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Indian Council of Agricultural Research

NBAIM
रा.कृ.उ.सू.ब्यू.

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो
NATIONAL BUREAU OF AGRICULTURALLY IMPORTANT MICROORGANISMS
Understanding and conserving our national heritage of agriculturally important microorganisms

Kusmaur, Mau Nath Bhanjan - 275 101 (U.P.)

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TABLE OF CONTENTS

Preface

Soil microbes are essential for decomposing organic matter and recycling old plant material. Besides being important in biogeochemical cycling of nutrients, microbes play vital role in maintenance of soil fertility and in crop protection. Microbes are also being exploited as biofertilisers, and creating new nitrogen-fixing organisms. Potential of *Rhizobium*, *Azotobacter*, *Beijerinckia*, *Azospirillum*, *Cyanobacteria*, such as species of *Aulosira*, *Anabaena*, *Nostoc*, *Plectonema*, *Scytonema*, *Tolypothrix* and *Azolla* as biofertilisers has been exploited so as these could serve as an alternative to chemical fertilisers. Several microbes are being developed as suitable biopesticides for management of insect and nematodal pests.

Applications of microbes to food, energy, industry and environment are interrelated in most situations. To cite an example, degradation of urban, municipal and industrial wastes by using a suitable strain of a microorganism result into (i) disposal of pollutant, (ii) biotransformation of a waste- into a byproduct, suitable for consumption as food, and (iii) production of energy during such conversions. Several microbes have been found helpful in solution of energy crisis. Synthetic fuels produced by activity of microbes include ethanol, methane, hydrogen and hydrocarbons. Cattle dung is fermented by methanogenic bacteria to biogas, an ideal biofuel in rural areas.

Complete genome sequences of many microbial species have revealed a tremendous amount of information on the physiology and evolution of microbial species, and providing the scientific community with information on more than 300,000 predicted genes. The benefits of comparative genomics in understanding metabolic diversity, virulence and pathogenesis, and the evolution of species have been unequivocally demonstrated and the usefulness of comparative techniques provides insight for more genomes sequencing projects for AIMs.

Microbial Resource Centre serves as an essential infrastructural function for scientific investigation with the aim to collect and long term conservation of genomic resources like biomaterials, microbial DNA, clones, novel gene constructs vectors which generates an important set of scientific pay-offs and future opportunities for biological science research. Keeping in view the future perspectives of MGRs, Indian Council of Agricultural Research (ICAR) has taken a prime lead and established Genomic resource repository for microbes at NBAIM which is not only the first microbial DNA bank in India but also in South East Asia.

With great pleasure, I extend my sincere thanks to Dr. S. Ayyappan, Secretary, Department of Agricultural Research and Education and Director General, Indian Council of Agricultural Research, New Delhi for guidance and support. I am thankful to Dr. S. K. Datta, Deputy Director General (Crop Science) and Dr. T. P. Rajendran, Assistant Director General (Plant Protection) for the cooperation received in the form of encouragement, suggestions and constructive criticism for developing the document.

At the end, I put into words my gratefulness and I wish to express my heartly thanks to Prof. Dilip K. Arora, ex-founder Director for his vision, creativity, zeal and consistent efforts to develop such a wonderful institution.

Director

Executive Summary

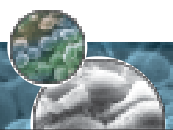
Microorganisms have adapted to inhabit almost every corner of the world. Their significance can be illustrated by the fact that the global active biomass of these usually unseen organisms is similar to that of all living plants and animals. A large part of all microorganisms lives in soils, sediments, aquifers and geological formations where they mineralize the organic compounds originating mainly from photosynthetic primary production. Microbes are important for the Earth System, playing a very important role in maintaining the wellbeing of our global environment. Despite the obvious importance of microbes, very little is known of their diversity, how many species are present in the environment, and what each individual species does i.e. its ecological function. Until recently, there were no appropriate techniques available to answer these important questions. They abound in habitats with extremes of temperature, pH and water 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. Since most have simple life cycles and can reproduce rapidly, they make ideal model organisms. Microorganisms have also gained importance as tools in the scientific world.

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 sciences and other fields as well. The various ways in which microorganisms have been used over the past 50 years to advance medical technology, human and animal health, food processing, food safety and quality, genetic engineering, environmental protection, agricultural biotechnology, and more effective treatment of agricultural and municipal wastes provide a most impressive record of achievements. Many of these technological advances would not have been possible using straightforward chemical and physical engineering methods, or if they were, they would not have been practically or economically feasible.

Nevertheless, while microbial technologies have been applied to various agricultural and environmental problems with considerable success in recent years, they have not been widely accepted by the scientific community because it is often difficult to consistently reproduce their beneficial effects. Microorganisms are effective only when they are presented with suitable and optimum conditions for metabolizing their substrates including available water, oxygen (depending on whether the microorganisms are obligate aerobes or facultative anaerobes), pH and temperature of their environment. Meanwhile, the various types of microbial cultures and inoculants available in the market today have rapidly increased because of these new technologies. Significant achievements are being made in systems where technical guidance is coordinated with the marketing of microbial products. Since microorganisms are useful in eliminating problems associated with the use of chemical fertilizers and pesticides, they are now widely applied in nature farming and organic agriculture.

Microbiology has experienced a transformation during the last 25 years that has altered microbiologists' view of microorganisms and how to study them. The realization that most microorganisms cannot be grown readily in pure culture forced microbiologists to question their belief that the microbial world had been conquered.

In India, till now very limited and isolated efforts were made for tapping of microbial diversity of Agriculturally Important Microorganism, their identification and preservation for different applications in agriculture and food sectors. The importance of microbial diversity in India was also



realized, as a result of which, the Indian Council of Agricultural Research established National Bureau of Agriculturally Important Microorganisms (NBAIM). NBAIM is a constituent organization under the aegis of Indian Council of Agricultural Research (ICAR). The Bureau started functioning from July, 2001 with one Officer-on-Special Duty (OSD) as Officiating Director and one assistant Finance & Accounts Officer as regular staff. However, the bureau made a modest beginning with the appointment of one Assistant Administrative Officer and few contractual staff. A regular Director for this Bureau was selected and joined NBAIM, New Delhi on 29th April, 2002. The Bureau was shifted to Mau Nath Bhanjan, Uttar Pradesh on 1st June, 2004. Initially the SFC agreed for 15 scientific posts through redeployment and 13 technical, six administrative and 8 supporting staff were agreed and approved in the IX Plan period for creation. In the X plan, three senior scientists, two scientists and one scientist (Sr. Scale) were redeployed. One scientist joined through ASRB selection. During XIth plan period 5 senior scientists and 6 scientists joined the Bureau, however, two scientist retired and three senior scientists were transferred to other institutes. At present the Institute has twelve scientific and seven technical staff against 30 approved positions for the scientists.

The mandate of the NBAIM is "To act as the nodal Institute at national level for acquisition and management of indigenous and exotic microbial genetic resources for food and agriculture, and to carry out related research and human resource development, for sustainable growth of agriculture".

NBAIM functions under the administrative control of the Crop Sciences Division of the ICAR. The Bureau is headed by the Director, who draws guidelines from the Crop Sciences Division, Bureau's Management Committee, Research Advisory Committee and Germplasm Advisory Committee. The Bureau has four divisions viz. "Microbial Conservation", "Microbiology", "Microbial Biotechnology" and "Microbial Isolation and Preservation". Microbial conservation division has the objectives to plan short-term and long-term conservation of different types of AIMs including the conservation of obligate parasites. Microbiology division has the objectives to identify, characterize and document the AIMs. This division also performs the work related to identification and utilization such as bio-fertilizers, bio-pesticides, growth promotion, bio-indicator, bio-degradation, bio-remediation, bio-composting, food processing etc. Microbial Biotechnology division will be vested with molecular characterization of AIMs (based on prioritization with expansion of IPR regimes), development of molecular markers and diagnostic tools. Microbial Isolation and Preservation division has the objective of isolation, characterization and preservation, and collection of exotic AIMs from different agro-climatic zones. Besides the above, the Bureau has HRD component under which training programs are organized for the researchers in the field of molecular identification of AIMs, technology development and its implementation. In addition, the Bureau has five cells namely Agriculture Research Information System (ARIS), Technical Cell, Culture Collection Cell, Planning Monitoring and Coordination Cell and Hindi Cell.

The Bureau is one of its kind, not only in India but also in Asia as it is set up to focus on the identification, characterization, conservation and preservation of microbial diversity of AIMs. After the year 2002 this newly established Bureau has gained momentum in organizing its mandated activities mainly in areas of microbial exploration, evaluation, conservation, preservation and surveillance of indigenous/ exotic AIMs.

NBAIM at Mau is having various buildings like administration and finance office, guest house, laboratories, hostel, residential quarters etc. for the need of the scientists and staff. A new laboratory wing was added to the Bureau in 2005-06 consisting state of the art facilities for biochemical and molecular diagnostics. The hostel facility at the Bureau is unique and has 118 rooms to house the Research fellows and the trainees.

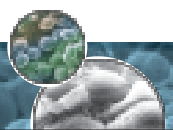
A landmark development was the establishment of the National Agriculturally Important Microorganisms Culture Collection (NAIMCC) in the year 2004. The NAIMCC consists of storage facility of 10000 AIMs. From 2001 to 2012 various facilities like PCR, Gel electrophoresis unit, Gel Documentation system, Lyophilizer, Microscopes, fermentor, Capillary DNA Sequencer, Biolog identification system, TGGE apparatus, Gas chromatograph, Automatic N-analyzer, Ultracentrifuge, High speed centrifuge, Sonicator, Freeze drier, Bead beater, ELISA reader with washer, water purification system, PCR four block, RT-PCR, Next Generation Pyrosequencer, Bioanlyzer, High Throughput

electrophoresis systems, robotic DNA Extractor, Scanning Electron Microscopes, Confocal laser microscope, FAME analyzer, etc. were added through different research projects funded by ICAR, DBT, DST, NATP and NAIP. The Bureau has HRD component in which training programs are organized as per the mandated activities of NBAIM. NBAIM is "Nodal Centre" for the registration of AIMs. The internet facility through VSAT was established at the Bureau in the year 2006-07. The website (www.nabim.org.in) of NBAIM was created and all the units of the NBAIM are linked with various ICAR research institutes.

The NBAIM foresees the following essential activities to be carried out in near future:

- Exploration and collection of AIMs from Soil, plants, fresh water etc. covering different agro-climatic regions.
- Isolation of extremophiles from exotic zones and extreme environments
- Collection of AIMs from existing culture collections
- Augmentation of cultures through exchange from various national MRCs
- To develop integrated electronic databases that includes habitat, geographic, phenotypic, genotypic, morphological, and accession information.
- To decipher the functional and structural genomics of a few AIMs for their better exploitation in agriculture and allied sectors
- To strengthen the state of the art microbial gene bank
- To strengthen the linkages with different Microbial Resource Centres (MRCs).

