch	+	+	+	+	+
Ammonia	+	+	+	+	+
Citrate	+	+	+	+	+
Urease	+	+	+	+	+
Oxidase	+	+	+	+	+
EPS Production	+	+	+	+	+
Zn-solubilisation	-	+	+	-	+

- Apart from the characterization of the samples, the strains previously isolated from sambar salt lake were further characterized and 14 *Bacillus* spp and 36 *Pseudomonas* spp were selected for the screening of salt tolerance level. Four strains of *Bacillus* were capable to tolerate upto 15% NaCl among 14 strains. Out of 36 *Pseudomonas* strains, only 2 strains (P-1010, P-1073) showed salinity tolerance upto 15% NaCl.
- Relative growth rate (RGR) also revealed that external NaCl concentration inhibit the growth of *Bacillus* spp and *Pseudomonas* spp. All four strains of *Bacillus* spp showed proline accumulation in response to NaCl stress. Maximum intracellular proline accumulation was measured in *Bacillus* spp (B-4) in response to NaCl stress. Proline content was found 0.2422 (µmolar/mg protein), which was 4.43 times higher in 15 % NaCl as compared to control.
- The strains of *Pseudomonas* (2) showed similar osmolytes accumulation in response to NaCl stress but intracellular proline accumulation was found to be lower than *Bacillus* spp. Maximum proline accumulation (0.0529 µmolar/mg protein) at 15 % NaCl had noticed in *Pseudomonas* spp (1010) and the magnitude of elevation was 2.2 time higher than control.

Project: Microbial Genomic Resource Repository (AMAAS)

PI : Arun Kumar Sharma
Co PIs : Alok Kumar Srivastava and Sudheer Kumar

Rationale

Microbial genetic materials (eg. genomes, plasmids, vectors, cDNAs) are very important tools for biotechnology and underpin the life sciences. The vast majority of microorganisms and their gene pool around the globe still remain hidden and need to be explored, identified, conserved and utilized for the benefit of humankind. Microbial genetic resources are established in many countries around the world having a variety of purposes. These range from small, specialized collections that support small groups of researchers to the large international public services to the scientific community and bio-industries. The huge gap between the discovery of new microorganisms and their potential numbers in nature has stimulated an interest in microbial diversity and the harnessing of their genes, properties and products. The operations of microbial collections have changed over the last twenty years as a result of the advancement of bioinformatics and the facility to present electronic data over the internet. This makes even the smaller collection resources more accessible.

Genetic material at MGRR can be deposited free of charge in the public collections and will be available for any third parties under the terms of the material transfer agreement. MGRR provides a safe deposit to the genes and genetic elements with associated information for long-term stable preservation of microbial genetic resources. In addition to repository services, MGRR is mandated to Nation wide survey and collection of information about microbial genetic resources, characterization, validation and molecular typification of reference microbial cultures, and generation of molecular dataset and generation of barcode as reference and exploration for collection of environmental microbial samples from different agroclimatic regions and direct DNA isolation through metagenomic approaches.

Objectives

- Nation wide survey and Collection of information about Microbial Genetic Resources
- Characterization, validation and molecular typification of reference microbial cultures, and generation of molecular dataset and generation of barcode as reference.
- Exploration for collection of environmental microbial samples from different agroclimatic regions and direct DNA isolation through metagenomic approaches
- Collection of DNA materials from micro-organisms and other relevant organisms which result from the various molecular genetics and genomics research programmes.
- Acquisition of gene constructs from various sources.
- Production/multiplication and quality control for distribution.

Achievements

- Under the explorations, sampling was done from *Picrorhiza* rhizosphere at Jammu during September 2013. The isolates obtained were assayed for heavy metals toxicity tolerance.
- In this study, higher altitude ($33^0$05.869'N/ $075^0$19.650' E 6576 ft) region of Jammu (Patnitop) was sampled for exploration of heavy metal degrading microbes from *Picrorhiza kurroa.* A total of 40 strains were isolated and designated as PR1 to PR40.
- All the isolates were characterized morpho-logically, biochemically (Gram test, carbo-hydrates, amines and carboxylic acid utilization, Nitrate reduction) and tested for their plant growth promotion (PGP) attributes (siderophore, phosphate, urease, oxidase, catalase, ACC deaminase and H_2S Production). They were also screened against Chromium (0.5 mM, 1.0 mM), Cadmium (0.5 mM, 1.0 mM), Mercury (0.1 mM, 0.25 mM, 0.5 mM) and Aluminium (5.0 mM, 10.0 mM) at respective Millimole concentration.
- All the screened strains were tested for antibiotic susceptibility against 13 different antibiotics at different concentrations. Evolutionary dynamics among the lineages were determined by the ARDRA generated clustering pattern of 16S rRNA and *rpo*B genes.

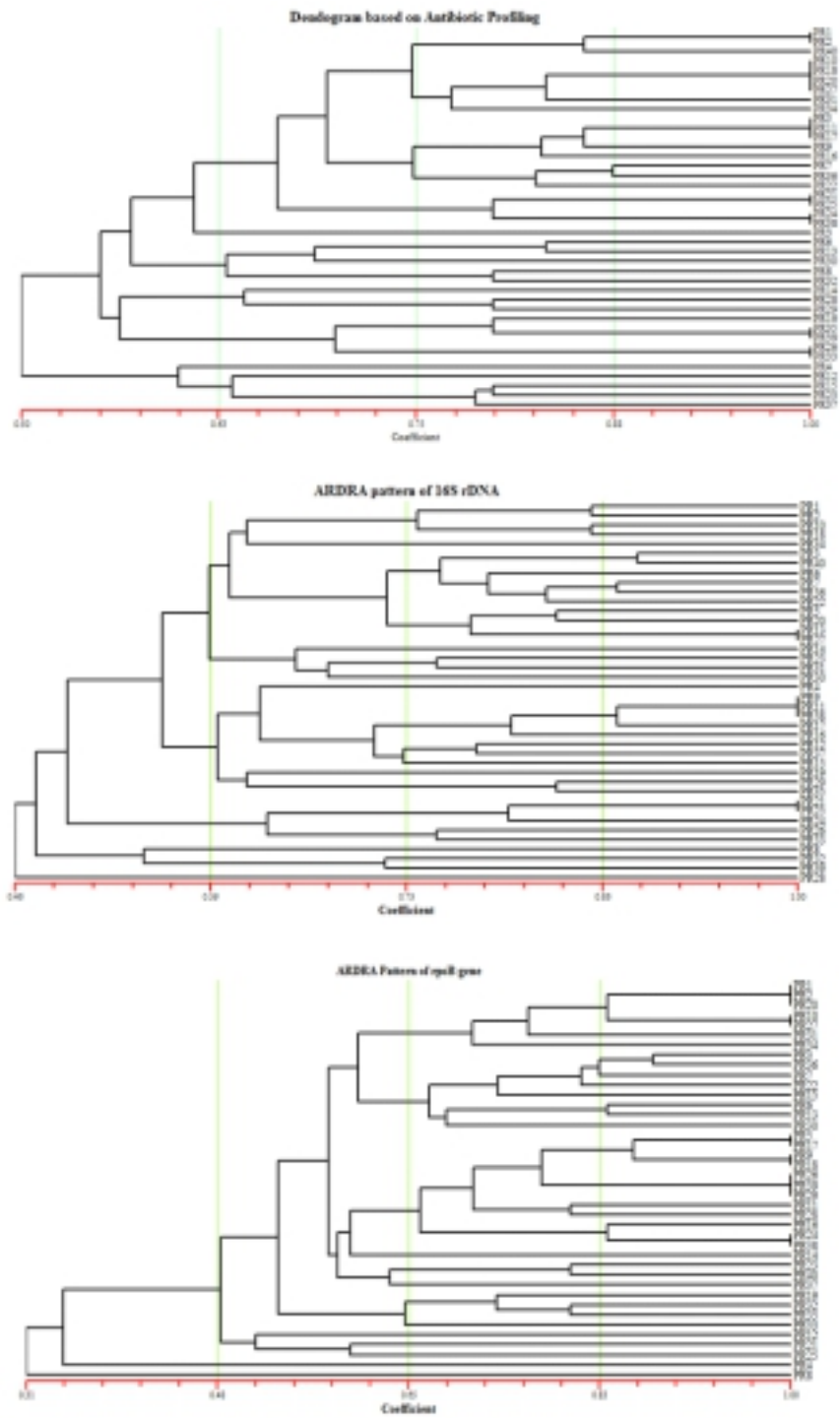

Fig: The ARDRA pattern analysis reflect the higher level of genetic diversity among the strains

Conclusion

The results revealed the dominancy of gram negative bacteria (only PR17 v^{+}). Among the all isolates, 39, 35 and 30 were positive to citrate, lysine and ornithine utilization, respectively. Only 7 isolates were urease positive and 18 were producing H_2S. All the isolates were siderophore producer. All the stains were able to produce significant amount of extracellular enzymes *viz.*starch hydrolysis, gelatin liquefaction, casein hydrolysis and siderophore production.

AMAAS : Overall Achievements

Microbial communities in agriculture have very wide range of roles to play in ecosystem management and sustainable crop production. Their potentials can be as wide as plant health promotion, soil nutritional balance, ecosystem function to control biotic and abiotic stresses, increased nutrients availability and acceleration of decomposition of organic materials and bioremediation in order to improve crop production and maintain sound environment for crop production. NBAIM is the nodal unit for the ICAR network project on 'Application of microorganisms in agriculture and allied sectors' (AMAAS) which 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, genomics and conservation of genomic resources. The project is running in seven thematic areas: Microbial diversity and identification; Nutrient management, Plant growth promoting rhizobacteria (PGPR), Antagonists, Biocontrol agents and Disease management; Microbial Management of Agrowaste; Bioremediation and Microbes in Post Harvest and Processing; Microbial management of abiotic stress; Microbial genomics; Human resource development and MGRR.

Centers of AMAAS

Theme 1: Microbial Diversity and Identification

1. National Bureau of Agriculturally Important Microorganisms, Mau
2. Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad
3. Kerala Agricultural University, Thrissur, Kerala
4. Department of Botany, Banaras Hindu University, Varanasi
5. Central Rice Research Institute, Cuttack , Orissa
6. Central Institute of Brackish Water Aquaculture, Chennai
7. National Dairy Research Institute, Karnal
8. National Research Center for Mushroom, Solan, Himachal Pradesh
9. National Research Centre for Groundnut, Junagadh, Gujarat
10. National Bureau of Fish Genetic Resources, Lucknow
11. Maulana Azad National Institute of Technology, Bhopal
12. Department of Microbiology, Chaudhary Charan Singh Haryana Agricultural University, Hissar, Haryana
13. Govind Ballabh Pant University of Agriculture and Techanology, Pantnagar, Uttarakhand
14. Rajendra Agricultural University, Pusa, Samatipur, Bihar
15. North East Institute of Science & Technology (CSIR), Jorhat, Assam
16. Department of Botany, University of North Bengal, North Bengal
17. Institute of Bioresources and Sustainable Development, Imphal, Manipur
18. School of Biotechnology, Banaras Hindu University, Varanasi
19. Mizoram University, Aizwal, Mizoram
20. Central Agricultural Research Institute, Port Blair, Andaman & Nikobar
21. University of Agricultural Sciences, Gandhi Krishi Vignyan Kendra, Bangalore
22. Central Institute of Freshwater Aquaculture, Bhubaneshwar
23. Punjab Agricultural University, Ludhiana
24. Central Marine Fisheries Research Institute, Ernakulam, Kochi, Kerala
25. Division of Biochemistry, Indian Agricultural Research Institute, New Delhi

26. Central Institute of Freshwater Aquaculture, Bhubaneshwar
27. College of Dairy & Food Science Technology, Maharana Pratap University, Udaipur