The ongoing research programmes of the Bureau have been reoriented by the ICAR in the light of the thrust areas and priorities identified by bringing all the activities in a network project entitled "Application of Microorganisms in Agriculture and Allied Sectors" funded by ICAR. Six thematic areas are identified: Microbial Diversity and Identification; Nutrient Management, PGPR and Biocontrol; Agrowaste Management, Bioremediation and Microbes in Post Harvest Technology; Microbial Management of Abiotic Stress; Microbial Genomics and Human Resource Development. In a near future, the NBAIM will be one of the India's largest holders of microbial germplasm and a readily available source of AIMs for the researchers and industry. The "National Gene Bank" facility could also be offered on regional scale to store germplasm of AIMs of South Asian countries. NBAIM would assume leadership to train the specialized manpower in the area of R&D activities at regional, national and international levels.



कार्यकारी सारांश

सूक्ष्मजीवों ने दुनियाँ के हर कोने में खुद को रहने के अनुकूल बना लिया है। उनकी उपयोगिता इन तथ्यों से बताई जा सकती है कि इनका विश्वव्यापी सक्रिय बायोमास, जिन्हें देखा नहीं जा सकता, को सभी जीवित पौधों और जन्तुओं के समान हैं। एक सूक्ष्मजीवों का बड़ा भाग जो मिट्टी, सेडीमैंट्स, एक्विफर और भौगोलिक निर्माणों में रहते हैं, जहॉ वो कार्बनिक योगिकों का अपघटन करते हैं, जो मुख्यतः प्रकाश संश्लेशित मूल उत्पादन से उत्पन्न होते हैं। सूक्ष्मजीव पृथ्वी निकाय के बहुत महत्वपूर्ण हैं। विश्वव्यापक पर्यावरण को बनाये रखने में एक बहुत महत्वपूर्ण भूमिका निभाते हैं। इन महत्वों के बावजूद उनकी विविधता के बारे में थोड़ा ही ज्ञान है। पर्यावरण में उनकी कितनी प्रजातियाँ हैं और व्यक्तिगत प्रजाति क्या करती है अर्थात् उनकी पारिस्थितिकी उपयोगिता क्या है, ज्ञात नहीं है। अब तक कोई उपयुक्त तकनीकी नहीं उपलब्ध है जो इन प्रश्नों का उपयुक्त जबाव दे। वे परम तापमान, पी0एच0 और लवण, जल दबाव परिस्थितियों में भी भरपूर संख्या में रहते हैं। गहन ताप जलवायु की पहचान, सूक्ष्मजीवों के संकलन, पोषकीय गतिकी, बहुमुखी और जीवन को बनाये रखने में उनकी बहुत महत्ता है। उनका सामान्य जीवन-चक्र और तीव्र प्रजनन-चक्र उन्हें आदर्श मानक जीव बनाते हैं। सूक्ष्मजीव वैज्ञानिक जगत में एक हथियार की तरह माने गये हैं।

सूक्ष्मजीवों की विलक्षणता और प्रायः अनुमानित न करने वाला स्वभाव और जैविक सामर्थ्य एक विशेष पर्यावरणीय वर्ग उन्हें जीव विज्ञान में एक उम्मीदवार बनाता है जो विशेष कठिनाइयों और समस्याओं को दूर करता है। विभिन्न रूपों में सूक्ष्मजीवों का उपयोग पिछले पचास सालों से नवीन चिकित्साशिल्प विज्ञान, मानव और जन्तु स्वास्थ्य, आहार प्रक्रिया, आहार सुरक्षा और विशेषता, अनुवांशिकी-अभियान्त्रिकी, पर्यावरण संरक्षा, कृषि जीव विज्ञान, अत्यधिक असरदार उपचार, एक अत्यधिक प्रभावशाली उपलब्धि दर्शाते हैं। इनमें से नई तकनीकी तब तक सम्भव नहीं है जब तक उन्हें प्रायोगिक रूप से संचालित न किया जाए। यद्यपि सूक्ष्मजीव तकनीकी का प्रयोग विभिन्न कृषि और पर्यावरणीय समस्याओं में किया जाता रहा है लेकिन इसे वैज्ञानिक वर्ग द्वारा स्वीकार नहीं किया गया है क्योंकि उनकी उपयोगिता को बार-बार दर्शाना कठिन है। सूक्ष्मजीव अपने अनुकूलतम अवस्था में सक्रिय रहते हैं और अपने स्रोत को अपघटित करते हैं। विभिन्न तरह के सूक्ष्मजीव बाजार में उपलब्ध रहते हैं और प्रायः उनकी उपलब्धता नई तकनीकी के कारण बढ़ रही है। सार्थक उपलब्धि प्रणाली में बनी है जहॉ तकनीकी मार्ग-दर्शन को बाजार के सूक्ष्मजीव उत्पादन से जोड़ा गया है। यद्यपि सूक्ष्मजीवों का उपयोग समस्याओं के निस्तारण में होता है अगर हम उनका प्रयोग रासायनिक खाद और कीट-नाशकों के साथ करें। उनका उपयोग अब बड़े स्तर पर कृषि क्षेत्र में हो रहा है।

पिछले 25 सालों में सूक्ष्मजीव विज्ञान के अध्ययन में क्रान्तिकारी बदलाव आये हैं तथा वैज्ञानिक अब यह मानते हैं कि सूक्ष्मजीवों के अध्ययन की प्रचलित विधियाँ उनके संसार को पूर्णरूप से जानने में असमर्थ है। भारत में कृषि उपयोगी सूक्ष्मजीवों की पहचान तथा उनका संरक्षण करके कृषि और खाद्य क्षेत्र में उपयोग करने के उद्देश्य से सूक्ष्मजैविक विविधता को जानने के बहुत ही सीमित तथा खण्डित प्रयास किये हैं। भारत में सूक्ष्मजीवों की अपार विविधता तथा उसके महत्व को ध्यान में रखकर भारतीय कृषि अनुसंधान परिषद् ने कृषि उपयोगी सूक्ष्मजीव ब्यूरो की स्थापना की। ब्यूरो ने जुलाई 2001 से कार्यकारी निदेशक की देखरेख में कार्य करना शुरू किया। अप्रैल, 2002 से पूर्णकालिक निदेशक मिलने के बाद 01 जून, 2004 को ब्यूरो को दिल्ली से मऊनाथ भंजन (उ0प्र0) में पुनः स्थापित किया गया। नौवीं पंचवर्षीय योजना में ब्यूरो के लिए 15 वैज्ञानिक, 13 तकनीकी सहायक, 06 प्रशासनिक तथा 08 सहायकों के पदों की संस्तुति की गयी। दसवीं पंचवर्षीय योजना में तीन वरिष्ठ वैज्ञानिक, 02 वैज्ञानिक तथा 01 वैज्ञानिक (सीनियर स्केल) का स्थानान्तरण ब्यूरो में किया गया। एक वैज्ञानिक की नियुक्ति कृषि वैज्ञानिक चयन बोर्ड द्वारा की गयी। 11वीं पंचवर्षीय योजना में 05 वरिष्ठ वैज्ञानिकों तथा 06 वैज्ञानिकों ने ब्यूरो में पदभार ग्रहण किया तथा इसी के साथ-साथ दो वैज्ञानिक सेवा निवृत्त हुए तथा तीन वरिष्ठ वैज्ञानिकों का दूसरे संस्थानों में स्थानान्तरण भी हुआ। वर्तमान समय में ब्यूरो में 12 वैज्ञानिक, 11 तकनीकी सहायक कार्यरत हैं जो कि स्वीकृत 30 पदों से काफी कम हैं।

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो का कार्य-अधिपत्र

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

ब्यूरो, भारतीय कृषि अनुसंधान परिषद् के फसल विज्ञान विभाग की प्रशासनिक देख-रेख में कार्य करता है। ब्यूरो में मुख्यतः चार विभाग हैं- सूक्ष्मजैविक संरक्षण, सूक्ष्मजैविकी, सूक्ष्मजैविक प्रौद्योगिकी और सूक्ष्मजैविक पृथक्करण तथा परिरक्षण। कृषि उपयोगी सूक्ष्मजीवों का संरक्षण लम्बे समय तक करना, सूक्ष्मजैविक संरक्षण विभाग का

दायित्व है। सूक्ष्मजैविकी विभाग का कार्य सूक्ष्मजीवों की पहचान करने तथा उनसे संबंधित सभी प्रकार के तथ्यों को रखना है तथा इसके साथ-साथ सूक्ष्मजीवों का उपयोग जैव उर्वरकों, जैव कीटनाशकों, फसल सहायकों, जैव कम्पोस्ट में करने के लिए उनकी पहचान भी करता है। कृषि उपयोगी सूक्ष्मजीवों के आण्विक गुणों की पहचान (प्राथमिकता तथा बौद्धिक सम्पदा के आधार पर) करना तथा ऐसी प्रौद्योगिकी का विकास करना जो आण्विक गुणों पर आधारित हो। ब्यूरो भारत ही नहीं अपितु एशिया भर में अपने प्रकार का अकेला संस्थान है क्योंकि इसे सूक्ष्मजैविक विविधता की पहचान, संरक्षण, गुण-चिहनंकन और परिरक्षण पर ध्यान केन्द्रित करने के लिए स्थापित किया गया है। वर्ष 2002 के बाद इन नवनिर्मित ब्यूरो ने अपनी अनिवार्य गतिविधियों मुख्यतः सूक्ष्मजीवों का अन्वेशण, मूल्यांकन, संरक्षण, परिरक्षण और राष्ट्रीय एवं अन्तर्राष्ट्रीय कृषि उपयोगी सूक्ष्मजीवों की निगरानी के कार्य को संयोजित करने में अप्रत्याशित गति हासिल की है।

मऊ में स्थित कृषि उपयोगी सूक्ष्मजीव ब्यूरो का यह नया परिसर विभिन्न प्रकार के भवन जैसे प्रशासनिक एवं वित्त विभाग, अतिथि-गृह, प्रयोगशाला, छात्रावास इत्यादि एवं वैज्ञानिक तथा कर्मचारियों की आवश्यकतानुसार आवास से युक्त है।

जैव-रासायनिक एवं आण्विक निदान के लिए वर्ष 2005-06 में अत्याधुनिक सुविधाओं से युक्त एक प्रयोगशाला को ब्यूरो में स्थापित किया गया। ब्यूरो में अनुसंधान कर्मियो एवं प्रशिक्षुओं के लिए 118 कमरों से युक्त अद्वितीय छात्रावास की सुविधा उपलब्ध है।

वर्ष 2004 में ब्यूरो ने एतिहासिक राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव संग्रहण केन्द्र की स्थापना की। रा0कृ0उ0सू0संग्रहण केन्द्र 10,000 (दस हजार) कृषि उपयोगी सूक्ष्मजीवों के भण्डारण की क्षमता रखता है। वर्ष 2000-2012 तक विभिन्न वित्तपोषित अनुसंधान परियोजनाएँ जैसे ICAR, DBT, DST, NATP एवं NAIP द्वारा विभिन्न सुविधाएँ जैसे पी0सी0आर0, जैल-वैद्युत कण संचलन इकाई, जैल-प्रलेखन इकाई, लाइफोलाइज़र, सूक्ष्मदर्शी किण्डवक, कोशिका डी0एन0ए0 अनुक्रमक, वायोलॉग पहचान प्रणाली, टीजीजीई उपकरण, गैस-क्रोमेटोग्राफ, स्वचालित डी0एन0ए0 विश्लेषक, अतिअपकेन्द्रित मशीन, उच्चगति अपकेन्द्रित मशीन, सोनीकेटर फ्रीजरड्रायर, गुरिया इटक यंत्र, एलिसा रीडर, जल-शोधन प्रणाली, पीसीआर चार ब्लाक, आर टी पीसीआर अगली पीढ़ी, अग्निछाया अनुक्रमक, जैव-विश्लेषक, उच्च-कालबद्ध निर्गम वैद्युतकण संचलन प्रणाली, रोबोट डी0एन0ए0 उद्धरणक, स्कैनिग इलैक्ट्रॉन माइक्रोस्कोप, कनफोकल लेजर सूक्ष्मदर्शी, फेम-विश्लेषक आदि को ब्यूरो में जोड़ा गया है। यह ब्यूरो मानव विकास संसाधन का घटक जिसके अन्तर्गत ब्यूरो की अनिवार्य गतिविधियों के तहत प्रशिक्षण कार्यक्रम आयोजित किये जाते हैं। रा0कृ0उ0सू0 ब्यूरो में कृषि उपयोगी सूक्ष्मजीवों के पंजीकरण हेतु नोडल केन्द्र का कार्य करता है। ब्यूरो ने वी-सैट के माध्यम से वर्श 2006-07 में इंटरनेट की सुविधा स्थापित की। ब्यूरो ने र0कृ0उ0सू0ब्यूरो को वेवसाइट www.nbaim.org.in को स्थापित किया जिससे रा0कृ0उ0सू0ब्यूरो की सभी इकाईयॉ तथा भारतीय कृषि अनुसंधान परिषद के विभिन्न संस्थान जुड़े हैं।

राष्ट्रीय कृषि उपयोगी सूक्ष्मजीव ब्यूरो द्वारा निम्नलिखित आवश्यक गतिविधियों को भविष्य में किये जाने की उम्मीद हैः

1. अन्तर्राष्ट्रीय क्षेत्र और अतिवादी परिवेश से अतिवादसह जीवाणुओं का पृथक्करण,
2. विविध कृषि जल-वायवीय क्षेत्रों से मृदा, वनस्पति, जल इत्यादि से कृशि उपयोगी सूक्ष्मजीवों का संग्रहण करना।
   I) मौजूदा बर्धन संग्रहण केन्द्रों से कृषि उपयोगी सूक्ष्मजीवों का संग्रहण करना
   II) पपद्ध विभिन्न राष्ट्रीय MRCs से आदान-प्रदान के माध्यम से सूक्ष्मवर्धकों की वृद्धि।
3. एकीकृत इलैक्ट्रनिक डाटावेस विकसित करना जिसमें वास, भौगोलिक प्रारूपिक, जीनोटिपिक, आर्कटिक और परिग्रहण जानकारी भामिल हो।
4. कृषि उपयोगी सूक्ष्मजीवों का कृषि एवं संरचनात्मक जीन विज्ञान का अध्ययन
5. सूक्ष्मजीव जीन बैंक की स्थिति सुदृढ़ करने के लिए प्राविधिक तरीके विकसित करना।
6. विभिन्न सूक्ष्मजीव संसाधन केन्द्रों के साथ सम्पर्क सुदृढ़ करना।

Infrastructure

Indian Council of Agricultural Research established National Bureau of Agriculturally Important Microorganisms (NBAIM) for exploration, evaluation and conservation of agriculturally important microorganisms (AIMs). The Bureau aims to excel in isolation and utilization of genes for conventional and unforeseen products of high economics and value in environment and agriculture. It is expected that NBAIM continues to fulfil its mandate to make Indian agriculture locally, regionally and globally competitive.

The bureau has well-equipped research laboratories, Central instrumentation facility, separate Fungal and Bacterial labs, Molecular Biology lab, Genomics unit with ultramodern instrumentation, Lyophilization unit, Culture collection facility, including cyanobacterial culture unit, newly developed Microbial Genome Resource Repository (MGRR), administration block, scientists' lobby, library, conference hall and miniconference rooms with state-of-the-art audio-visual equipments and Agricultural Research Information Service (ARIS) cell, etc. The campus as well as laboratories has been put under the electronic surveillance system.

To ensure regular water and electricity supply, tube-wells facilities and power generators have been installed within the campus. Electricity supply is being substantially enhanced and provided with new high-power DG sets to run the controlled working environment in the laboratories.

The administrative, finance and Director's personal section are equipped with all modern equipments like computers with internet facility, printers (black & white as well as coloured), photocopier machines, colour xerox machines, lamination machine, poster printing machine, fax machine etc.

National Agricultural Important Microbial Culture Collection (NAIMCC)

The NAIMCC was created with the inception of Bureau as a mandated activity for characterization and long term conservation of agriculturally important microorganism. It was initiated with mineral and glycerol storage, further enhanced its capability to lyophilization and now it has all the conservation facility including cryogenic storage under ultra low temperature (-196°C). NAIMCC is well equipped will microbial imaging systems like light microscopy, scanning electron microscopy and laser confocal microscopy. The NAIMCC has state of art facility for isolation, conservation, maintenance and storage facility for nearly 100000 microbial holdings.

The bureau follows strict quality control and biosafety standards in the culture collection as well as in laboratories. Various types of microorganism including filamentous fungi, bacteria, actinomycetes and yeasts are maintained under the long-term preservation. Each culture is preserved by at least two methods according to the type of microorganism. At present NAIMCC has a collection of more than 4500 microbial cultures. It has published its first catalogue of strains in 2009 and second edition in 2011. The relevant information regarding cultures like source of isolation, place of isolation, growth conditions, depositor and year of deposit are also given with each accession. In addition to that catalogue also has composition of different culture media, deposition forms, long term storage protocols and information for registration of microbial cultures.

NAIMCC have been digitized and put-up all the information about cultures in a database in retrievable format. The software is on MySQL database management system. A variety of data can be accommodated in fields like information on passport data of a culture like its geographical location of isolation , name of the donor (person or Institute), name of the depositor, cultural details, the form in which it is preserved, etc. It has space for images, maintains inventory for lyophylization,

generates Bar codes, reminder for revival time of culture, etc. The format is organized to permit rapid searches and to facilitate communication between database and user.

Biodiversity Authority of India recognized the NAIMCC as the National Repository for microbial germplasm. 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.

Research facilities

The bureau has different well-equipped research laboratories, Central instrumentation facility, separate Fungal and Bacterial labs, Molecular Biology lab, Genomics unit with ultramodern instrumentation. All the laboratories are equipped with most modern instruments required to carry-out research work in the molecular biology and microbial biotechnology. To name a few are

- Scanning Electron Microscope (SEM)
- Confocal Laser Microscope
- Automated Lyophilization Unit
- Liquid Nitrogen Plant and Cryopreservators
- FLX454 pyroseqencing unit with hydroshear, emulsion PCR and cluster analysis
- 16 capillary ABI 3130*xl* automated genetic analyser
- Mini high performance computing system with bioinformatic softwares
- Workstations for data annotation and analysis
- Real Time PCR
- Chemiluminiscence gel imaging system
- Robotic liquid handing system
- DNA bar-coding system
- Pulse field gel electrophoresis
- DGGE unit
- Chip based high throughput electrophoresis
- 2D gel eletrophoresis
- Metabolic growth kinetic analyzer (Bioscreen)
- Fatty acid methyl ester (FAME) analyzer
- High performance liquid chromatography (HPLC)
- Gas liquid chromatography (GLC)
- Thermocyclers
- Atomic absorption spectrophotometer
- BIOLOG microbial identification system
- RT-PCR
- Automated Media Preparator
- Spectrophotometer
- Ultra centrifuge
- Refrigerated incubator shaker
- Deep freezers (-80°C)
- Walk-in cold rooms
- Lyophilization
- Glass house

Library and Agricultural Research Information System (ARIS) CELL

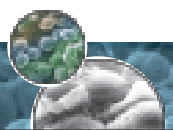
The NBAIM library has subscribed many periodicals related to microbial research and also has internet facilities. It has collections of fourteen scientific journals/periodicals and a number of books belonging to various subjects like bacteriology, biochemistry, bioinformatics, bioinstrumentation, biotechnology, botany, environmental sciences, integrated pest management, microbiology, molecular biology, phycology, plant pathology, virus, mycology, genetics, genomics, administration and miscellaneous literature. The library has facility to access many national and international journals through Consortium of e-Resources in Agriculture (CERA).

IPR and Bio-safety Cell at NBAIM

NBAIM has established an IPR cell for the management of intellectual knowledge and technologies generated at NBAIM, which 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 as described by them, is also being applied as guidelines for the Bureau.

NBAIM website

The new website of NBAIM (www.nbaim.org.in) designed based on the ICAR guidelines for uniformity of website, contents 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.

National Genomic Resource Repository

Microbial Genomic Resource Repository (MGRR) has aims to collect 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 utilize for further research in agriculture in many ways like, for the increment of the soil fertility, crop production, crop quality and their resistance to diseases. MGRR will provide all those genetic material to the researchers/scientists, working in the field of molecular biology and microbial genomics. MGRR is maintaining the genes responsible for nitrogen fixation, nitrogen assimilation, root nodulation, bioremediation, phosphate solubilization, disease resistance, salt tolerance, stress resistance and biocontrol, etc. could be exploited to enhance crop productivity. MGRR DNA Bank has developed its guidelines for submission and distribution of the genetic resources under appropriate material transfer agreement (MTA)

Major thrusts at NBAIM

The unique genetic and biosynthetic capabilities of microorganisms has made them likely candidates for solving particularly difficult problems in the agriculture. For many years, soil micro-biologists and microbial ecologists have tended to differentiate soil microorganisms as beneficial or harmful according to their functions and how they affect soil quality, plant growth and yield, and plant health. Despite the well recognized importance of microorganisms, only less than 5% of the world's microorganisms are on record. In India, very limited and isolated efforts were made for tapping of microbial diversity of AIMs, their identification and preservation for different applications in agriculture and food sectors. The importance of microbial diversity in India was also realized, as a result of which, the Indian Council of Agricultural Research established National Bureau of Agriculturally Important Microorganisms (NBAIM) for exploration, evaluation and conservation of agriculturally important microorganisms (AIMs). The Bureau aims to excel in isolation and utilization of genes for conventional and unforeseen products of high economics and value in environment and agriculture.