Theme 2: Nutrient Management, PGPR and Biocontrol

1. Indian Institute of Soil Science, Nabi Bagh, Bhopal
2. Central Research Institute for Dryland Agriculture, Hyderabad
3. Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora
4. Indian Institute of Spice Research, Calicut, Kerala
5. Central Plantation Crops Research Institute, Kasaragod, Kerala
6. National Bureau of Agriculturally Important Microorganisms, Mau
7. National Bureau of Agriculturally Important Insects, Bangalore
8. Division of Microbiology, Indian Agricultural Research Institute, New Delhi
9. Central Rice Research Institute, Cuttack, Orissa
10. Tamil Nadu Agricultural University, Coimbatore
11. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh
12. Indian Institute of Vegetable Research, Varanasi
13. Central Tuber Crops Research Institute, Thiruvananthapuram
14. Indian Institute of Pulses Research, Kanpur
15. Directorate of Oilseeds Research, Rajendranagar, Hyderabad
16. National Research Centre for Grapes, Pune, Maharashtra
17. Central Marine Fisheries Research Institute, Kerala
18. Central Inland Fisheries Research Institute, Barrackpore
19. Indian Institute of Horticulture Research, Bangalore
20. Central Plantation Crops Research Institute, Kasargod

Theme 3: Agrowaste management, Bioremediation and microbes in PHT

1. Division of Microbiology, Indian Agricultural Research, Institute, New Delhi
2. National Bureau of Agriculturally Important Microorganisms, Mau
3. Department of Zoology, University of Delhi, Delhi
4. National Research Center for Mushroom, Solan
5. Central Institute of Post Harvest Engineering and Technology, Ludhiana
6. Central Rice Research Institute, Cuttack , Orissa
7. Central Institute of Fisheries Education, Mumbai
8. Central Soil Salinity Research Institute, Zarifa farm, Karnal
9. Central Institute of Brackishwater Aquaculture, Chennai
10. Indian Institute of Vegetable Research, Varanasi
11. Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore
12. Central Institute for Subtropical Horticulture, Lucknow

Theme 4: Microbial Management of Abiotic Stress

1. National Bureau of Agriculturally Important Microorganisms, Mau
2. Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad
3. Central Research Institute for Dryland Agriculture, Hyderabad
4. Vivekananda Parvatiya Krishi Anusandhan Sanstha, Almora

Theme 5: Microbial Genomics

1. National Bureau of Agriculturally Important Microorganisms, Mau
2. Department of Microbiology, Govind Ballabh Pant University of Agriculture and Techanology, Pantnagar, Uttarakhand
3. Indian Institute of Vegetable Research, Varanasi
4. National Research Center for DNA Finger-printing, National Bureau of Plant Genetic Resources, New Delhi
5. Madurai Kamaraj University, Madurai, Tamilnadu
6. National Research Center on Plant Bio-technology, Indian Agricultural Research, Institute, New Delhi
7. Division of Microbiology, Indian Agricultural Research, Institute, New Delhi
8. Centre for Cellular and Molecular Biology, Hyderabad
9. Indian Institute of Horticulture Research, Bangalore, Karnataka

Theme 6: Human Resource Development

1. National Bureau of Agriculturally Important Microorganisms, Mau

Theme 7: Microbial Genomics Resource Repository

1. National Bureau of Agriculturally Important Microorganisms, Mau

Achievements

Theme1: Microbial Diversity & Identification

- Analysis of 16S rDNA clone libraries highlighted the dominance of genus *Pseudomonas* (>50%) along with a wide range of other diversified groups *viz. Chloroflexi, Chlorobi, Planctomycetes, Gemmatimonadetes, Verrucomicrobia, Fermicutes* in Kumaun Himalayan agroecosystems .
- First report of Himalayan psychrophilic diazotroph *P. migulae* S10724 strain and its proteome analysis has been completed .
- MALDI–TOF–MS based identification revealed the involvement of two crucial proteins at low temperature nitrogen fixation process: *Nif*U family SUF system FeS assembly protein and copper-sensitivity suppressor protein.
- Nitrogen fixation and energy production associated proteins were up-regulated at 10°C, while energy consuming processes were found to be down-regulated .
- Potential growth promoting psychrotrophic (*P. jessenii* strain MP1, B-01444; *Dyadobacter* sp. strain B2 TB-1684) and psychrophilic (*P. migulae* strain S10724, TB-1685) diazotrophs have been submitted in NBAIM microbial repository.
- A total of 120 culturable fluorescent pseudomonads were isolated from vegetative as well as reproductive growth phase of green gram rhizosphere. PCR based techniques, i.e. Rep PCR, amplified ribosomal DNA restriction analysis (ARDRA) and ribosomal intergenic space analysis (RISA) revealed significant intrageneric diversity among the isolates associated with reproductive growth phase of green gram. Based on the genotypic analysis, 16S rRNA based diversity study was carried out among 85 distantly related fluorescent pseudomonads isolates. 16S rRNA partial sequencing analysis identified *Pseudomonas aeruginosa* as the dominant group.
- Functional diversity among the 120 isolates were investigated by screening for the production of enzymes and hormones such as phosphatase, indole-3-acetic acid (IAA),1-aminocyclopropane-1-carboxylate (ACC) deaminase, chitinase and antimicrobial metabolites. Real time qPCR based relative expression of the three important bacterial stress responsive genes, i.e. *acdS*, *KatA* and *gbsA* in *P. aeruginosa* GGRJ21 were analysed due to its stable growth kinetics under osmotic stress conditions. High production efficiency of different plant-growth promoting (PGP) traits as well as consistent up-regulation of bacterial stress responsive genes under osmotic stress condition could promote GGRJ21 as a potent plant growth regulator
- Biofloc of nitrifying bacteria was made and applied to 50 litre of fish culture tank. The fish culture was maintained without water exchange and zero level of ammonia for a period of 60 days.
- Two metagenomic libraries constructed from wastewater samples amended with ammonia & hydroxylamine (WS3) and nitrate & nitrite (WS2) revealed high abundance and diversity of nitrifying and denitrifying genes in the wastewater samples .
- Comparative analysis of WS2 and WS3 metagenome showed that *Acinetobacter, Pseudomonas, Achromobacter, Xanthomonas, Methylobacterium* and *Acidovorax genus* were present in high abundance.
- Comparative pathway analysis showed that of WS2 and WS3 metagenome that enzyme class such as oxidoreductases, transferases , hydrolases are in high abundance. Also enzymes muconate cycloisomerase, butyleneglycol dehydrogenase, butyrate kinase exclusively present in WS2 sample and enzymes caffeoyl-CoA methyltransferase, thioglucosidase, dolichyl-phosphate beta-glucosyltransferase were exclusively present in WS3 sample.
- Gene encoding 24 differentially expressed proteins, expressed differentially at 20% and 35% of NaCl stress in Haloferax volcanii H5 as compared to those expressed at 10% NaCl were amplified using custom designed primers. Out of the 24 genes targeted for amplification, 17 genes were successfully amplified to the nearest expected size of the amplicons. The amplified products were cloned to pUC18/pUC19 cloning

vectors for studying the expression and validation in *E. coli* to identify probable candidates genes in imparting extreme osmotolerance in *Haloferax volcanii* H5.

- Twenty-three extreme halophilic (all grow upto 35% of NaCl) were characterised for presence of industrially important enzymes like amylases, proteases, lipases, etc. Six isolates (*Halorubrum chaoviator* H10-DGR and *Halorubrum chaoviator* H3-DGR; *Halorubrum orientale* SBN1-DGR; *Halobacterium noricense* H4-DGR; Haloarchaeon 3A1-DGR and Haloarchaeon H9-DGR) did not show any amylolytic, proteolytic and lipolytic activities.
- *Haloarcula argentinensis* SB45-2-DGR and *Haloarcula salaria* H5-DGR and *Haloarcula salaria* 5A9-DGR produced all the three enzymes viz. amylases, proteases and lipases. Quantification and characterization of the enzymes produced at near saturated NaCl concentration is underway at present.
- The genome of *Bacillus megaterium* MSP20.1 (growth:~27.5% NaCl; genome:4.45- Mbp; CDSs:4,255; stress response:110 genes) and *Bacillus* sp. NSP22.2 (growth:4-22.5% NaCl; genome:4.03-Mbp; CDSs:4,251; stress response:105 genes) were sequenced and the sequence data are being used to understand the mechanisms of osmotolerance. Both the genomes have been released under the accession numbers, AVBB00000000 and AVCV00000000, respectively.
- Three metagenome samples (two from Little Rann of Kutch and one from White desert) were sequenced to know the structural and functional profiles of the organisms present in the crystallizers with near saturated NaCl concentration. The data indicated that archaea were predominant in the crystalizer wherein concentration of NaCl is near to saturated level. There was variation in the number of species found in the samples. Whereas 20208 species found in LR1 (35.8% salts), there were 15254 species in the sample LR2 (49.4% salts) and 22654 species were found in sample obtained from white desert (38.8% salts).
- Out of 58 multi potential Plant Growth Promting Rhizobacteria (PGPR's) tested against virulent isolate of rice bacterial blight pathogen (*Xanthomonas oryzae pv oryzae*) under *in vitro* conditions, six isolates (SA1, SA2, SA3, SA8, SA12 and SA29) were found best in inhibiting the pathogen growth under *in vitro* conditions. The molecular characterization of the screened isolates using 16S rDNA amplification revealed that all the isolates belong to the genus *Bacillus* and *Psuedomonas.*
- Thirty five *Trichoderma* isolates from different locations of South Andaman were characterized for their morphological, biochemical and antagonistic activities against three plant pathogens (*Sclerotium oryzae, Fusarium oxysporum* and *Pythium aphanidermatum*). The isolates TDK2, TRC3, TNB6 and THB3 were most efficient in percentage inhibition of mycelial growth of test pathogens. TRV1 and TRC3 were showed highest chitinase whereas TDK2 was recorded with highest cellulose and protease activities. The ITS and *tef* gene characterization showed that four species (*T. harzianum, T. aureoviride, T. asperellum* and *T. koningiopsis*) were prevailing more among all the isolates.
- Out of the nine multipotential *Trichoderma* isolates tested *in vitro* using paper towel method, TRC3 and THB3 showed higher germination percentage in both chilli and tomato seed treatments and followed by TRV1. The highest shoot length of chilli and tomato seedlings was showed in treatment of TRC3 (6.43 and 3.60 cm, respectively) followed by NRT2 (5.57 and 3.53 cm, respectively) and THB3 (5.30 and 3.53 cm, respectively). Also the same pattern was observed in root growth *i.e.* TRC3 produced longer roots (12.43 and 15.30 cm) followed by NRT2 (12.30 and 14.90 cm) and THB3 (11.40 and 14.87 cm) in chilli and tomato seedlings, respectively.
- Microbial biodiversity in the aquaculture ponds was examined using metagenomics.
- 16S rDNA from pond sediment was amplified using bacterial fD1 and rP2 universal primers and the amplified PCR products were purified and cloned in to pTZ57R/T vector in *E. coli* DH5α.
- The clones were screened using M13F and M13R primers for inserts.
- 137 clones fell into 10 major phyla of the bacterial domain and a number of so far unreported bacteria such as proteobacteria (Alpha-, Beta-, Gamma-, and Delta-), the bacteroides group, actinobacteria, chloroflexi, firmicutes,

acidobacteria group, and planctomycetes, cyanobacteria / chloroplast, chlorobi, verrucomicrobia and unclassified bacteria were identified in aquaculture pond sediment.

- The brackishwater aquaculture pond sediment had a higher abundance of Gamma- and delta-Proteobacteria, mainly involved with sulfate reduction in anaerobic conditions.
- Out of 12 efficient diazotrophs identified based on the results of 16S rDNA sequencing and its nucleotide-nucleotide BLAST (blastn) analysis, 7 showed closest affiliation to species of *Pseudomonas*; 4 to *Rhizobium* sp. and 1 to *Acinetobacter schindleri*.
- Twenty three AIMs which showed excellent biocontrol activity against four fungal pathogens (*Alternaria carthami, Fusarium oxysporum* f. sp. *Carthami, Sclerotium rolfsii* and *Rhizoctonia bataticola)* and 3 bacterial plant pathogens *(Xanthomonas campestris, X. axonopodis, Rolstonia solanacearum*) were characterized by 16S rDNA sequencing. . Based on the results of 16S rDNA sequencing and its nucleotide-nucleotide BLAST (blastn) analysis, 5 showed closest affiliation to *Pseudomonas* sp., 4 to *Pseudomonas fluorescens*, 2 to *Pseudomonas plecoglossicida*, 7 to *Pseudomonas putida* and one each were identified as *Acinetobacter bereziniae, Klebsiella pneumoniae, Chryseobacterium indologene*s and *Rhizobium* sp.
- These putative isolates with biocontrol activity produced HCN, siderophores and volatile metabolites like Phenazine and Phluoroglucinol.
- *In vitro* assay on inhibitory activity of these individual antimicrobial metabolites showed that metabolites suppressed the growth and sporulation of fungal plant pathogens.
- Field evaluation of five each of FP and PSB isolates on groundnut crop indicated significant increase in nodulation, dry matter production, nutrient uptake and yield over the un-inoculated controls. While PSB isolates enhanced the pod yield by 12.02 to 26.9 %, fluorescent pseudomonads enhanced the pod yield by 5.75 to 26.69%.
- A total of 135 bacterial isolates were obtained from the rhizosphere of plantation and agricultural crops as well as forest's soil of North Bengal including high altitude regions of Darjeeling Hill areas. RAPD analysis of these bacterial isolates were performed. All reproducible polymorphic bands were scored and analyzed following UPGMA cluster analysis protocol and computed into similarity matrix using NTSYS computer program to prepare a dendrogram. Analysis of dendrogram revealed that similarity coefficient ranged from 0.55– 0.97. On the basis of initial screening twelve isolates were found to be most efficient phosphate solubilizer. These PGPR isolates were further identified as *Bacillus pumilus* BRHS/C1, *Bacillus altitudinis* BRHS/P22, *Bacillus altitudinis* BRHS/S73, *Enterobacter cloaeae* BRHS/R71, *Bacillus pumilus* BRHS/T382, *Bacillus pumilus* BRHS/T384, *Burkholderia symbiont* BRHS/P92, *Bacillus aerophilus* BRHS/B104, *Paenibacillus polymyxa* BRHS/R72, *Bacillus methylotriphicus* BRHS/P91, *Pseudomonas fulva* E/B/P/1 and *Enterobacter ludwigii* E/B/S/T/1 by 16S rDNA sequences and have been deposited [JF 836847, HQ849482, JF899300, KC703974, JQ765579, JQ765580, JQ765578, KC603894, KC703775, JQ765577, JX399583 & JX 411945] to the National Center for Biotechnology Information (NCBI) GenBank and the cultures have been deposited to NAIMCC, NBAIM [NAIMCC-B 01483, NAIMCC-B 01484, NAIMCC-B 01485, NAIMCC-B 01486, NAIMCC- B01487, NAIMCC-B 01488, NAIMCC-B 01489, NAIMCC-B 01490, NAIMCC-B 01491, NAIMCC-B 01492, NAIMCC-B 01493 & NAIMCC-B 01494]. Multiple sequence alignment of ITS gene sequences of all the selected PGPR isolates was conducted. There were quite a number of gaps that were introduced in the multiple sequence alignment within the region that were closely related and similar sequence indicated the relationship among the isolates. Their phylogenetic placement using UPGMA analysis were also studied.
- Genetic relatedness among the potential eight PGPR isolates- *Bacillus pumilus* (BRHS/C1), *Bacillus altitudinis* (BRHS/P22), *Enterobacter cloacae* (BRHS/R-71), *Paenibacillus polymyxa* (BRHS/R-72), *Bacillus altitudinis* (BRHS/S-73), *B. methylotrophicus* (BRHS/P-91), *Bukholderia symbiont* (BRHS/P-92) and *B. aerophilus* (BRHS/B-104) obtained from the high altitude regions of Darjeeling hills were conducted using DGGE formats. Analyses of genetic relatedness among these PGPR isolates were conducted on the basis of

the differences in their conserved sequences. The DGGE electrophoresis yielded a unique and uniform banding pattern of each bacterial isolate. In case of 0-100 % denaturant, no major difference among the bacterial isolates was noticed; however variations in the banding patterns were observed when the denaturing gradient was 20-60%, run time 8 hours in 100 V. All the obtained bands were scored individually in the form of 0/1 matrix and analysed in NTSYS-PC software.

- *Bacillus pumilus, B.altitudinis, B. methylotrophicus, B. aerophilus and Burkholderia symbion*t showed good growth promotion in two varieties of Okra plants. However, *Bacillus pumilus* (NAIMCC-B01483), *Bacillus altitudinis* (NAIMCC-B01484) and *Burkholderia symbiont* (NAIMCC-B01489) promoted better growth in comparison to control even after two months of application.
- Biopriming seeds of *Vigna radiata* separately with two isolates of Non Streptomyces Actinomycetes (NSA 3 and NSA 7) and an isolate of *Streptomyces griseus* (S-26) at a concentration of 108 cfu/ml showed comparatively better growth as evidenced by increase in shoot, root length as well as average height and total plant fresh weight in comparison to untreated control. POX activities increased more in NSA 3 and NSA 7 treated plants. In case of PAL acitivity, plants treated with S-26 showed better results in comparison to other isolates (NSA-3 and NSA-7) and control. Similarly, chitinase and glucanase activities were enhanced following biopriming seeds with NSA- 3 isolate as well as foliar application of plants.
- Three PGPR isolates (*B. Methylotrophicus. B. aerophilus* and *Burkholderia symbiont*) also showed growth promotion in six varieties of wheat and Champasari variety of rice. In GN variety, *B. methylotrophicus* showed better growth in comparison to control as well as other PGPR treatments. In wheat varieties GY, PBW343, KD, MW and KW *Bacillus aerophilus* showed increase in shoot fresh biomass where as in KD and KW, *B. methylotrophicus* showed increase in shoot dry biomass in comparison to control.
- Surveys for collection of wild mushrooms were undertaken during rainy season in the forest areas of Arunachal Pradesh, Gujarat (GIR Forest) and Himachal Pradesh. A total number of 139 specimens were collected from these forest areas which have never been explored. Tissue cultures of 73 specimens were deposited in the Gene Bank of DMR, Solan.
- Twelve wild specimens of *Lentinus* spp. were collected which have been studied in detail and several of them could be new reports from India and world. There DNA sequencing of ITS region is under studies for establishing as new records.
- Cultures of indigenous *Herticium coralloides* and Hericium sp.(medicinal mushroom) were cultivated on wheat straw and both the cultures form fruiting bodies in liquid culture and malt extract agar medium.
- A wild specimen with green colored lamellae is very interesting and it could be a new mushroom species from the world.
- Genomic DNA of *Auricularia* spp. (19 cultures), *Hericium* and *Sparaciss* spp. have been isolated for their authentication.
- The diversity and functional activities of free-living culturable diazotrophic bacteria in the rhizospheric soil of citrus-poplar cropping system was assessed. Morphological, biochemical and molecular techniques were used to study the diversity of diazotrophs in the citrus – poplar cropping system. The physiochemical properties of soil samples were also studied using standard methods.
- The physico- chemical properties such as pH, electrical conductivity, organic carbon, ammoniacal nitrogen and nitrate nitrogen content of the soil samples ranged from 7.5-8.6, 0.17-0.29 dSm-1, 0.40-0.85%, and 46-67 ppm and 35.5-48.5 ppm, respectively.
- One hundred thirty, free living diazotrophs were characterized using cultural, morphological, biochemical, and functional characteristics. Seventy four isolates were assessed based on the amplification of two *nifH* primers as *nifH*1 and *nifH*2. The *nifH* positive isolates were further characterized using restriction fragement length polymorphism of 16S rDNA and the isolates were identified as *Stenotrophomonas maltophilia*, *Stenotrophomonas rhizophila*, *Rhizobium* sp., *Pseudomonas fulva, Xanthomonas* sp., *Agrobacterium* sp., *Achromobacter* sp., *Ensifer adhaerens, Bacillus amyloliquefaciens, Xanthomonas theicola, Azotobacter* sp. Diverse groups of diazotrophic bacteria were

observed in the rhizospheric soils of citrus-poplar cropping system.

- A total of eighteen methanotrophic bacteria were isolated from rice rhizospheric soil using nitrate mineral salt (NMS) medium by serial dilution spread plate technique. The petri plates containing inoculum were incubated in an atmosphere of methane in jars.
- The culturable population of methanotrohic bacteria varied from 39.5 - 84 x 10^5cfu/g of soil in the treatment I (Residue + different levels of nitrogen). The population of methanotrohic bacteria in the treatment II (transplanting rice, direct seeded rice, conventional tillage, zero tillage) varied from 14 - 55 x 10^5 cfu/g of soil. The population of methanotrophic bacteria in the rice crop having treatment III (zero% residue, full residue, zero tillage, conventional tillage) ranged from 34 - 78 x 10^5 cfu/g of soil. All the methanotrophic bacterial isolates were characterized for various biochemical tests as oxidase, catalase, MR-VP, indole production, citrate utilization, and triple sugar iron assay using standard methods. All the bacterial isolates were found to be positive for citrate utilization whereas negative for indole production and VP test. All the isolates except AJ10, AJ16, AJ17 and AJ18 were found to be positive for MR test. Variable results were observed for triple sugar iron assay.
- Total 635 LAB isolates, isolated from milk samples; characterized at morphological, biochemical and molecular levels.
- On the basis of ARDRA profiles, 16 bacterial species obtained *viz Lb. rhemnosus, E. faecalis, Lb. helveticus, E. faecium, Lc. lactis sub. lactis, Lb. plantarum, Leu. mesenteroides, E. durans, Lb. fermentum, Lc. garviae, Lb. casei, Lb. bulgaricus, Lb. acidophilus, Lb. brevis, Lactobacillus ssp., Leu. spp., Strep. spp., E. hirae, Pedio. pentosaceus,* and *Pedio. acidilactici* showing more than 95% similarity with the GenBank sequences. We have got 11 new strains, submitted to GenBank .their accession numbers are from JQ929642- JQ929652.
- Thirty most efficient LAB isolates were submitted to NBAIM Culture Collection and remaining isolates are preserved as glycerol stocks.
- Out of 635 isolates, 74 were screened showing antibacterial activity. Five isolates out of 74 were bacteriocin positive and were studied for their chemical nature (protein). Antibacterial factor were confirmed through gel permeation chromatography. Metabolite of these 5 isolates was precipitated with ammonium sulphate to concentrate the protein. One isolate was selected for the purification and characterization of bacteriocin. Purification of bacteriocin was done by using gel permeation chromatography. The purified bacteriocin exhibited inhibition against various food borne pathogens and spoilage microorganisms including both Gram positive and negative bacteria. They were tested against various food spoilage organisms viz., *Micrococcus luteus, Staphylococcus aureus, Listeria monocytogenes, E. coli, Citrobacter* sp., *Klebsiella pneumonia.* They were heat stable and exhibited activity in a pH range of 2-8 with maximum activity between pH 4 and 5. Molecular weight of bacteriocin was found to be ~4.95 kDa using SDS PAGE. HPLC profile showed a single peak further attesting the purity of the bacteriocin.
- Purified bacteriocin was obtained from *Lactobacillus rhamnosus* (L34) and *Lactococcus lactis* ssp. *lactis* (Sa1) was applied for enhancing the shelf life of natural food products like fruit juice, vegetable and paneer. It enhanced the shelf life of these food products. EPS and bacteriocin positive isolates significantly improved shelf life of dairy products compared to marketed products in laboratory.
- Twenty five (25) cyanobacterial strains were fully characterized by 16S rRNA methods for correct identification and sequences have been submitted to NCBI and obtained their accession numbers.
- Fifty (50) fast growing heterocystous nitrogen fixing strains have been screened for ARA activity for biofertilizer applications, also their growth pattern were recorded.
- Enhancement of natural pigments like Chl-a, phycobiliproteins and carotenoids under different pH at different time intervals was done for potential strains.
- Fifteen (15) microalgal strains were characterized for lipid profiling and fatty acid compositional analysis by GC-FID methods and five (05) were found at par with commercialized strains.
- An analysis of the 28 soil samples resulted a total of 53 agriculturally important microbes including 7 rhizobium, 18 PSB, 10 actinomycetes, 15 fungi and

3 unidentified microbes were recovered.

- RAU 820, RAU-832, RAU-833, RAU-856, RAU-901and RAU-905 were separately tested against moong crop. Result indicated that germination percent of moong ranges 90-100%. But RAU-835 (*Bacillus amyloquefaciens*) and RAU-820 (*Burkholderia* sp.) was found most effective under pot study and given higher yield i.e. 102 grains and 110 grain per plant, respectively.
- The effect of seed treatment and soil application of *Bacillus amyloquefaciens* (RAU-833), *Burkholderia* sp. (RAU-820) , *Ochromobacter intermedium* (RAU-905) was studied against rice crop as seed treatment and soil application. Results indicated that seed treatment with all microbes was found most effective and superior to soil application. The yield was recorded 2770, 2646 and 2500 kg/ha respectively.