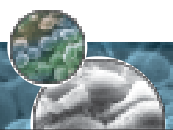
The NBAIM since its developmental phase has played an important role in isolation, preservation and characterization of AIMs, diversification of agriculture in India through exploration of agriculturally important microorganisms, identification, characterization and documentation of AIMs, conservation, maintenance and utilization of AIMs, microbial biodiversity and systematics, Human Resource Development. From being an institute primarily concerned with microorganisms today NBAIM plays a key role in the overall management of microbes in India. The Bureau is one of its kinds not only in India but whole in South East Asia as it focuses on the conservation and preservation of microbial diversity of agriculturally important micro-organisms. It is expected that NBAIM continues to fulfill its mandate to make Indian agriculture locally, regionally and globally competitive.

Isolation and Identification of AIMs

- The national priority of isolation, identification and utilization of AIMs in the processes of biofertilization, bioprocessing and bio-remediation or addressing the pathogens causing either diseases or spoilage in agri-products has been attended in the ongoing agricultural research and education programmes in the NARS to quite an extent. However, the absence of a national mechanism to maintain and document the promising AIMs isolates has been an important concern since a large number of isolates resulting from previous studies in the country may not be available for posterity.
- A mechanism may be developed for sending and receiving referral samples of AIMs for maintenance, cataloguing and facilitated access for use in public interest.
- Need of this hour is to facilitate the activites pertaining to exploration, characterization, evaluation, maintenance, conservation and documentation of various categories of microbes important to agriculture/animal science/ fisheries in the national system.

Diversity of exotic AIMs in different ecological niches

- Modern technologies, in the field of diagnostic of AIMs are valuable tools in the process of planning and management of microbial diversity, allowing the incorporation of multiple criteria for a better use of AIMs. This is particularly important in developing countries like India, where ecological imbalance has reached to alarming proportion.
- Conservation and characterization the variable AIMs for its optimum utilization by the coming generations is essentially required. A better understanding of microbial diversity promises to



provide an array of new products and processes as well as a better awareness of the microbial biosphere the earth's life support system. The understanding of microbial diversity will be a critical aspect of future agricultural since microbial diversity is the basis for emergence of plant diseases and so the control of their productivity, as well as providing new ways to identify products of microbial origin.

- The microorganisms present in the diversified agro-ecosystems of India will also provide a valuable source of novel bioactive compounds.
- NBAIM will pool all the available resources and upgrade the facilities to meet the current and future requirement for the conservation and characterization of AIMs in the country. The characterization of microbes is of paramount important, not only from the point of view of protecting the important gene-pool resource, but also for supporting integrated pest and disease management programmes. The identification of indigenous species, strains, races and types of microorganisms would also help in identifying and developing suitable biocontrol agents, which in the present and coming century, will be the main armory for the eco-friendly management of biotic stresses. The Bureau will provide good opportunities for isolating and utilizing genes for conventional and unforeseen products of high economic, environmental and agricultural values. The effort will greatly strengthen the national capability in quarantine and other regulatory matters. The Bureau will also perform an important function of depository of AIM, which will facilitate the process of registration and patenting. Above all, the Bureau will help in understanding and conserving our national heritage of microorganisms, not well understood and conserved so far.

Biosystematics of AIMs

- The discipline of "Microbial Taxonomy and Biosystematics" has been losing ground in universities/ research institutes and agricultural universities, though its importance has increased with the changing IPR scenario. There is an urgent need to revive it and it is in this context, work on "Microbial Taxonomy", "Bio-systematics" and "Evolution" has become more relevant.
- Due to enormity of biodiversity of AIMs in the country, the existing facilities are far from adequate, as a result of which many scientists send their collections outside the country for identification, and in the process, the country looses valuable gene pool resource and also foreign exchange for the services which can be easily provided in India with required infrastructure.
- Biosystematics of microbial isolates of Indian origin is urgently needed. NBAIM is the only national body which can take lead in this matter and scientists and researchers from all over the country could get identification and diagnostics of AIMs.
- Development of National Culture Collection Centre as per Budapest Treaty with the state-of-the-art Facilities for identification and taxonomic studies of Agriculturally Important Microorganisms. This is utmost important, as at present no Centre/Institute/University in India is providing the services of identification of AIMs to the scientists engaged in agriculture, pure sciences and industry. NBAIM may act as a nodal centre for developing a National Facility for the Identification of AIMs.

Exploration and Survey

- NBAIM will act as a nodal agency, responsible for taking appropriate measures for system wide management of AIMs e.g. (i) constituting microbial genetic resource advisory committees, (ii) preparing national exploration maps, (iii) developing and widely disseminating guidelines for handling and storage of microbial isolates, (iv) registration and notification of microbial deposits, (v) developing/implementing coordination, linkage and cooperation mechanisms, (vi) technical backstopping for the development of national policy and its implementation, and (vii) handling matters/ concerns related to biosafety, biopiracy and IPR issues, etc.

Enhancing the productivity of crop plants:

Following activities would be performed at NBAIM to enhance the production of crop plants:

a) Development of biofertilizers, microbial bioinsecticides, bioherbicides, biofungicides; biocontrol agents for enhancing crop yields.

b) Identification of microbes involved in nutrient cycling.

c) Deciphering the role of marine micoflora in agriculture. Salt tolerant strains can be a source of novel genes that can provide tolerance to crop plants.

d) To enhance scientific understanding/ development and application of use of various microorganisms, strains and isolates both in terms of quantity and quality.

e) To improve technology for isolation, purification, rapid multiplication and production of various productivity enhancing microorganisms in agriculture and allied sector.

f) To build/enrich and maintain/strengthen biodiversity of microorganisms important to agriculture, including a national repository system for various soil, aqua, plant, animal, fish and post harvest/industrial processing related microbes.

g) To undertake Human Resource Development (HRD) and training for trainers and scientific/technical personnel for evolving technologies in relation to research, management and application of microorganisms, and for addressing the issues of biosafety, management of intellectual property, facilitated access, and equitable benefit sharing.

Utilization of AIMs

- Deciphering functional diversity of bacteria with respect to abiotic stresses (soil salinity, drought, temperature) for use in agriculture.
- Microbial strategy for improving crop nutrition
  - Developing BGA biofertilizers technology
  - Use of asymbiotic nitrogen fixers
  - Use of mycorrhizal fungi
  - Use of plant growth promoting rhizobacteria (PGPR) e.g. *Pseudomonas fluorescens, P. stutzeri, P. aerouginosa, Bacillus subtilis, Bradyrhizobium japonicum*
- Microbial management in horticulture
  - Microbial analysis of biodynamic preparations, biodynamic compost and microbial liquid manures
  - Use of AM-based bio-fertilizers and bio-preparations
- Plant Growth Promotiing Microorganisms (PGPM)
  - Use of *Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* for disease control
  - Use of entomopathogenic fungi for control of insect pests
  - As growth promoters
  - For induced systemic resistance
  - As detoxificants of xenobiotics
  - As nutrient solubilizer and mobilizer
- Microbes for agro-waste management/ composting
  - Use of microorganism that can hasten the process of composting
  - *Trichurus spiralis, Paeciliomyces fusisporus, Trichoderma viride* and *Aspergillus* sp. are used as compost accelerator after supplementation of rock phosphate and urea to narrow down the C: P and C: N ratio of substrates technology developed to prepare microbial enriched compost
  - Use of mesophilic/ thermophilic cultures for agricultural residue management
  - Use of *Bacteriovorous* nematodes *Cephalobus persegnis, Mesorhabditis cranganorensis* and *Panagrolaimus* sp. for aerobic composting
  - Large scale microbial-based vermi-composting of coconut wastes using a local species of epigeic earthworm (*Eudrilus* sp.)

Research Achievements
Institute Projects

Project 1 : Characterization of beneficialrhizobacteria and its role in induced systemic resistance (ISR) and horizontal resistance in plants

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

Rationale

The interspecies relationships in different group of beneficial rhizobacteria need further definition. This may be understandable, considering the fact that many geno-species are very similar phenotypically and therefore, are not readily distinguishable. Sequencing of housekeeping genes in general and protein-encoding genes in particular and sequencing of 16S rRNA gene have been used in the identification of species or strains and delineation of phylogenetic relationships among different strains and species. Compared to 16S rRNA genes, housekeeping genes provide a higher degree of resolution as the latter genes evolve faster than the former. It may not therefore be surprising that various protein-encoding genes, including *recA*, *rpoB*, *rpoD*, and *gyrB*, have been used for the classification of several unrelated bacteria at the intrageneric level. Therefore, the present approach will lead to generate reliable information regarding the phylogenetic relationship of various beneficial rhizobacteria.

The present project was started with the broad objectives for the year "Selection of potential rhizobacterial strains (rhizobia); Extraction of total genomic DNA andPCR amplification of 16S rDNA and 16-23S IGS rDNA; Amplification of 16-23S rDNA; *rpo*B amplification; gyrB amplification; rpoD amplification".

Objectives

- Molecular identification of bacterial community structure in the rhizosphere of higher plants through sequence information from specific groups of genes.
- Decipher the genetic relatedness among members of the rhizospheric bacterial community through Multilocus Sequence Typing of rhizobacteria with following genes:
- 16S rRNA gene, *rpo*B, *rpo*D, *gyr*B, *atp*D, *gac*A, and *nif*H
- Use of rhizobacteria to induce and record the expression of multigenic resistance, higher consititutive levels of specific isozymes of hydrolytic and/or antioxidant enzymes.

Significant Achievements

- Total 64 nodules were collected from 5 different locations (Faizabad, Ayodhya, Maunath Bhanjan). The rhizobia were isolated from the nodules collected on YMA medium and the developing colonies were purified, subcultured, characterized and maintained in slants.
- Morphologically isolates were rhizoid and mucilaginous, small and whitish colonies were observed. All the isolates were found gram –ve.
- The isolates were also tested for antibiotic resitance against six different antibiotics including Kanamycin, Streptomycin, Choloramphenicol, Ampicillin, Tetracyclin and Rifampcin. The tolerance limit varied from 5 to 110µg/ml concentration. The carbon substrate utilization profiling was generated using BIOLOG and phyllogentic tree was constructed on the basis of substrate utilization pattern using UPGMA.
- The primers were designed for further molecular caharcterization of the isolates based on multilocus sequence analysis. Further work to validate the primers in wet lab is in progress.