Theme 2: Nutrient Management, PGPR and Bio control

- Potassium containing rock powder and dolomite ($CaCO_3MgCO_3$) have been found carrier materials for potassium solubilizing bacteria and bio control agent (*Trichoderma* spp.), respectively as they maintained the viability and efficiency in the bioformulation. These bioagents were effective on elephant foot yam.
- The expression of genes of cell wall associated protein and protein associated with biofilm formation with potent K solubilizer, *B. subtilis* was identified.
- Identified the expression of genes which were involved in mycoparasitism during interaction of *T. harzianum* and *S. rolfsii.*
- Soil and tuber treatment of *Trichoderma asperellum* is effective in managing greater yam anthracnose in field
- Tuber treatment of *T. harzianum* could manage collar rot of elephant foot yam
- Out of 5408 bacterial isolates from 15 different ecological niches of southeast & southwest coast of India, 95 possess a significant antibacterial activity against most of the aquaculture pathogens. The majority of the antagonistic bacteria belonged to *Bacillus* spp. and *Pseudomonas* spp. Apart from these bacteria, isolates from Actinobacteria and *Vibrio* with potential antagonistic activity against aquaculture pathogens were also identified.
- The antagonistic isolates characterized are also efficient in producing different varieties of enzymes, tolerating environmental stresses and inability in resistance to common antibiotics.
- Developed a molecular screening tool-colony multiplex PCR- for rapid identification of the genus *Bacillus* and *Pseudomonas*.
- *Bacillus subtilis* MBTDCMFRI Ba37 and *Pseudomonas aeruginosa* MBTDCMFRI Ps04 were selected as candidate bacteria for further studies.
- Two media (glycerol-alanine medium and modified bacillus medium) have been standardized for *Pseudomonas* and *Bacillus* spp. which helps in the increased production of bioactive compounds and biomass.
- The bioactive compounds obtained from *Pseudomonas aeruginosa* MBTDCMFRI Ps104 on spectroscopic analysis were found to be N-substituted methyl octahydro-1-phenazinecarboxylate and propyl 2-oxoacetate and the potential molecules from *Bacillus subtilis* MBTDCMFRI Ba37 by NMR spectroscopy was found to be Octahydro isopropyl dimethyl methyl cyclopenta phenanthrene, methyl-pentyloxymethoxy, trimethyl benzoannulenyl-methoxybutenone, and actoxyeth acetyl dimethyl benzo annulene carboxylate.
- Large scale production up to 10 L in New Brunswick BioFlo/ CelliGen 115 of *Pseudomonas aeruginosa* MBTDCMFRI Ps04 was optimized to yield 40 g dry weight per litre and to yield maximum bioactive compounds.
- The *Pseudomonas aeruginosa* MBTDCMFRI Ps04 was heat inactivated and spray dried to develop a microbial product (MPs'). The nutritional content of the MPs' were evaluated to be used as fish feed additive.
- Five *Trichoderma* isolates *viz., T. asperellum* TaS1, *T. asperellum* TaS2. *T. harzianum* Th4d, *T. asperellum* TaDOR7316 and *T. asperellum* Tv5 tolerant to salinity identified.
- Sunflower seed treatment with *Trichoderma* isolates *viz., T. harzianum* Th4d, *T. asperellum* TaS1 and *T. asperellum* TaDOR7316 resulted in better seed germination and seedling growth in saline soil at EC 4 in comparison to control.
- Seed treatment with *Trichoderma asperellum* TaDOR673 and *T. asperellum* TaDOR7316 in castor

able to impart drought tolerance and improved seed yield.

- Liquid formulation of *Trichoderma harzianum* Th4d 20% SC with longer shelf life (more than 18 months) and with broad host range has been developed and MOU signed with 6 private firms for technology transfer.
- Identified a new strain of *Bacillus thuringiensis* NAIMCC-B01463 effective against the polyphagous pest *Spodoptera litura*
- Mycorrhizal fungal inoculation improved the maize grain micronutrients (Fe & Zn) besides bioavailability
- Inoculated maize plants produced grains fortified with bio-available micronutrients as a result of reduced phytic acid and enhanced phytase activity
- Indigenous mycorrhizal fungal spores enumerated twice (46 / 100g soil) in integrated nutrient management treatment (100% NPK + FYM) in comparison to NPK alone treatment
- Mycorrhizal fungal association saved 25% water soluble fertilizer in precision farming in sugarcane.
- Mycorrhizal spores were examined under high resolution microscopes and encapsulated with polymers
- DNA fingerprints of five elite cold tolerant 'P' solubilizing bacterial strains (*Pseudomonas fragi* CS11RH1, *Pseudomonas* sp. CS11RP1, *Pseudomonas poae/ trivialis* CT4RH2(2), *Pseudomonas poae* RT5RP(2) and *Pseudomonas poae* RT6RP) were obtained using RAPD-PCR techniques. RAPD markers specific for each strain were identified. Primers for highly specific sequence-characterized-amplified-regions (SCAR) were then designed from nucleotide sequences of specific RAPD markers. These primers efficiently amplified SCAR markers unique to CS11RH1, CS11RP1, CT4RH2(2), RT5RP(2) and RT6RP, respectively.
- Restriction analysis (ARDRA) with nine endonucleases pattern allocated the fourteen cold tolerant 'P' solubilizing bacterial strains into five major types.
- Inoculation with (T5) *Pseudomonas poae* PB2RP1(2) recorded 1.69 and 1.96 fold higher dehydrogenase enzyme activity at 30 and 60DAS, respectively as compared to control (1.01 and 1.13 µg TPF g^{-1} dm $24h^{-1}$) in lentil.
- *Pseudomonas* sp. PCRP7(2) (T9) recorded maximum (1.42 and 3.42 fold) enhancement in total (acid+alkaline) phosphomonoesterase enzyme activity at 30 and 60 DAS, respectively over the uninoculated control (52.6 and 46.4 µg NP g^{-1} dm h^{-1}) in lentil .
- Carrier based formulation of eight cold tolerant 'P' solubilizing bacterial consortia were tested for P uptake and growth in wheat (VL804) under field conditions. Inoculation with 'P' Solublizing bacterial consortia significantly enhanced wheat grain %P content ranged from 3.77 to 32.1% and maximum %P content was recorded in C5 (32.1%) followed by C6 (11.3%) and C7 (7.6%) as compared to uninoculated control.
- The total 'P' content (stover+ grain) enhanced (4.41 to 22.1% except C1, C2 and C9) and maximum increment in %P content was recorded in C5 (22.1%) followed by C6 (10.3% and C7 (8.8%) over uninoculated control
- Bacterization with cold tolerant 'P' solubilizing bacterial consortium C8 {CS11RH4, CS11RH1, PB2RP1 (2)} enhanced wheat (VL 804) yield (average of three years) by 17.6% followed by C3 {(PB2RP1 (2), NS12RH2 (1), CS11RH4)} 14.9% and C4 {CS11RH1, CS11RP1, CS11RH4} 13.8% over uninoculated control (27.3q/ha) under field conditions
- To formulate a mixed consortium of the organisms, the compatibility of actinomycetes with PGPR and *Rhizobium* was tested. PGPR strains *Bacillus megaterium* P3, *Bacillus subtilis* P10 and *Lysinibacillus fusiformis* P25 and soybean rhizobia: *Rhizobium* R10, R11, R32, R50 and R51, *Bradyrhizobium* R16, R33 and R34, chickpea rhizobia- *Rhizobium* R 40 and R56 were co-inoculated with 5 different strains of *Streptomyces* sp. (A1, A2, A6, A10 and A17) on humic acid vitamin agar, nutrient agar and tripticase soy agar. The results showed that all the 5 strains of *Streptomyces* were compatible with all the three PGPR strains P3, P10 and P25 when inoculated together. No soybean and chickpea *Rhizobium* strain was compatible with any of the *Streptomyces* strains. So it was concluded that consortia of actinomycetes can be prepared with PGPR.

- Compatibility among five different strains of *Streptomyces* sp. (A1, A2, A6, A10 and A17) was studied by co-inoculating the five strains on Humic acid vitamin agar and starch casein agar. All the strains were compatible with one another and none of them inhibited each other.
- In order to improve the quality of inoculants, modifications were made to nutrient broth to obtain high cell densities of *Bacillus* sp. Peptone was removed and in its place, glucose, K_2HPO_4 and $MgSO_4$. $7H_2O$ were added. Glucose is an easily utilizable carbon source and phosphate is a source of P and also increases the rate of glucose uptake. Concentration of yeast extract was increased so as to compensate for peptone and also decrease the C: N ratio of the medium which serves to increase the cell counts. Calcium carbonate was added so as to protect the bacteria from pH fluctuations which adversely affect bacterial growth. Two strains of *Bacillus* P10 and P25 were grown in nutrient broth as well as the modified broth for 48h at 28°C under continuous shaking at 125 rpm. The cell counts improved many fold in the new medium . Another variant of yeast mannitol broth with glucose at a concentration of 10g/l and mannitol reduced to 5g/l instead of 10 g/l did not give any difference in counts of rhizobia.
- Field evaluation of 17 carrier based actinomycetes inoculants on maize (var. JM-216) in kharif 2013 in a Vertisol at Jabalpur (Fig 1) showed that the best isolates A1, A2, A6, A10, A16 and A30 gave average yield of 3843 kg ha^{-1} (control yield 2279 kg ha^{-1}). Liquid formulations of the same isolates were also evaluated on chickpea (JG-16). Isolates A1 A2 and A6 and A17 gave average yield of 2292 kg ha^{-1} over control (1389 kg ha^{-1}. Liquid formulations A10, A17 individually and in mixed consortium (CRP) of *Rhizobium* (R40, R56) and PGPR (P3, P10, P25) strains were evaluated separately and in combination on chickpea. Combination of A10 + A17 + CRP gave the highest yield of chickpea (2972 kg ha^{-1}) which was 65% higher over uninoculated control (1805 kg ha^{-1}).

Theme 3: Agrowaste management, bioremediation and microbes for post harvest management and Processing

- The microbial consortium consisting of *Serratia marcesencs* L-11, *Streotomyces rochei* PAH-13 and *Phenarochaete crysosporium* VV-18 was identified for bioremediation of PAH under controlled condition as well as under microcosm.
- A microcosm experiment of unsterilized amended soil with different co-substrates was carried out for *in-situ* degradation and metabolic profiling of PAH mixture by inoculation of microbial consortium.
- High rate of PAHs degradation (Flourene 97%, Phenanthrene 88%, Anthracene 94% and Pyrene 95%) was observed during third to fifth day in compost amended soil.
- GC-MS analysis of treated soil extract was carried out to detect non-polar and polar metabolites produced during degradation of PAH. Total 20 polar and non-polar metabolites were identified depicting complexity of degradation mechanism.
- The microbial consortium had the capacity to degrade a mixture of Anthracene, Fluorene, Phenanthrene and Pyrene @ 200 ppm under microcosm within 7 days. This consortium has the potential to bioremediate oil contaminated sites as an environmental friendly option. Maximum removal and uptake of heavy metals (Zn, Ni, Cu, Cd, Pb) from aqueous solution was observed with sterilized and unsterilized pressmud and pressmud plus rice husk along with microbial consortium at 25 ppm concentration after 3hrs of contact time.
- Optimum inoculum rate for maximum removal of heavy metals by agrowastes along with microbial consortium of six fungi and one bacterium was in the range of 0.6 to 1.0 g of agrowaste along with microbial consortium.
- Optimum time interval for maximum removal of heavy metals from aqueous solution by agrowastes along with microbial consortium was during 1-5 hours. Maximum removal of heavy metals from aqueous solution of 25 ppm by agrowastes along with microbial consortium was in the pH range of 4-6.
- Pressmud, rice straw individually and in combination with rice husk along with microbial consortium removed substantial amount of Ni and Cu from industrial wastewater containing 302 ppm of Ni and 7 ppm of Cu in plastic drums of 60 litres capacity. These agrowastes along with microbial consortium can be used for removal of heavy metals from industrial wastewater

- Environmentally important groups of bacteria viz. chemolithotrophic nitrifiers, aerobic denitrifiers, heterotrophic nitrifiers, chemolithotrophic and heterotrophic sulfur oxidizers isolated and characterised.
- These bacteria were evaluated for their efficiency to oxidize ammonia, nitrite and sulfide *in vitro*.
- Two new methods *viz*., a differential filtration-micro irrigation method for rapid enrichment of chemolithotrophic ammonia oxidizing bacteria (AOB) and a simple method for qualitative confirmation of denitrifying bacteria was developed and evaluated.
- Large-scale AOB-NOB enrichments (80 L) developed and concentrated to 6 L by TFF for treatment to mitigate ammonia ponds.
- Survey of HCH contaminated agricultural site adjoining to the industry and dump sites was done followed by sampling.
- Enrichment and residue analysis of HCH isomers and various metabolites from HCH dump site, industrial soil/sediment and water samples has been completed.
- Isolation and characterization of noble HCH degrading bacteria especially Sphingomonads has been done.
- Characterization of isolated organisms in terms of *lin* genes and rate of HCH degradation has been done.
- Bacteria capable of degrading lignocellulose were isolated from the sediment of Mahul mangroves and sugarcane bagasse from Versova using R_2A agar medium. The identified bacteria (using 16S rDNA technique) were *Pseudomonas alcaligenes, Bacillus cereus, Bacillus vietnamensis, Bacillus selenatarsanitis, Bacillus thuringiensis, Bacillus pumilus, Bacillus subtilis, Bacillus firmus, Bacillus megaterium, Bacillus endophyticus, Bacillus* sp., *Alcaligenes feacalis, Nocardia farcinica, Proteus mirabilis* and *Schawnella* sp. The gene sequences were submitted to NCBI-GenBank under accession numbers, KF573632 to KF573639. *Bacillus pumilus, Bacillus cereus, Bacillus subtilis* and *Proteus mirabilis* were found to show higher cellulose and xylanase activity compared to other isolates.
- Consensus degenerate hybrid oligonucleotide primers (CODEHOP) were constructed using aligned eubacterial protein sequences of betaine aldehyde dehydrogenase (*BetB*) from the NCBI databank. Expected size of the amplicons was 700 – 720 bp. The ability of the primers to retrieve the desired sequences of *BetB* from community DNA was checked with the community DNA isolated from intertidal seawater, an algal mat growing in a salt pan in Bhayander, and enrichment cultures from various samples. Amplicons from these areas were all of the expected size.
- One millilitre of each water sample from sea [DC] (44‰), Madh Island intertidal zone [M] (25‰), marine inlet water leading to the Bhayander saltern [I] (78‰) and a saltern pool from a saline pond at the Bhayander saltern system [P] (300‰) was inoculated in nutrient broth containing 50, 100, 150, 200, 250 and 300‰ NaCl. Enrichment was done in 2 replicates over 1 week. No growth was observed in any of the samples at 300‰. DNA was extracted from each enrichment and 50 ng of DNA was used for amplifying the *BetB* gene in duplicates for each enrichment. Sample from sea and Bhayender saltern showed the presence of *BetB* gene at all salt concentrations at identical intensity on agarose which may be considered as semi-quantitative data. Samples from intertidal zone and Bhayender saltern showed a decline in *BetB* copy number with a rise in NaCl concentration.
- Different methods were employed for the identification of the new isolates. O3 was identified using Sherlock-MIDI GC-FAMES peak profiling as *Bacillus cereus*. Ten isolates were analyzed by amplified rDNA restriction analysis (ARDRA) with three restriction enzymes namely, *HaeIII, MboI* and *AluI*. B5 was identified as *Enterobacter hormaechei*, and B6 and B8 as *Pseudomonas alcaligenes*. Eight other isolates were analsed by 16S rDNA gene sequencing. They were found to be *Bacillus licheniformis, Enterobacter* sp., *Enterobacter hormaechei, Microccous* sp., *Alishewanella* sp., *Lysinibacillus fusiformis, Kocuria palustris* and *Shwanella* sp.
- *Bacillus cereus* was replaced with *Bacillus subtilis* in Consortium Ca (referred to as Ca*) and was evaluated for activity as well as stability. Of all the crude consortia evaluated till date, Ca* has given the best results with higher xylanase activity compared to the individual members. Cellulase activity of *Bacillus subtilis* was significantly higher

and that of *Bacillus megaterium* was slightly higher when compared to the other isolates.

- Ability to degrade pentachlorophenol is widely distributed among phylogenetically distinct bacteria in agricultural soils irrigated with effluent discharged from pulp and paper mill.
- Out of selected 8 isolates, *Lysinibacillus fusiformis*, *Ensifer adhaerens* and *Pseudomonas putida* were used in consortium for PCP removal from soil.
- Formation of intermediates TeCH and DCP indicated that two different pathways were operating i.e reductive dehalogenation and hydrolytic dehalogenation during PCP degradation.
- Bacterial isolates utilizing PCP up to 500 ppm obtained in this study can work in consortium for effective removal of PCP from pulp and paper mill effluent contaminated sites.
- Consortium of *Ensifer adhaerens, Pseudomonas putida* and *Lysinibacillus fusiformis* having PGP activities and ability to degrade PCP in soil significantly increased yield of wheat and reduced PCP load of effluent contaminated soil in a pot experiment.
- Among unculturable diversity, OTUs corresponding to the phylum Proteobacteria were most abundant followed by Acidobacteria and Firmicutes.

Theme 4: Microbial Management of Abiotic Stress

- Functional characterization including production of plant growth hormones *viz*, IAA, GA, Cytokinin and production of exopolysaccharides and ACC deaminase activity, of four salt tolerant N_2 fixing AIMs namely ASANFXI S173E: *Azospirillum irakense,* ASFPI S4 (1) S: *Pseudomonas sp.,* ASPSBIX S124E: *Bacillus subtilis and* ASPSBIX S122R: *Bacillus endophyticus* exhibiting 12.5 to 15% salt tolerance was carried out.
- The strains produced 6.2 – 8.3 g IAA and 2.6 – 2.9 g GA/mg protein; 20-35 g cytokinin/L; 35 to 58 g exopolysaccharides/mg protein; ACC deaminase activity in the range of 152 to 239 nmol ketoglutarae /g. biomass/h.
- The amount of osmolytes *viz*., proline and glycine-betaine produced by these salt tolerant AIMs were quantified and ranged from 1.5 to 10.5 and 0.5 to 0.8 ug/mg protein respectively.
- The salt tolerant AIMs and their consortia enhanced the seed germination by 23 to 33.6%, dry matter production by 12.9 to 85.4% and grain yield of wheat (DWR 162) by 50 to 183% over un-inoculated control under field condition at a salinity level of 14.3 dS/m in a salt affected soil at Agricultural Research Station at Gangavati (Karnataka).
- Identification of genes encoding salt tolerance *viz*., *proBA, gbsTIBA, otsAB, ectABC* in 21 salt tolerant isolates, revealed that these AIMs were found to possess combination of two to four genes responsible for salt tolerance
- Multiple alignment (CLUSTAL-W) of amino acid sequences produced by cold tolerant *Pseudomonas lurida* NPRp15, *Pseudomonas putida* PGRs4 and *Pseudomonas fluorescens* PPRs4 shared 42.5, 54.5 and 63.6% respectively, identity with major cold shock proteins (*Csp*A) from *Bacillus cereus* ATCC 14579.
- Comparison of deduced partial *Csp*A amino acid sequences gained from the 3 bacterial species showed that two RNA-binding motifs RNP-1 and RNP-2 were present. The conservation of these two RNP motifs suggests their structural importance for the binding of nucleic acids on the surface of *Csp*A.
- Restriction analysis (ARDRA) with endonucleases (Alu I, Hha I, Rsa I and Bsu I) allocated the twelve cold tolerant bacterial strains into the four major types.
- Inoculation with cold tolerant bacterial consortium C3 (PBRs7+PCRs4+PGRs1) recorded 2.77 and 1.08 fold higher dehydrogenase enzyme activity at 30 and 60DAS, respectively as compared to control (0.60 and 2.00 µg TPF g^{-1} dm $24h^{-1}$).
- Consortium C3 recorded maximum (1.2 and 1.1 fold) enhancement in total (acid+alkaline) phosphomonoesterase enzyme activity at 30 and 60DAS, respectively as compared to control (149.0 and 86.5 µg NP g^{-1} dm h^{-1}).
- Under field conditions bacterial consortium C7 (PPRs4+NARs9+PBRs7) enhanced wheat (VL Gehun 804) yield by 8.6% over uninoculated control (30.9q/ha).

Theme 5: Microbial Genomics

- The efficacy of the formulations (containing

cyanobacteria exhibiting fungicidal activity) was evaluated in fungi infested fields (sick plots) of cotton at Sirsa. Significant reduction in mortality, and enhanced plant defense enzyme activity and growth were recorded using in cotton, with lowest values of 13% with Anabaena laxa based formulation.

- Cyanobacteria inoculated treatments recorded enhanced plant vigour, with 15-20% increase in plant growth parameters and activity of hydrolytic and pathogenesis related enzymes in field experiment of BLB challenged cotton crop at CICR, Nagpur.
- PCR based assays and SEM analyses confirmed colonization by cyanobacteria and amplification of endoglucanase gene in rhizosphere soil samples, which correlated positively with enhanced hydrolytic enzyme activity in root tissues.
- *Anabaena* and *Calothrix* based formulations proved promising as biocontrol agents against root knot nematodes in tomato at IIVR, Varanasi, besides improving plant growth, soil microbial activity and yield.
- *Bacillus thuringiensis* strains were isolated and identified from Andhra Pradesh soil samples using five approaches *viz.,* analysis of crystal protein production with microscopy, detection of *cry* gene content by PCR, SDS-PAGE profiling, Cloning, sequencing, and toxicity test.
- Molecular characterization and elucidation of *cry26* full length gene from native *Bt* strains.
- Toxicity analysis of native *Bt* strains purified toxins against Aracanut root grubs *Leucopholis lepidophora* Blanch 3rd instar larvae.
- 3D homology modelling of new Cry26 toxin using Phyre2 bioinformatics tool
- *Bt* isolates were isolated from root nodules of leguminous crop. and were characterized through phenotypic and microbiological studies.

Human Resource Development

NBAIM provides training on cutting age technologies in the area on microbial identification, diversity analysis through culture dependent and culture independent techniques, and microbial interactions for its exploitation in abiotic and biotic stress management. The bureau has made significant achievements to train the human resources in microbial identification, characterization, and its application in crop stress management. During 2013-14 the following training were organized by bureau:

Sthapna Divas Samaroh and Kisan Sangosthi held on 1st June, 2013

Subject Matter Training: "Computational tools for microbial research, 12 Days from Nov., 19-30, 2013. Number of participants- 25.

A Group Meeting of Culture Collections of Importance in Agriculture and Allied Sectors

A group meeting on "Culture Collections of Importance in Agriculture and Allied Sectors" was organized at NBAIM, Mau on 10th Dec., 2014 with an aim to develop a consensus on issues pertaining to culture collections of India. The meeting began with the welcome of Chief Guest Prof. C. Manoharachary, Chairman RAC, NBAIM, Dr. Manjit Singh, Director, DMR, Solan, Dr. S. Rajendra Prasad, Project Director DSR, Mau, Dr Arun K. Sharma, Director, NBAIM, Mau, and other researchers from different culture collections of India (MCC, Pune, NFCCI, Pune, VTCC, Hissar, ITCC, New Delhi, CUBGA, New Delhi, DMR, Solan, NBAII, Bangalore, IIHR Bangalore, etc.). Many issues were deliberated during meeting at length and some of the important recommendations emerged out were (1) development of uniform/standard passport data form, MTA and guidelines for microbial deposition in compliance with NBA, WFCC and IDA, (2) development of panel of taxomonists of the country for identification of microoraganisms, (3) encouragement for registration of potential microbial cultures and their safe deposit at NAIMCC, (4) establishment of reference isolate/type isolates for each species of plant pathogenic fungi for understanding changes in population structure responsible for disease epidemics in future, (5)

retrieval of cultures from other repositories of India, (6) development of passport data for plant pathogenic cultures involving ecological, morphological, molecular, biochemical and aggressiveness data/features, and (7) development of network (e.g. National Network on Microbial Culture Collection) involving leading culture collections of India.