Table. Locations of sampling and physicochemical properties of the soil

Location	Nodule	Soil Type	Soil pH	Soil EC
Raunahi	10	Sand	7.70	0.721
Agathuwa	14	Loam	6.7	0.779
Tamsa	16	Clay	6.1	0.802
Ayodhya	12	Loam	6.3	0.699
Mau	12	Alkaline	8.6	0.614

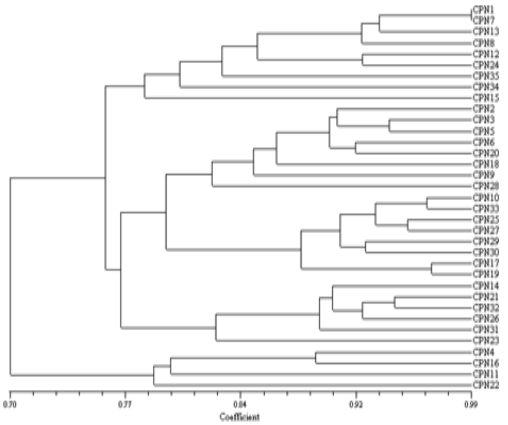


Fig. The phylogenetic tree based on carbon utilization was developed

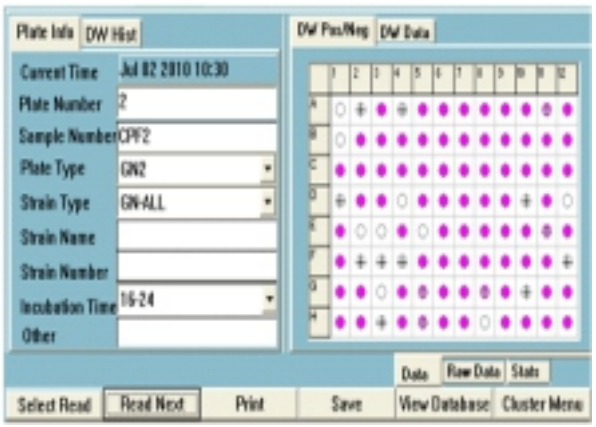


Fig. Carbon substrate utilization based on BIOLOG

Primers used for molecular characterization of the isolates

Aspartate – semialdehyde dehydrogenase	asd forward	F 5′-CGG CCG GGA GAT GCT GAACA-3′
	asd reverse	R 5′-ATG CGC TTG GTG AAC TTCTTG-3′
Phosphogluconate dehydratase	edD forward	F 5′-GGC ATC ATC ACC TCC TACAA-3′
	edD reverse	R 5′-CGG CGT GCC GGG ATT-3′
Glyceraldehyde3 –phosphate dehydrogenase	gap forward	F 5′-CGG TCC GGT CGA GACCAA-3′
	gap reverse	R 5′-CGG TAG AGA TCC TTG TGCAT-3′
protein-PII uridylyl transferase	glnD forward	F 5′-GTG CGC TGC CAC ATG CAYTT-3′
	glnD reverse	R 5′-CCG GRT CRC GCT TGA A-3′
6-phosphogluconate dehydrogenase	gnd forward	F 5′-GGG CCG GCT CAA CTCCTA-3′
	gnd reverse	R 5′-CGG CAT CGG CAG GTT-3′
NADH dehydrogenase I chain E protein	nuoE1 forward	F 5′-GCG CGC KCA GGA GCAGGA-3′
	nuoE1 reverse	R 5′-CGC AGG CGC CCT GACATT-3′
Putative oxidoreductase protein	ordL2 forward	F 5′-GCG GCG CGG TCG TCA Tx-3′
	OrdL2 reverse	R 5′-CGC CAT GGC CGG AAT A-3′
DNA strand exchange and recombination protein	recA forward	F 5′-CCG GTT CGC TCG GCC TCGATA-3′
	recA reverse	R 5′-CGC CCA TCT CGC CCT CGATTT-3′
2-oxoglutarate dehydrogenase E1	sucA forward	F 5′-GCT CGG CCT CGA ATA-3′
	sucA reverse	R 5′-CCG TCA GCG ACA GGT-3′
Glucose – 6 –phosphate 1-dehydrogease	zwf forward	F 5′-GGG GGC ACC GGC GAT CTTG-3′
	zwf reverse	R 5′-AGC GCA GTG CCA TCA GATTCT-3′

Project 2 : Genotypic diversity and rhizosphere competence of potent antagonists of soil borne pathogens of vegetables

PI : Sudheer Kumar
Co-PI : Alok Kumar Srivastava

Rationale

Recently, an environmentally friendly approach to protecting plants from fungal pathogens through rhizobacterium-mediated biological control is in practice. Antagonistic bacteria comprise a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots. Within the last few decades, a large array of bacteria including species from the genera *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligens*, *Arthobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance plant growth. As biocontrol agents, isolates of *Pseudomonas fluorescens* and *Bacillus* have been the most studied and exploited. To be an effective antagonistic strain, bacteria must be rhizospherically competent, and capable of surviving and colonizing the rhizospheric soil. Unfortunately, the relationship between antagonistic bacteria and plants can be unstable. Although promising results might be obtained *in vitro*, they are not necessarily reproducible under field conditions. The variability with the performance of antagonistic bacteria may be attributable to various environmental factors that can determine antagonistic bacteria growth and as a result, affect their ability to exert a beneficial effect on the plant. Such environmental factors include climate, weather conditions (temperature, wind, humidity, etc.), soil characteristics and the composition or activity of indigenous soil microbial flora. To achieve optimal growth conditions promoting interaction between antagonistic bacteria and nursery seedlings, it is important to determine how rhizobacteria exert their effects on plants and whether these effects are influenced by various environmental factors, including the presence of other microorganisms. Therefore, it is necessary to develop efficient biocontrol strains under appropriate field conditions. One possible approach is to explore soil microbial diversity for antagonistic bacteria with plant growth promoting activities as such bacteria are assumed to be well adapted to the soil environment from which they are isolated.

Rhizosphere competence is a dynamic process by which introduced bacterial inoculants make use of nutrients excreted by the seed and/or plant root, proliferate, efficiently colonize the root system, and survive over a considerable time period in the presence of indigenous microorganisms. Rhizosphere competence is a crucial element in beneficial plant-microbe interactions as inadequate root colonization leads to decreased biocontrol activity. A major factor contributing to the inconsistent colonization by the bacterial inoculants remains their variable ecological

Antibiotic resistance assay of antagonistic strains from tomato rhizosphere

Characteristics		MB7	MB14	MB69	MB89	MB91	MB99	MB101	MPRO1	MUN1	MPM1	M10A	MPF14	MB21	MPF37	MPF47	MB65	MB123
Antibiotic resistance	Ampiciline	+C	+C	-	-	+B	+A	+E	-	-	+E	+A	+E	+C	+E	+C	+E	+B	+E
	Chlorophenichol	+C	+C	+A	+A	-	-	+C	+C	+C	+C	+C	+C	+C	+E	+C	+E	+D	+E
	Rifampicine	+B	+B	-	-	-	+C	+C	+B	+B	+D	+C	+E	-	+E	+C	+E	+D	+E
	Tetracycline	+C	+D	+C	+C	+C	+C	-	-	-	-	+C	-	+B	+E	-	+D	-	+E
	Streptomycin	+C	+D	+C	+C	+D	+C	+C	-	-	+E	+B	+E	+C	+E	+E	+D	+B	+E

Different concentration supplemented with NA as µg/ml of antibiotic represent by the different letters A=20, B=40, C=60, D=80 and E=100

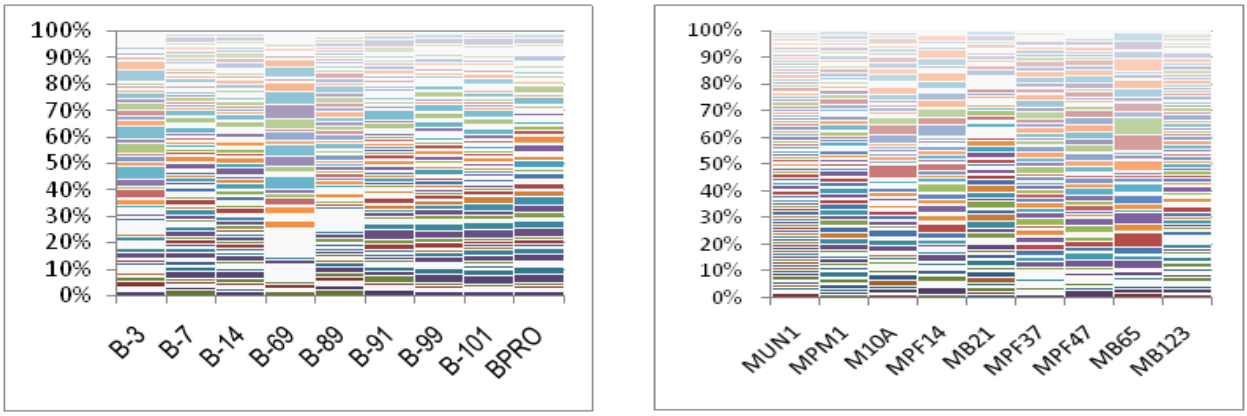
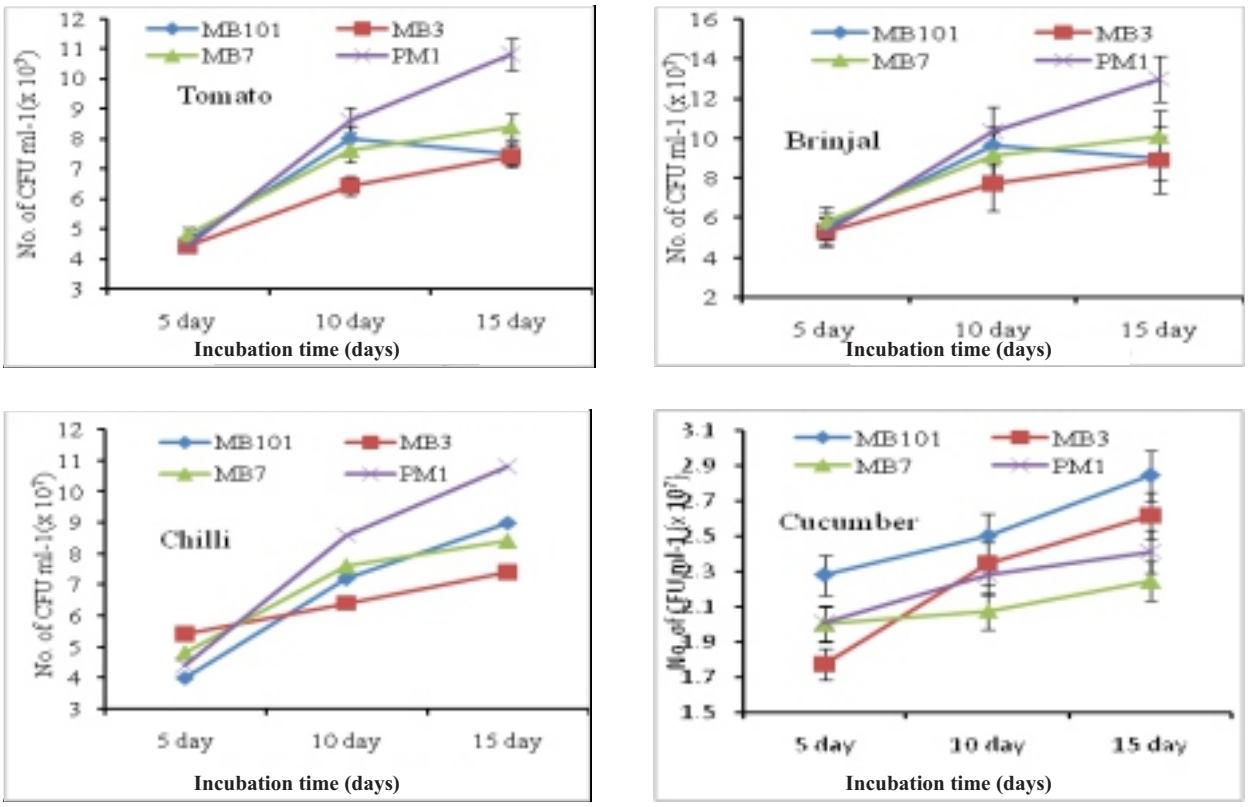


Fig. Phylogenetic tree based on the carbon utilization of antagonists isolated



performance. Numerous studies have been performed in order to identify traits and factors that contribute to successful establishment, spread and survival of bacterial inoculants in the rhizosphere. The present investigation is undertaken to understand the host preference in rhizospheric competence of potent antagonists.