- **One day Patent Awareness Workshop with special reference to Microbial Genetic Resources**

A one day Patent Awareness Workshop with special reference to Microbial Genetic Resources was organized on 5th Feb, 2014 at NBAIM Mau as an activity under ICAR plan scheme "Intellectual Property Management/Commercialization of Agriculture Technology". It was organized by Dr. Renu, Senior Scientist cum ITMU, Member Secretary as Workshop Coordinator. The Chief Guest of the occasion, Dr. Rajendra Kumar, Director General, Uttar Pradesh Council of Agricultural Research (UPCAR); expert guests, Sh. R. Saha, Former Advisor, DST & Ex Director, PFC, TIFAC, GOI; Dr. H. S. Chawla, Professor & Head, Genetics & Plant Breeding, GBPUAT, Pantnagar and Dr. H. B. Singh, Professor, BHU, were welocomed by Director NBAIM. The workshop started with the briefings about origin of NBAIM, its developmental stages, activities and achievements. Deliberations were made on concept, objectives and importance of IPR in microbial genetic resources context. A Technical Bulletin on "Intellectual Property Rights: Facts and Procedure in India" was released by Hon'ble chief guest Dr. Rajendra Kumar, DG UPCAR, followed by his address. Sh. Saha presented, "Intellectual Property: General view", followed by lectures on "Patent filling system in India and Abroad, with special reference to Patenting of microorganisms" and "Implementation of PPV & FR Act in India" by Dr. H. S. Chawla. Dr. H. B. Singh gave detailed and interesting insight into "Commercialization of bioinoculants, regulatory requirements and IPR issue". Dr. Sushil Kumar Sharma, Principal Scientist and I/C NAIMCC also presented details on "Registration of microbial germplasm".

- **National Training on Microbial Characterization and Nanoformulations: Methods and Applications**

A ten days National training programme on **"Microbial Characterization and Nanoformulations: Methods and Applications"** was successfully organized by National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, from 4th March, 2014 to 13th March, 2014. Dr. Arun Kumar Sharma, Director, NBAIM was the course director and Dr. Prem Lal Kashyap, scientist, NBAIM was the course co-ordinator for the training. The training focused on microbial identification and characterization using conventional genomics and metagenomics approaches. The hands-on-research experience and exposure to cutting edge research on microbial nanotechnology as a suitable system for the synthesis of different kinds of nanoformulations for advanced diagnostics, biosensors, and targeted delivery systems for agrochemicals was also provided. A total of 27 participants from different ICAR institutes, state agricultural universities, central

universities, CSIR institutes and private sectors across the country, were trained in this programme. Resource experts from reputed ICAR institutes (National Bureau of Agriculturally Important Insects, Bengaluru; Directorate of Wheat Research, Karnal), CSIR institutes (National Chemical Laboratory, Pune; Microbial Culture Collection, NCCS, Pune) and Central universities (Banaras Hindu University, Varanasi) were invited for delivering lectures and providing hands-on training on their respective areas of expertise. Apart from invited resource persons, in-house scientists of the Bureau were actively involved in the training by delivering lectures and demonstrating practical experiments.

A National workshop on "Bioinformatics-assisted biological research : Microbial perspective" was organised on 24-25 March, 2014. A total of 65 participants attended the workshop from different part of Country.

Hindi Chetana Maas

वर्ष 2013 में ब्यूरो में विगत वर्षों की भाँति ही हिन्दी चेतना मास का आयोजन 14 सितम्बर से 13 अक्टूबर 2013 को आयोजित किया गया। मास पर्यंत चलने वाले इस हिन्दी जागरूकता कार्यक्रम के दौरान वाद-विवाद, हिंदी टंकण, लेखन, टिप्पणी लेखन, वैज्ञानिक निबंध लेखन आदि प्रतियोगितायें आयोजित की गईं। समापन दिनांक 13 अक्टूबर 2013 को किया गया जिसमें निदेशक, एनबीएआईएम, मऊ के द्वारा प्रतियोगिता के प्रतिभागियों को पुरस्कार वितरित किया गया।

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17. शंकर लाल, रेनू, उदय भान सिंह, अरूण कुमार, रमेश चंद्र यादव, राजीव कुमार सिंह एवं ए. के. शर्मा । 2013। कृषि के मित्र जीवों का संरक्षण। खेती, जनवरी, पेज सं 23–24।
18. आशा साहू, नीशा साहू, उदय भान सिह एवं एम. सी. मन्ना 2013। फास्फेट युक्त जैविक खाद बनाने की सरल विधि। मेला विशेषांक–2013। बीज अनुसंधान निदेशालय, कुशमौर, मउनाथ भंजन–275 101।
19. उदय भान सिंह, पवन कुमार शर्मा, रेनू, वसीउल्लाह, पाण्डियन के., कार्तिकेयन एन., अल्का सिंह, सुशील कुमार शर्मा, अरुण कुमार शर्मा बायोपेस्टीसाइड्सः पर्यावरण अनुकूल प्रकृति का अमुल्य उपहार। स्मारिका–मेला विशेषांक–2014। बीज अनुसंधान निदेशालय, कुशमौर, मउनाथ भंजन–275 101।

Training Manuals / Technical Bulletin / Brochures/Leaflets/Books:

1. Sharma, A. K., Singh D.P. and **Renu** (2013). A Laboratory Manual on Molecular Microbiology and Pathology. Pubished by National Bureau of Agriculturally Important Microorganisms, Mau Nath Bhanjan, U.P. INDIA. Pp 252.
2. Prem Lal Kashyap, Alok Kumar Srivastava, sudheer Kumar and Hillol Chakdar (2014) A Training manual on Microbial characterization and Nanoformulations: Methods and Applications. National Bureau of Agriculturally Important Microorganisms, Mau. p. 106.

3. Renu, Manish S. Bhoyar and Udai Bhan Singh (2014). Intellectual Property Rights: Facts and Procedures in India. National Bureau of Agriculturally Important Microorganisms, Mau. pp 75.
4. A bilingual Technical brochure entitled Intellectual Property Rights: An Overview edited by Renu, Manish S. Bhoyar and Udai B. Singh and published by NBAIM, Mau. pp. 4.
5. Leaflet on Microbial Genetic Resources (MGR) Portal by Renu, Udai B. Singh and Hillol Chakdhar (2013) Pubished by National Bureau of Agriculturally Important Microorganisms, Mau Nath Bhanjan, U.P. INDIA.
6. Training manual of NABG Subject training on "Computational tools for microbial research" on 19-30 Nov., 2014.
9. COMPOST HANDBOOK - research- production -application by Drs. MC Manna, A. Subba Rao, Asha Sahu and Udai B. Singh. Fertilizer Development and Consultation Organization, New Delhi, India.

Book Chapter

1. Udai B. Singh, Asha Sahu, Dhananjaya P. Singh, Renu, Nisha Sahu, M. C. Manna, B.K. Sarma, H.B. Singh, S. Rajendra Prasad and A.K. Sharma (2014). Microbial community in rhizosphere and their impact on plant Biology: An Overview. In: *Microbial Biodiversity: A boon for Agriculture Sustainability"* Asha Sinha, Sweta Srivastava and Ravindra Kumar (Eds) *published by Biotech Books, New Delhi*. (Accepted)
2. Udai B. Singh, Asha Sahu, Renu, Dhananjaya P. Singh, Nisha Sahu, M. C. Manna, B.K. Sarma, H.B. Singh, S. Rajendra Prasad and A.K. Sharma (2014). Microbial diversity and biodegradation of organic pollutants and heavy metals from rhizosphere ecosystem: A mechanistic approach. In: *Microbial Biodiversity: A boon for Agriculture Sustainability"* Asha Sinha, Sweta Srivastava and Ravindra Kumar (Eds). P*ublished by Biotech Books, New Delhi*. (Accepted)
3. J. K. Thakur, Asha Sahu, Udai B. Singh, A. Mandal and M.C. Manna (2014).Molecular Techniques in Soil Biodiversity Study. In: *Microbial Biodiversity: A boon for Agriculture Sustainability"* Asha Sinha, Sweta Srivastava and Ravindra Kumar (Eds). P*ublished by Biotech Books, New Delhi*. (Accepted)
4. Alka Singh, Ruchi Singh, Udai B. Singh, Renu, Sudheer Kumar and A.K. Sharma (2014). Molecular methods for studying soil microbial diversity: Tools and Techniques. In: *Microbial Biodiversity: A boon for Agriculture Sustainability"* Asha Sinha, Sweta Srivastava and Ravindra Kumar (Eds) *published by Biotech Books, New Delhi*. (Accepted)
5. M.C. Manna, Asha Sahu, Udai B. Singh, J. K. Thakur, A. Mandal and A. Subba Rao (2013) Microbial Biodiversity: Present and Future of Soil Health. In Soil Microbial Ecology. Dhananjaya P. Singh and Harikesh B. Singh (Eds). Published by Studium Press LLC, USA. pp. 279-321.

Meetings

Meetings attended by Director, NBAIM

1. Attended the meeting of National Biodiversity Authority, Hyderabad on 8th April, 2013.
2. Attended the meeting of submission of NBAIM, QRT report to the Hon'ble D.G. Indian Council of Agricultural Research, New Delhi on 30th April, 2013.
3. Attended the meeting of EFC with DDG (CS) at ICAR, New Delhi on 8th May, 2013.
4. Attended the meeting of Directors on Performance Indicators at NCAP, New Delhi on 15th July, 2013.
5. Attended the 85th ICAR Foundation Day and Farming Research System at NASC, New Delhi on 16th July, 2013.
6. Attended 4th NABMGR meeting at NBAII, Bangalore on 10-11 October, 2013.
7. Attended meeting of Agrinnovate at AP Shinde Hall, NASC, New Delhi on 19th October, 2013.
8. Attended meeting of the Brainstorming on Seed marketing- role of Agrinnovate India Ltd, held at NASC conference facility, New Delhi on 8th November, 2013.
9. Presentation of 12th plan budget proposal before the SFC in ICAR, New Delhi on 13 November, 2013.
10. Attended the Vice Chancellors and Directors Interface Meeting at NIASM, Malegaon, Baramati and Pune on 19-20 January, 2014.
11. Attended the 35th Executive Council Meeting at SHIATS, Allahabad (Deemed University) on 7th February, 2014.

By Scientists

- Dr. Pawan Kumar Sharma, Principal Scientist, Attended 16th Indian Agricultural Scientists & Farmers' Congress on "Nanobiotechnological Approaches for Sustainable Agriculture and Rural Development" at Integral University, Lucknow from 22-23 February 2014.
- Dr. Alok Kumar Srivastava, Senior Scientist, attended the meeting for 14th regular Session of CGRFA at ICAR New Delhi on April 3, 2013. Attended the meeting for finalizing the guidelines to manage the genetic resources on April 29 and 30th 2013.
- Dr. Alok Kumar Srivastava, Senior Scientist, Attended the QRT report submission meeting with DG, ICAR on April 30, 2013
- Dr. Alok Kumar Srivastava, Senior Scientist, Attended the NAAS Brainstorming Session on "Role of Root Endophytes in Agriculturalal Productivity" on July 5, 2013 at NASC, New Delhi
- Dr. Alok Kumar Srivastava, Senior Scientist, Attended the Meeting of Performance Indicators on July 15, 2013 at NCAP, New Delhi.
- Dr. Alok Kumar Srivastava, Senior Scientist, Attended the review meeting of RFD Nodal officers on October 29, 2013, at ICAR, New Delhi,
- Dr. Dhananjay Pratap Singh, Sr. Scientist attended State Level Sanctioning Committee (SLSC) meeting with Chief Secretary, UP Govt on 30 May 2013 at Lucknow regarding RKVY project
- Dr. Dhananjay Pratap Singh, Sr. Scientist attended NABG review meet and discussions of "Mega Projects" at IASRI, New Delhi in 10-11 July 2013
- Dr. Dhananjay Pratap Singh, Sr. Scientist attended Brainstorming session on "Bioinformatics in Agriculture : Way Forward" at NASC, New Delhi on 12 July 2013
- Dr. Renu attended and repoteure in International Conference on Impact of Technological Tools on

Food Security under Global Warming Scenario (ITTFS-2012) to be held at Shobit University, Meerut from 11-12 May, 2013 and presented paper on Global Scenario in Patenting System: Microbiological Perspective

- Dr. Renu attended Sensitization Programme on Intellectual Property Rights on 18/02/2013 organized by Directorate of Seed Research, Mau
- Dr. Renu attended National Symposium on Abiotic and Biotic Stress Management in Vegetable Crops held at Indian Institute of Vegetable Research from April 12 – 14, 2013 and presented paper on Detection and Genetic Diversity of *Xanthomonas campestris* pv. *campestris* Isolates from India based on Molecular Techniques.
- Dr. Renu, Senior Scientist, NBAIM cum I/c IPR cell, NBAIM delivered a lecture on "General Information about Intellectual Property Rights in India" as a guest lecturer in PPV & FRA training on 05/03/2013 organized by NEFORD, Lucknow at DSR, Mau.
- Mr. Udai Bhan Singh, Scientist, attended SAS (Statistical Analysis Software) training at Directorate of Seed Research (Indian Council of Agricultural Research), Kusmaur, Maunath Bhanjan-275 101, India organized by Indian Veterinary Research Institute (IVRI), Ijjatnagar, Bareily, UP on 3-8 March, 2014.
- Mr. Udai Bhan Singh, Scientist, attended Sensitization workshop on SAS (Statistical Analysis Software) (2013) at National Bureau of Agriculturally Important Microorganisms (Indian Council of Agricultural Research), Kusmaur, Maunath Bhanjan-275 101, India organized by Indian Agricultural Statistical Research Institute (IASRI), New Delhi.
- Mr. Udai Bhan Singh, Scientist, attended National Workshop on "Capacity Building Workshop on Agropedia and Open Access Institutional Repository" (2013) organized at ICRISAT, Patancheru, AP.
- Mr. Udai Bhan Singh, Scientist, attended International Conference on Impact of Technological Tools on Food Security under Global Warming Scenario (ITTFS-2012) held at Shobhit University, Meerut May 11-12, 2013.
- Dr. Lalan Sharma attended 21 days International Training programme on "Plant Biosecurity and Incursion Management" at National Institute of Plant Health Management, Hyderabad held from April 8th to 28th, 2014.
- Dr. Lalan Sharma attended review meeting of NABG project at Indian Agricultural Statistics Research institute, New Delhi held on July 10 and 11, 2013.
- Dr. Lalan Sharma attended Brain Storming on Bioinformatics in agriculture, away forward at National Academy of Agricultural Sciences, Complex, New Delhi held on July 10 and 11, 2013.
- Dr. Lalan Sharma attended progress meeting of NABG project at Indian Agricultural Statistics Research institute, New Delhi held on November 7 and 8, 2013.
- Dr. Lalan Sharma attended Workshop on integration and mainstreaming of the activities under the three mega projects of the council at National Academy of Agricultural Sciences, Complex, New Delhi held on November 9, 2013.
- Dr. Lalan Sharma attended final review meeting of NABG at National Academy of Agricultural Sciences, Complex, New Delhi held on March 8, 2014.

Award and Recognition

ISO Certification to NBAIM

National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, obtained ISO9001:2008 (certificate No NAT09D) on 28.03.2014. This certification to NBAIM is awarded for 'Provision of services to carry out Research & Development (R&D) Activities in terms of conducting basic and applied research on agriculturally important microorganisms including crops, horticulture, animal husbandry and fisheries sciences'


CERTIFICATE

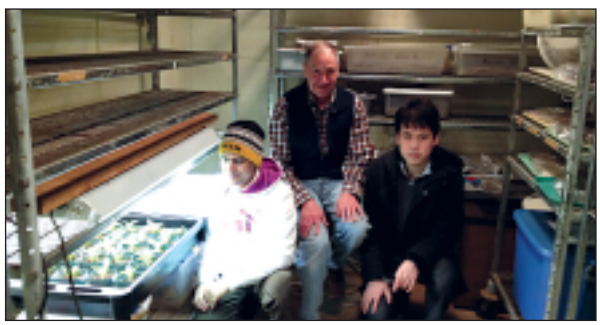
Awards

- Dr. Arun Kumar Sharma, Director, NBAIM was presented the 'Plant Pathology Leadership Award' of the Indian Phytopathological Society (ME2) during its Zonal Meeting and National Conference on managing threatening disease of Horticultural , Medicinal Aromatic and Field Crops in relation to changing climatic situation, held at Indian Institute of Sugarcane Research, Lucknow and organised by Indian Phytopatholocal Society, New Delhi and Association of Plant Pathologists of India, Lucknow in collaboration with ICAR, CSIR, INSA, DBT, DST, IRRI, NABARD and CABI.

- Dr. Arun Kumar Sharma, Director, NBAIM was conferred the 'Honorary Fellowship Award -2014' by the Bioved Research Institute of Agriculture and Technology, Allahabad and The Integral University, Lucknow, during the 16th Agricultural Scientists and Farmers Congress, organized at Integral University, Lucknow on Feb. 20-23, 2014.

- Dr. Renu, Senior Scientist, NBAIM participated in International Conference on Impact of Technological Tools on Food Security under Global Warming Scenario (ITTFS-20) organized by Hi-Tech Horticultural Society, Meerut from 11-12 May 2013 at Shobit University, Meerut and presented poster on"Global Scenario in Patenting System: Microbiological Perspective. The poster got award in poster presentation category.

- Major RS Yadav SRDA Award 2012 conferred to Dr Renu, Senior Scientist (Biotechnology) for outsanding contributions in field of Biotechnology by Society for Recent Development in Agriculture.
- Mr. Udai Bhan Singh, Scientist was awarded DST Young Scientist: 2012-13 (under Fast Track Program, DST)
- Mr. Udai Bhan Singh, Scientist was made the fellow of the Society for Applied Biotechnology, Tamil Nadu
- Dr. Prem L. Kashyap, Scientist (Plant Pathology) attended three months international training on "Development of nanoparticles based formulation to manage tomato root rot" from September 1, 2013 to December 29, 2013 NBAIM, Mau in the lab of Prof. Patricia Heiden, Department of chemistry, Michigan Technological University (MTU), Houghton. The training was sponsored by NAIP, ICAR India. During the three months training, he learnt synthesis of chitosan based nanoparticles, gold nanoparticles, quantum dots by using various chemical approaches. He got hand-on training on various instruments like scanning electron microcopy (SEM), FTIR, Dynamic light spectrophotometer (DLS) instruments etc. required for the characterization of nanoparticles and nanoformulations. In addition, he used NMR for characterization and structure prediction of peptide based nanoformulations.



- Udai B. Singh, Renu, Dhananjaya P. Singh, Jatindra K. Pradhan, Wasiullah, Manish Roy and Arun K. Sharma (2014). Bio-prospecting of microbial bio-agents from rhizosphere eco-systems triggering plant defence responses provide protection against sheath blight disease in rice (*Oryza sativa* L.). Paper presented in National Seminar on Indian Agriculture and Rural Development in Changing Global Scenario" organized by Krishi Vigyan Kendra, Institute of Agricultural Sciences, Banaras Hindu University at Rajiv Gandhi South Campus, Barkachha, Mirzapur, U.P., India on 7th February, 2014 was awarded Best Poster Award.

- Anjney Sharma, Rajeev Kaushik, Alok K. Srivastava, Sudheer Kumar, P.L. Kashyap, Hillol Chakdar and A.K.Sharma (2014) Multifaceted beneficial effects of stress tolerant rhizosphere microorganism *Serratia nematodiphila* CG39 on plant health. National Conference on "BIOFUTURITY : Current Scenario and Future Trends in Biotechnology held at Bundelkhand University Jhansi (U. P.) from March 26-27, 2014. was awarded with Best Poster Presentation Award.
- Aditya Pratap Soni, Pragya saxena, Prem Lal Kashyap, H. Chakdar, A. K. Srivastava, T. P. Rajendran, A. K. Sharma (2014) Isolation and diversity analysis of cold adaptive bacteria from the high altitude Himalayan regions of Arunachal Pradesh, India. In: National Conference on "Perspectives & Trends in Plant Sciences and Biotechnology" (PTPB-2014 from February 21-23, 2014 at Punjab University, Chandigarh was awarded with Best Poster Presentation Award
- Renu, Manish S. Bhoyar, Udai B. Singh, Ramesh Chandra Yadav and Arun Kumar Sharma (2013). Global Scenario in Patenting System:

Microbiological Perspective. Paper presented in International Conference on Impact of Technological Tools on Food Security under Global Warming Scenario (ITTFS-2012) held at Shobhit University, Meerut on May 11-12, 2013. Theme 7, pp. 2, was awarded Best Poster Presentation Award.

Linkages

Scientists of the leading Culture Collections of India were brought together at NBAIM and effort was made to develop linkages among them. The Culture Collections that participated included, ITCC, New Delhi; MCC, Pune; ARI Pune; MTCC, Chandigarh; VTCC, Hisar, individual scientists holding Culture Collections like those at IIHR, Bangalore, Director DMR, Solan and others. The Chairman, RAC, NBAIM chaired one of the sessions. These linkages have led to participation of Scientists/Researchers from various Culture Collections in training programmes at NBAIM, three months attachment training of newly recruited scientists of NBAIM at some of these institutes; participation of the scientists of some of these Culture Collections scientists in National Workshop organized at NBAIM, and other such activities. Efforts are on to transfer some cultures from the smaller Culture Collections which are not designated repositories, to NAIMCC. Proposal is also underway to make Culture Collection of Mushrooms at DMR, Solan as a node of NAIMCC.

1. Microbial Type Culture Collection, Chandigarh
2. National Chemical Laboratory, Pune
3. Agarkar Research Insititute, Pune
4. Microbial Culture Collection, Pune
5. UPCAR (Uttar Pradesh Council of Agricultural Research), Lucknow
6. Through sub-scheme AMAAS, the linkages have been developed with following categories of Research Institutions in the Country:

(i) ICAR institutes

(ii) SAU's

(iii) Central Universities

(iv) Traditional Universities

(v) Vetinerary and Animal Science Universities

(vi) CSIR institutes and others

MOU's Established

MOU's were signed with the following Universities/Institutions for scientific collaboration, imparting training to their students and other related aspects:

1. Sher-e-Kashmir University of Agricultural Sciences & Technology – Jammu.
2. Integral University, Lucknow.
3. Sam Higginbottom Institute of Agriculture, Technology and Science (Deemed University), Allahabad.
4. Chatarpati Sahuji Maharaj University, Kanpur.
5. IFTM University, Moradabad

Library Information and Documentation

Books	No.
Administration	133
Bacteriology	66
Biochemistry	40
Bioinformatics	61
Bioinstrumentation	01
Biotechnology	233
Botany	40
Biocontrol	07
Biostatistics	07
Biofertilizers	03
Environmental science	30
Enzymology	06
Food science	13
Genetics & genomics	92
Hindi book	72
Integrated pest management	19
Intellectual property Right	70
Microbiology	463
Molecular biology	205
Mycology	177
Nanotechnology	04
Other	28
Phycology	12
Plant pathology	109
Plant virus	04
Proteomics	09
Soil biology	19
Virology	38
Yeast	05
Total	**1966**

Books Procured During 2013-2014:	66
Journal List	**No. of Issues**
Annual Review of Microbiology	19
Annual Review of Phytopothology	17
Applied and Environmental Microbiology	24
Agricultural Research	4
Asian agri-history	4
Biotechnology and Environmental Sciences	3
Biology and Fertility of Soils	21
Canadian Journal of Microbiology	43
Clinical Microbiology	4
Current contents	50
Current Science	159
Eukaryotic Cell	11
FEMS Microbiology Reviews	1
Fungal Genetics and Biology	10
Indian Journal of Experimental Biology	24
Indian Journal of Microbiology	16
Indian Journal of Sugarcane Technology	21
Indian Phytopathology	74
Indian Horticulture	6
Indian Farming	12
Journal of Bacteriology	100
Journal of Biosciences	24
Journal of Biotechnology	10
Journal of Eco-friendly Agriculture	5
Journal of the Indian Botanical Society	9
Journal of Mycology and Plant Pathology	3
Journal of Scientific and Industrial Research	42
Kheti	12

Krishika Shodh Patrika	2
Molecular Plant Microbe Interaction	29
Molecular Plant Pathology	13
Mycobiology	10
Mycologia	12
Mycological Research	18
Microbiological Research	17
Nature	171
Pestology	11
Plant Diseases	12
Plant Pathology	23
Phal Phul	6
Science	97
Soil Biology and Biochemistry	11
The Indian Journal of Agricultural Science	12

Miscellaneous literature

1. Annual report of ICAR institutes
2. Complete solution for biotech Research
3. Advanced Biotech
4. Current content of life science
5. Catalogues & Dictionaries
6. ICAR News/ Bulletins
7. Laboratory Manuals
8. News Papers- Following daily newspapers are being received

   Hindi : Dainik Jagran , Hindustan, Amar Ujala

   English : The Times of India

   Weekly : Employment News

Digital E Resources

1. CD version of various DARE/ICAR publications
2. Annual Reports CD of year 2012-13
3. CD ROM of various training conducted at NBAIM
4. Specialized training on molecular microbiology and pathology.