Objectives

- To study the genetic diversity of potential antagonist of soil borne disease of vegetables
- To study influence of the host plant species on the population dynamics of antagonists in the rhizosphere.

Achievements

- After the preliminary screening under laboratory against different soil borne pathogens of vegetable crop and further under green house condition against the Rhizoctonia root rot of tomato a total 18 bacterial strains were selected. These isolates were characterized on the

molecular level for variability, genetic relatedness and identified on the basis of 16S rDNA sequencing.

- These bacteria were also evaluates for intrinsic antibiotic resistance and further for root colonization potential with solanaceous and cucurbits crop.
- These isolates were also differentiated on the basis of their carbon utilization pattern and a phylogeny was drown on the basis of metabolic fingerprinting.
- Four rhizospheric bacterial strains (*B. megaterium* MB3, *B. subtilis* MB7 and *Enterobacter* sp. PM1) were evaluated for their comparative root colonization in different vegetable crops (tomato, chilli, bringal and cucumber)
- Different isolates showed the differential preference to the host rhizosphere. Isolate PM1 has more root colonization potential over other tested isolates and showed more preference toward solanaceous crops and less preference to cucumber.
- These isolates were further analyzed for the presence of different antibiotic gene which may help to compete with other rhizospheric microbes.

Conclusion

Different isolates has differential preference to the host rhizosphere. So while formulating and using the biocontrol agents the host preference must be taken care for the better results.

Project 3 : Isolation and Identification of cyanobacteria from saline soil habitats, characterization of their salt-adaptation mechanisms and application for stress-tolerance in rice

PI : Dhananjaya Pratap Singh
Co-PI : Anurag Chaurasia

Rationale

Cyanobacteria inhabit almost all kinds of natural habitats right from soil, water, sediments, agricultural fields, freshwater ecosystem and eutrophicated ponds, lake, rocks, sea and even the walls of old buildings. Many diazotrophic heterocyst-forming cyanobacteria possess the ability to form associations with vascular/non-vascular plants and produce growth-promoting substances. Some cyanobacterial species fix atmospheric nitrogen while others form symbiotic associations with plants and fungi. Looking into their primitive existence, cosmopolitan occurrence and great potentialities in terms of bioactive metabolite production, plant growth promotion and soil health improvement, this project was undertaken to explore the diversity of these fascinating blue-green microbes in different habitats including the saline soil systems and in the eutrophicated ponds, ditches and rivers. Since these microorganisms are continuously exposed to different kinds of stress conditions, analysis of their adaptation mechanisms and finally their application in the stress alleviation of rice crop are the other major issues to be deciphered under this project.

Objectives

1. To isolate, identify and characterize agriculturally important cyanobacterial strains for saline soil habitats.
2. To evaluate molecular, biochemical, morphological and cellular mechanisms of adaptation and survival of selective cyanobacterial strains under salt stress conditions.

Fig. Cyanobacterial isolates collected from the soils of different salt-habitats

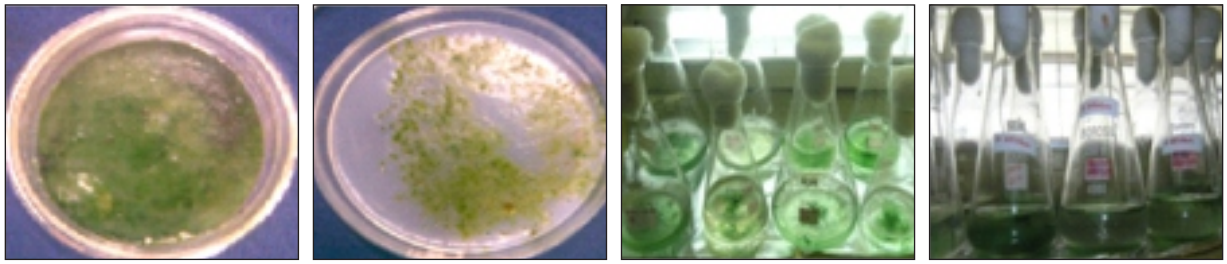
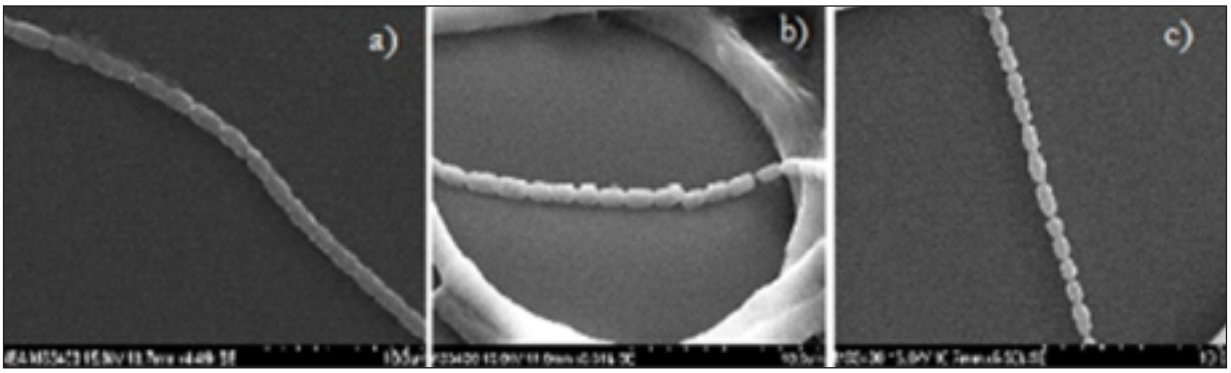


Fig. Scanning electron micrograph (SEM) of *Anabaena doliolum* grown under different salt (NaCl) concentrations showing cellular deformation. Under salt concentrations cells tend to loose; a) control; b) 200mM NaCl; c) 400mM NaCl



3. To assess the role of efficient cyanobacterial strains in the tolerance of rice plants to salt stress conditions.

Significant achievements

- Morphological characterization of the isolates led to the characterization of 23 isolates of the genera *Anabaena, Nostoc, Aulosira, Cylindrospermum, Hapalosiphon, Tolypothrix, Oscillatoria* and *Scytonema*. Molecular identification based on 16S rDNA gene sequencing is under way.
- Induced accumulation of polyphenolics and flavonoids in cyanobacterial strains grown under different salt concentrations is correlated with their growth under stress conditions and enhanced antioxidant activity. *Plectonema boryanum*, *Hapalosiphon intricatus, Anabaena doliolum* and *Oscillatoria acuta* grown for 21 days under different salt concentrations (80, 160, 240, 320 and 400 mM) in BG11 medium showed differential growth responses in terms of biomass, total protein, chlorophyll content, total content of polyphenol, flavonoid and carotenoid, accumulation of phenolic acids (gallic, caffeic, chlorogenic, ferullic and vanillic) and flavonoids (rutin and quercetin).
- Induced accumulation of phenolics, flavonoids and antioxidant activity in cyanobacteria under salt-stress is correlated. Cyanobacterial extracts showed prominent free radical scavenging antioxidant activity. Highly significant correlation was found between phenol content and antioxidant activity. Phenylpropanoids accumulation can act as alternative mechanism to overcome salt-stress.

Project 4 : Mapping and utilization of structural and functional culturableactinobacterial diversity from mangrove ecosystem of India

PI : Mahesh Yandigeri
Co-PI : Dilip K. Arora

Rationale

The present investigation to map structural and functional actinomycetes diversity from mangrove ecosystem and their evaluation for utilization for plant growth promotion and nutrient management.

Objectives

- To characterize the actinomycetes isolated from mangrove ecosystems of India for different physiological and biochemical traits.
- Molecular characterization and mapping of structural and functional culturableactinobacterial diversity in mangrove ecosystems.
- Evaluate the role of some isolates for their plant growth promotion activities and nutrient management.

Achievements

A total of 116 Streptomycetes isolates were procured from Chilika lagoon by employing various media

and enrichment techniques and sorted out into 59 different morphotypes based on colour of aerial and substrate mycelium, pigmentation and microscopic examination. Further screening of alkali-halophilic nature of different morphotypes revealed that a total of 21 isolates possessed the ability to grow at pH 9.0 and 1.71 M NaCl (w/v) respectively. The population frequency of streptomycetes in Chilika lagoon was shown to be different in all three sectors. Central sector harboured maximum population (Chadheiguha 28.1% and Nalabana 23%), followed by South sector (Rambha, 21.2%; Badakuda, 10.53%), while least population frequency was recorded in Sea Mouth sector (Manikapatana, 10.32%; Sea mouth, 6.6%). Sediment samples had highest streptomycetes population frequencies than lake water while, colonization frequencies of alkali-halophilic streptomycetes (pH 9.0; NaCl 1.71 M) shown to be decreased from marine habitat to fresh water lake.

Characterization of 21 alkali-halophilic actinomycetes revealed that South sector harboured maximum percentage of siderophore producers (48.5%) while Central sector had highest IAA (45%), and extracellular protease enzyme (45.1%) producers. Sea mouth sector was enriched with nitrate reductase activity (42.3%) and had biocontrol attributes as well (antimicrobial activity (38.8%) and chitinase enzyme (39%) production). Chitinase and protease enzyme producing isolates were procured from sediment samples while cellular siderophore, IAA, antimicrobial activity and nitrate reductase potential activity was found to be highest in water samples. Interaction studies with phytopathogenic fungi captured using scanning electron microscopy,

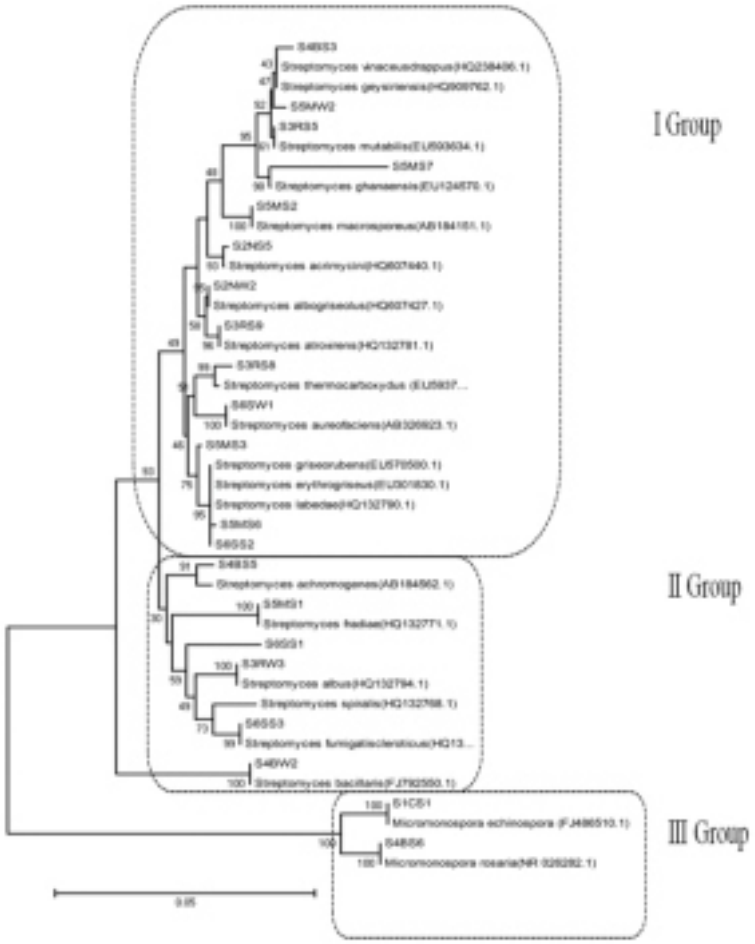


Fig. Phylogenetic tree based on the 16S rRNA gene sequences of alkali-halophilic actinomycetes isolates and their closest phylogenetic relatives. The tree was created by the neighbor-joining method. The boot strap values from 5000 pseudoreplications are shown at each of the branch points on the tree. bar indicates % similarity.

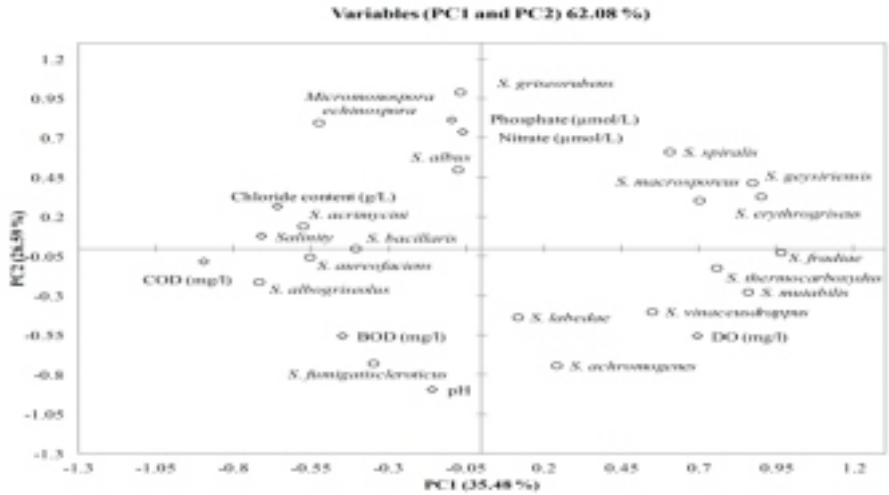


Fig. Principal component analysis of between biogeochemical characters in diamond shape (◊) and population distribution indicated in round shape (○).

exhibited rupturing of fungal mycelium, colonization and finally complete destruction. Catabolic carbon assimilation pattern was analysed based on utilization/ non-utilization of 95 substrates studied by the BIOLOG™ system, and conversion of values into binary matrix (1/0) followed by clustering (dendrogram) for all isolates. At a 70% similarity level, all isolates were grouped into one major and eleven minor groups. All actinomycetes isolates showed different types of carbon substrate utilization pattern ranging from 9-82 out of 95 substrates.

- Diversity analysis and identification of Alkali-halophilicactinomycetes using molecular tools revealed isolates were belonging to *Micromonosporaechinospora, M. Rosaria, Streptomyces albogriseolus, S. acrimycini, S. albus, S. mutabilis, S. thermocarboxydus, S. atrovirens, S. bacillaris, S. geysiriensis, S. achromogenes, S. vinaceusdrappus, S. fradiae, S. macrosporeus, S. griseorubens, S. labedae, S. ghanaensis, S. aureofaciens, S. spiralis, S. erythrogriseus, S. fumigatiscleroticus*.
- The PCA plot was based on the biogeochemical properties as well as frequency of species distribution into two principal component factors, which explained 59.95% of the total variances. Results implied that, the pH, BOD, OD, COD, chloride and salinity factors played key roles in their ecological distribution in the hyper-saline lake.

Conclusion

In conclusion, *Streptomyces* population in different sectors of Chilika lagoon were considerably varied with variation in their physiological as well as biochemical profiles, which enable us to understand behavioural, ecological, as well as specific substrate requirement in a particular brackish niche.

Project 5 : 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

Rationale

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. In order to achieve sustainable agriculture and addressing to additional concerns about food safety and environmental protection it is necessary to develop safer, more specific and environment-friendly of pathogen controlling agents in which bacteriophages offers a great potential. Bacteriophages provide highly specific control opportunities for bacterial diseases by specifically infecting and destroying the disease-causing bacteria.

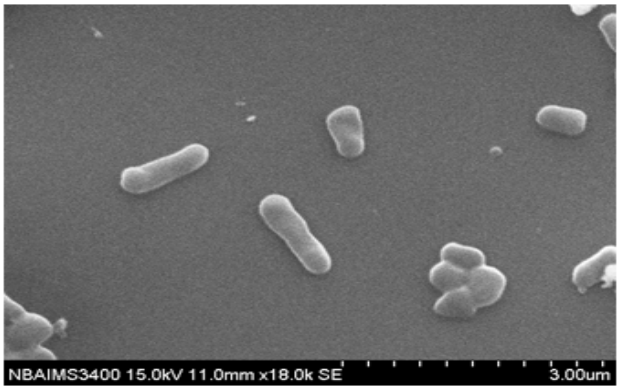
The aim of the proposed research project is to collect, characterize and conserve phages of phtyopathogenic bacteria , *Xanthomonas campestris pv. campestris* (*Xcc*) a causal organism of black rot of crucifers and to look out for their possible role in disease management programme.

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.

Significant achievements

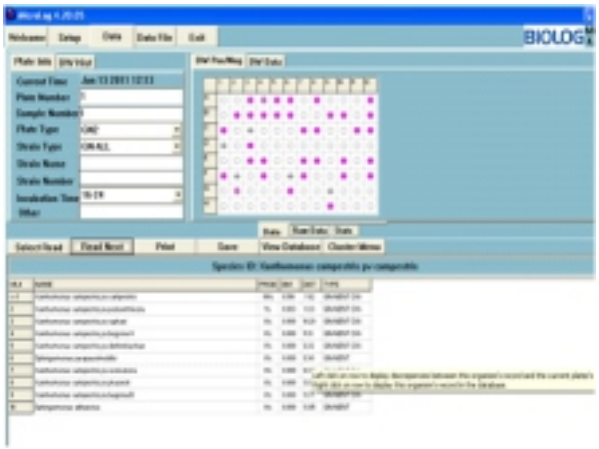
- Surveys from different geographical locations in India like of Ghazipur (Jangipur; neaby Ghazipur city; Ishupur, Mohamdabad; Nand Ganj, Saidpur), Varanasi (IIVR farm and nearby villages, area nearby Varanasi city), Lucknow, U.P.; Delhi; Haryana; Simla, H.P. ; vegetable fields at Pantnagar Hill Campus, Ranichauri, Uttrakhand; Shillong, Meghalaya were carried out and collection of diseased plant samples from various cruciferous hosts like cauliflower, cabbage and rai was done.
- Isolation of 12 strains of *Xcc* was done and their pure cultures were subjected to morphological characterization, Scanning electron microscopy, hypersensitivity reaction on pepper plant, pathogenecity assay, host range, *Xcc* determinative biochemical characterization as well as subjected to metabolic fingerprinting utilizing Biolog GN microtiter plates (Microlog2, Version4.2, BiologInc, Hayward, CA) and 16S rRNA gene sequencing.
- Morphologically all isolates were gram negative rods with an average length of 1.9µm and 0.8µm width. Distinct colonies appear after 48 hours incubation at 28°C on nutrient agar.
- For routine pathogenicity tests, plants were inoculated 5 weeks after sowing of respective susceptible hosts cabbage cvs. Golden Acre , cauliflower **cv** Kataki 1 and mustard cv Pusa bold growing on a greenhouse bench at 24 to 28°C by cutting the edge of the leaves and applying bacterial cultures to the wounds. Bacterial cultures were grown in liquid nutrient broth , adjusted to 0.1 optical density, diluted to contain 10^6 CFU/ml. For inoculation, two leaves per plant were cut 2 cm from each side of the central vein with sterile scissors and bacterial cultures were applied. Plants were incubated at 26 ± 5°C under high relative humidity. The plants were observed daily for symptom appearance during 2 weeks. Typical V shaped lesions were observed after 7-10 days of inoculations.
- All isolates were tested for their host range on various crucifer plants. *Xcc* 11 and *Xcc* 7 were able to produce symptoms on all three hosts tested i.e. cauliflower, cabbage and mustard.



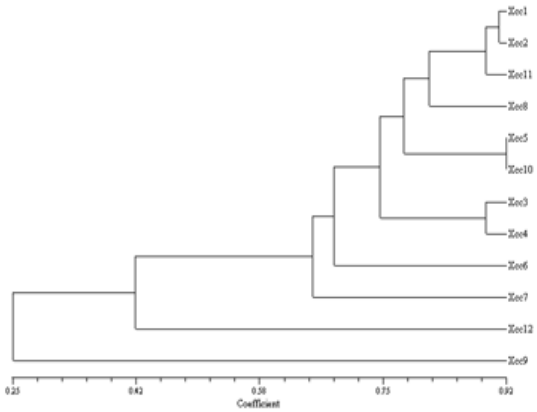
SEM of Xcc 4 isolate

Pathogenecity test of Xcc4 isolate on susceptible cauliflower host

- All 12 isolates tested were identified at least to the Xanthomonas genus level by BIOLOG. Of these, 11 isolates were identified to the species level (*X. campestris*) and 4 strains were identified to the pathover level (*X. campestris* pv *campestris*). Jaccard's similarity coefficients based on metabolic fingerprinting data ranged from 0.15 to 0.91 among 12 isolates of *Xcc* indicating a high genetic diversity among them, Isolate *Xcc*5 was found to be closest to *Xcc*10 (91%) and *Xcc*9 is highly dissimilar to *Xcc*12 (15% similarity).
- Technique for isolation of phage was standardized. For isolation of bacteriophage of *Xcc*, collection of soil and plant samples from



Characterization based on carbon utilization pattern based on BIOLOG



Dendrogram obtained on the basis of carbon source utilization pattern of 12 isolates of *Xcc*

Isolate No.	Biolog identification	
Xcc1	Xcc (0.516)	
Xcc2	Xcc (0.518)	
Xcc3	Xcr (0.583)	
Xcc4	Xcr (0.581)	
Xcc5	Xcp (0.711)	
Xcc6	Xcr (0.802)	
Xcc7	Xanth.	Xcd (0.421)
Xcc8	Xcc (0.668)	
Xcc9	Xcp (0.833)	
Xcc10	nd	Xcp (0.158)
Xcc11	Xcc (0.514)	
Xcc12	nd	

The names in the adjacent column refer to closest matched identity given by Biolog system whenever similarity value was below 0.5.
Xcc - Xanthomonas campestris pv. campestris;
Xcp - Xanthomonas campestris pv. poinsettiicola;
Xcr - Xanthomonas campestris pv. raphani;
Xanth – Xanthomonas
Nd – not determined

black rot infected cabbage field at Shillong was done. Enrichment of soil samples was done with the indicator bacterial hosts and plaque assay was carried out by soft agar overlay method. Clear plaques were selected for further 3-4 successive reselection steps for isolation of lytic phage. Further characterization of phage will be done by organic solvent sensitivity test, plaque morphology and host range. Attempts for isolation of more phages is in progress.