ITMU

Establishment of Intellectual Property Management Unit of NBAIM marked the beginning of IPR regime primarily focusing towards management of IPR portfolio and transfer/commercialization of technologies at institute level. An attempt has been made for promotion of creation, dissemination, use and protection of intellectual property for socio-economic prosperity as one of the activities of IPR cell established in the institute. An ICAR Plan Scheme on Intellectual Property Management and Transfer/Commercialization of Agricultural Technology Scheme (Up-scaling of existing components i.e. Intellectual Property Right (IPR) under ICAR Headquarters Scheme on Management and Information Services)" is carrying out awareness, guidance and assistance facilities to scientists and researchers at the Institute in relation to IP management.

Institute Technology Management Committee:

1. Dr. A. K. Sharma, Director, NBAIMChairman
2. Dr. Sushil Sharma, Principal Scientist, NBAIM Member
3. Dr. Alok Kumar Srivastava, Senior Scientist, NBAIM Member
4. Mr. Udai B Singh, Scientist, NBAIMMember
5. Dr. A. N. Singh, Senior Scientist, DSR, Mau Member (External)
6. Dr Renu, Senior Scientist, NBAIM Member Secretary

Objectives

- Proper implementation of ICAR guidelines for IP management and technology transfer/ commercialization
- Increase awareness and enhancing literacy for IPR and capacity building
- Disclosure of IP contemplated or generated,
- Patent/IPR/prior art search,
- Patent/IPR application writing,
- Filing of applications at the concerned granting authorities,
- Pre-grant and post-grant follow up,
- Technology evaluation and screening

Microbial Genetic Resource Portal

Microbial Genetic Resource Portal (MGR Portal; *www.mgrportal.org.in*) of NBAIM was launched by Dr. R.S. Paroda, Chairman, of National Advisory Board for Management of Genetic Resources and Dr. S. Ayyappan, Co- Chairman, of National Advisory Board for Management of Genetic Resources and Secretary, DARE & Director General, *ICAR in* 4th Meeting of National Advisory Board for Management of Genetic Resources at National Bureau of Agriculturally Important Insects (NBAII), Bengaluru on 10th October 2013. The MGR portal is developed by in-house team of ARIS cell/AKMU at NBAIM

Microbial Genetic Resource Portal contains information pertaining to conservation of microbial genetic resource with special reference to agriculturally important microbes in National Agriculturally Important Microbial Culture Collection (NAIMCC) housed at National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, Uttar Pradesh. The portal is informative in various aspects of microbial conservation. It includes latest valuable information on microbial diversity with special reference to India, need for microbial genetic resources conservation and International and National status of conservation of microbial genetic resources. Latest listings on different federations, societies and networks of Microbial Resource Centers in the world, some leading culture collections in the world, some important microbial culture collections in India and microbial repositories in India recognized by National Biodiversity Authority (NBA), India are available. Besides this, the portal gives detailed insight into conservation and management of AIMs at NAIMCC, various services offered by NAIMCC, dynamic database search engine, and accessibility to catalogue, microbial registration facility, easily downloadable forms for various purposes and linkages to important sites. NBAIM also houses Microbial Genomic Resource Repository (MGRR) running in the form of project under ICAR funded networking project on Application of Microbes for Agriculture and Allied Sectors (AMAAS). MGRR maintains genetic materials like whole genome shotgun and cDNA/EST libraries, PAC/BAC/YAC clone vectors, component cells from sequencing projects, promoter DNA-fragments with reporter genes, RFLP probes specific for different microbes and expression vectors. Alongwith other details, an account on deposition and procurement of genetic material has been provided in the portal. Both ICAR and NBA have link provided to portal on their respective site.

Committees

The Research Advisory Committee and Institute Management Committee have boon reconstituted in view of the completion of term.

Research Advisory Committee

1. Dr. C. Manoharachary, Chairman, RAC, NBAIM, Emeritus Professor, Department of Botany, Osmania University, Hyderabad
2. Dr. B.N. Johri, Acharya PC Ray Fellow (MPCST), Department of Biotechnology, Barakatullah University, Bhopal 462 026 (M.P.)
3. Dr. R.C. Kuhad, Professor, Department of Microbiology, Delhi University, South Campus, Benito Juarez Marg, New Delhi 110 021.
4. Dr. D.L.N. Rao, Pr. Scientist and Coordinator, All India Network Project on Soil Biodiversity-Biofertilizer, Indian Institute of Soil Sci ence, Nabi Bagh, Berasia Road, Bhopal 462 038 (M.P.)
5. Dr. S.S. Dudeja, Department of Microbiology, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125 005, Haryana.
6. Dr. Arun Kumar Sharma, Director, NBAIM, Mau, U.P.
7. Assistant Director General (PP), ICAR, Krishi Bhavan, New Delhi 110 001.
8. Dr. Alok K. Srivastava, Senior Scientist, NBAIM, Member Secretary

Institute Management Committee

1. Director, NBAIM, Mau (Chairman)
2. Dr. Rajinder Kumar Director General Uttar Pradesh Council of Agricultural Research Lucknow, U.P.
3. Dr. R.D.Rai, Head, Division of Biochemistry, IARI, New Delhi
4. Dr. S. Kumar Head, Regional Station, ICAR Research Complex for Eastern Region, Ranchi
5. Dr. R.D. Prasad, Pr. Scientist, Division of Plant Pathology, DOR, Hyderabad
6. Dr. Chandish R. Ballal, Pr. Scientist, Division of Insect Ecology, NBAII, Bangalore.
7. ADG (PP), ICAR, New Delhi
8. The Finance & Accounts Officer Indian Institute of Sugarcane Research, Lucknow, U.P.
9. The Director Research ND University of Agriculture & Technology Kumarganj Faizabad Uttar Pradesh
10. The Joint Director (Agriculture) Government of Haryana Chandigarh
11. Administrative Officer NBAIM Kushmaur, Mau (Member Secretary)

PME Committee

1. Dr. Alok Kumar Srivastava, In-charge
2. Dr. Sushil K. Sharma, Member
3. Dr. P.L. Kashyap, Member
4. Dr. Hillol Chakdar, Member
5. Dr. N. Karthikeyan, Member

RFD Committee

1. Dr. Alok K. Srivastava, Nodal Officer
2. Dr. P.L. Kashyap, Co-Nodal Officer
3. Sh. S.N. Yadav, Member

Institute Joint Staff Council

1. Ashutosh Kumar, Secretary (Staff Side)
2. Rehan Asad Khan, Member
3. Amar Nath Singh Patel, Member
4. Anchal Kumar Srivastava, Member & Member, CJSC
5. Chetan Singh, Member
6. Ashish Kumar Singh, Member

Hindi Committee

1. Dr. Arun Kumar Sharma, Director, Chairman
2. D.P. Singh, Hindi Adhikari
3. Dipak T. Nagrale, Member
4. Lalan Sharma, Member
5. Anchal K. Srivastava, Member
6. Ashok Kumar, Member
7. Administrative Office, Member
8. Chandra Kishore Gaud, Member

NBAIM Personnel

Scientific staff

Dr. Arun Kumar Sharma - Director

Dr. Sushil Kumar Sharama- Principal scientist

Dr. Pawan Kumar Sharma- Principal Scientist

Dr. Alok K. Srivastava – Senior Scientist

Dr. D. P. Singh – Senior Scientist

Dr. Renu – Senior Scientist

Mr.Anurag Chaurasia –Scientist

Mr.Udai Bhan Singh –Scientist

Dr. P. L. Kashyap –Scientist

Dr. Lalan Sharma –Scientist

Mr. Sanjay Goswami –Scientist (on study leave)

Dr. Dipak T. Nagrale –Scientist

Dr. Hillol Chakdar –Scientist

Dr. K. Pandiyan- Scientist

Mr. N. Kartikeyan- Scientist

Technical staff

Manish Roy - Technical Assistant

Anchal Kumar Srivastava - Technical Assistant

Mahesh Yadav - Technical Assistant (Driver)

Amit Rai - Sr. Technician

Alok Upadhyay - Sr. Technician

Ashutosh Rai - Technician

Shabana Khan - Technician

Administrative, finance and other supporting staff

Ajay Kumar Soni - AO*

Samar Nath Yadav - AAO

Shyamji Shukla - Assistant

Rehan Asad Khan - Assistant

Abhishek Kumar - Assistant

Ashok Kumar - Assistant

Siddarth Arora - Jr. Stenographer

Satish Pal - Sr. Clerk

Amar Nath Singh Patel - Sr. Clerk

Manoj Kumar - Skilled Supporting Staff

Bali Ram - Skilled Supporting Staff

Chetan Singh - Skilled Supporting Staff

Rekha Gupta - Skilled Supporting Staff

Ram Gopal - Skilled Supporting Staff

Ram Avadh Singh - Skilled Supporting Staff

Chandra Kishore - Skilled Supporting Staff

Anil Kumar Rana - Skilled Supporting Staff

Ashish Kumar - Skilled Supporting Staff

Ajay Vishwakarma - Skilled Supporting Staff

Subhash Kushwaha - Skilled Supporting Staff

*(Ajay Kumar Soni, Administrative Officer, DSR, Mau with additional charge of A.O., NBAIM, Mau)

Staff transferred

Dr. Sudheer Kumar selected as Principal Scientist (Plant Pathology) and Joined Directorate of Wheat Research, Karnal, Haryana, *w.e.f.* August 2014

Staff Joined

Dr. K. Pandiyan and **Mr. N. Kartikeyan** joined as Scientist (Ag. Microbiology) after completing the FOCARS training. (joined on 10.10.2013)

Dr. Sushil Kumar Sharma joined the Bureau as Principal Scientist (Agri. Microbiology) from Directorate of Soybean research, Indore, M.P. (joined on 01.08. 2013)

Dr. Pawan K. Sharma joined the Bureau as Principal Scientist (Plant Pathology) from ICAR Research Complex, NEH, Manipur).(joined on 28.09.2013)

Photo Gallery

Dr. S. Rajendra Prasad, Project Director, DSR, Mau welcoming Dr. A. K. Sharma, Director, NBAIM, Mau by presenting a Bouquet on the occasion of Kisan Mela.

Dr. A. K. Sharma, Director, NBAIM, welcoming Sh. A. Purkayastha, Dy. Secretary, Seeds

Dr. A. K. Sharma, Director, NBAIM, addressing the farmers gathering.

Dr. P. Chakrabarty, A.D.G. (P.P.), visiting microbial culture storage facility at New Delhi.

Dr. H. Chakdar, Chief - De - Mission, NBAIM Sports team, receiving the momento

NBAIM Sports Team participation in Zonal Sports Meet held at Bhopal during September, 2013

Photo Gallery

Flag Hosting by Dr. A. K. Sharma, Director, NBAIM on Independence Day and Republic Day.

Participants during AMAAS Annual Review Meeting on 23-24 October 2014

NBAIM stall during 'Kisan Mela' at NBAIM Campus, Mau organized by DSR, Mau

Research Advisory Committee (9th RAC) meeting held at NBAIM, Mau

Institute Management Committee (IMC) at NBAIM Culture storage facility at New Delhi

QRT chairman and members visiting NBAIM culture storage facility at New Delhi

Photo Gallery

Chairman RAC and participants of CC group meeting visiting the NAIMCC

Smt. Kumud Lata Srivastava, District Magistrate, Mau inaugurating exhibition of microbial cultures on foundation day of NBAIM

Trainees working in NBAIM Laboratories