Conclusion

The present study characterizes various strains of *Xanthomonas campestris* pv. *campestris* (*Xcc*), a causal agent of black rot in crucifers, collected from different hosts plants like cauliflower, cabbage and rai and from different locations in India. Identity of *Xcc* was confirmed by morphologically, biochemically as well as at molecular level. Pathogenecity tests with all isolates on their respective susceptible hosts were able to produce typical V-shaped lesions. BIOLOG was found to be very effective method of identification of this bacteria which could identify it even at pathovar level. 16S rDNA sequencing of all isolates were done, matched with other sequences at NCBI and were submitted to NCBI genebank. The isolated strains will serve as host source for isolation of *Xcc* specific bacteriophages.

Project 6 : Exploration, preservation and evaluation of endophytic actinomycetes from Indo-Gangetic plain

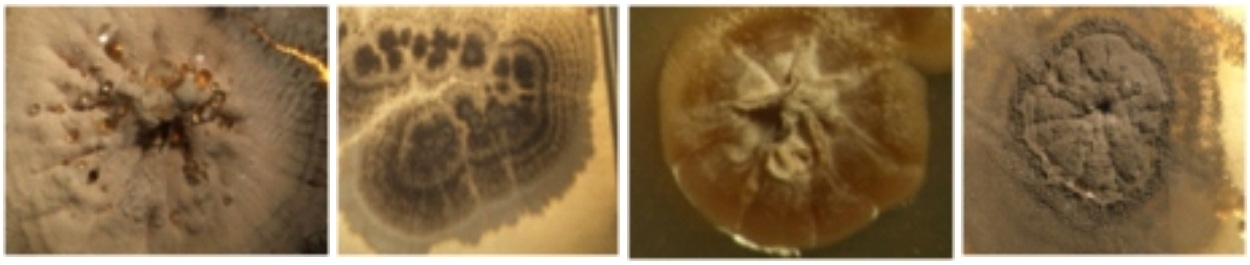
PI : Anurag Chaurasia
Co-PI : Dhananjaya Pratap Singh

Rationale

Endophytic microbes are ubiquitous in most plant species, especially in field-grown plants. Hallmann & coworkers defined any bacterium as an endophyte if it does not visibly harm the plant and it can be isolated from surface disinfected plant tissues or extracted from inside the plant. As of 1997, bacteria isolated from the internal plant tissue of healthy-looking plants were comprised of over 129 species representing over 54 genera. Although some of the endophytes are pathogenic to host plants and can locally or systemically colonize plant tissues, others latently reside in the internal tissue of nonsymptomatic plants without causing any adverse effects to the plants. Consequently, intimate associations between endophytes and host plants can be formed without harming the plant. Endophytes have been demonstrated to improve and promote growth of host plants as well as to reduce disease symptoms caused by plant pathogens and/or various environmental stresses. Management of beneficial microbial communities to favour plant growth could be realized by a deeper understanding of the physiological and molecular interactions between microbes and plants. This research also may have broader economic and environmental impacts. Indo-Gangetic plains are a major crop production region of the country and have rich biodiversity, hence this project has been formulated to isolate, utilize and conserve the endophytic actinomycetes from various crops grown in this region which will be further used for enhancing agricultural productivity. Endophytic actinomycetes also produce bio-active metabolites and have been found responsible for the medicinal properties of the various medicinal plants. Actinomycetes strains having PGPR and biocontrol potential will be of immense use for enhancing the agricultural productivity.

Objectives

- Isolation of endophytic actinomycetes from the crops growing in Indo-Gangetic plain.



- Preservation and identification of the isolates.
- Evaluation of the endophytic actinomycetes isolates for enhancing agricultural productivity and human welfare.

Significant Achievements

- Endophytic actinomycetes were isolated from the root, stem and leaves of wheat, *Brassica juncea*, *Punica granatum*, *Carica papaya*, *Anethum sowa*, *Aloevera* and *Karonda* using actinomycetes isolation agar & starch casein agar.
- Rare endophytic actinomycetes were also isolated using specific isolation media.
- Actinomycetes strains were found to have different morphology when grown on different media.
- All the isolates have been preserved on the slant and in 20% glycerol for short term storage.
- PGPR and biocontrol potential evaluation of the isolates have been conducted.
- Isolates are being identified by polyphasic approach.
- Limited field evaluation of wheat endophytic actinomycetes (strain – 20) has been conducted. Various genera of actinomycetes associated with cyanobacteria (Cyanomycetes) have also been isolated.

Project 7 : Diversity analysis of methylotrophic bacteria from different ecological zones of India

PI : Kamlesh Kumar Meena
Co-PI : Mahesh S. Yandegeri

Rationale

Mangroves are known to be highly productive ecosystems and have immense ecological values. The microbial community in the mangrove sediment is strongly influenced by bio-geographical, anthropological and ecological properties. Bhitakanika is second largest mangrove forest in India but not studied well for its microbial diversity and useful microorganisms. Geographically Bhitarkanika is located between 20°4'-20°8'N Latitudes and 86°45'-87° 50' Longitudes and is spread in 268 km^2 area. In this study we tried to explore the methylotrophic bacterial community from the sediments of the mangrove.

Significant achievements

- Physiochemical properties of the mangrove sediment were determined. Five composite sediment samples were subjected to the estimation of physical properties and the range of pH was 7.4 to 8.0 while the electrical conductivity from 4.04 dSm^{-1} to 6.67 dSm^{-1} with organic carbon range from 0.7 to 1.5.
- The nitrate mineral salt medium (NMS) was used for the enrichment of methylotrophs (gl^{-1}): with methanol (0.1% v/v) (Patt *et al.* 1974). The pH of NMS was adjusted according to the sample pH, by addition of a mixture of $NaHCO_3/Na_2CO_3$. Antifungal cycloheximide (40 mg ml^{-1}) was added 500µl per litre media. Enriched samples were serially diluted and plated on NMS agar. 45 isolates were selected from one season and thus total 90 isolates were screened on the basis of salt tolerance and different morphotypes.
- DNA of all 90 isolates was extracted and then subjected to the 16S rRNA gene PCR and were amplified using primers pA 5'-AGAGT TTGATCCTGGCTCAG3' (*E.coli* position 8-27)

Sample	Sample Type	Season I (June-July)			Season II (Feb-March)		
		pH	E.C. (dSm1)	Organic carbon	pH	E.C. (dSm-1)	Organic carbon
BN1	Sediment	8.6	7.22	1.0-1.5	7.8	6.67	1.0-1.5
BN2	Sediment	8.9	3.571	1.0-1.5	7.4	5.84	1.0-1.5
BN3	Sediment	9.1	7.379	1.0-1.5	8.0	4.19	0.7-1.0
BN4	Sediment	9.0	7.143	1.0-1.5	8.0	4.04	0.7-1.0
BN5	Sediment	8.0	4.945	1.0-1.5	7.8	5.53	1.0-1.5

Methylotropic bacteria from mangrove

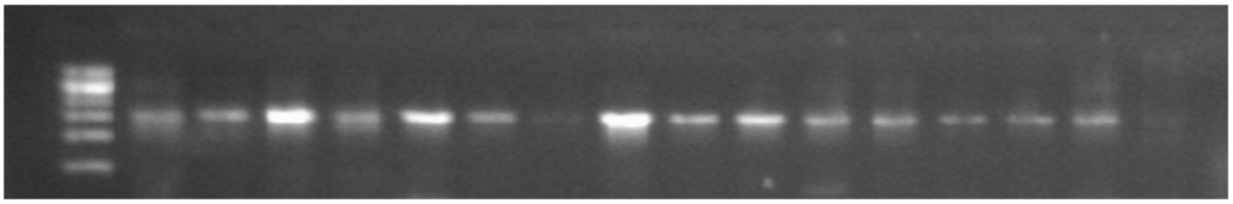


Fig. 16S rRNA gene amplification (isolates BN1 to BN16)

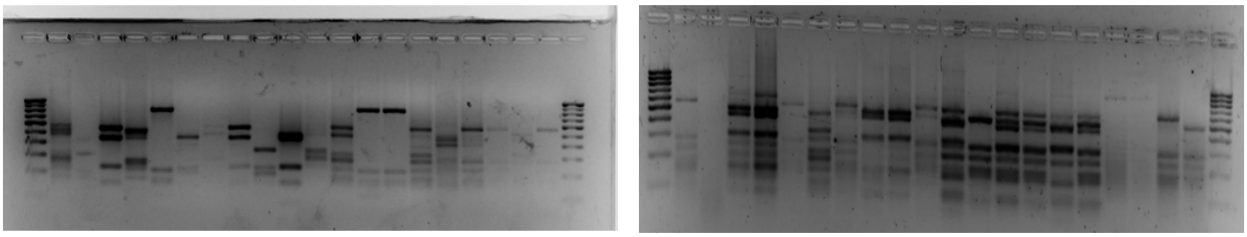


Fig. DGGE profiling and variant bands cut down to reamplify the v3-v5 region.

and pH 5'-AAGGAGGTGATCCAGCCGCA3' (*E.coli* position 1525-1544).

- PCR products were separated on 1.5% agarose gel stained with ethidium bromide and documented in Alpha Imager TM1200 analysis system.
- Following 16S rDNA amplification, the products were digested with selected restriction enzymes having different restriction sites. Approximately 1 µg of PCR-amplified 16S rDNA fragments were restricted with three different endonucleases *HaeIII* (Fermentas) separately, incubated at 37^{0}C for overnight and resolved on 2% agarose gels.
- Under culture independent assessment of methylotrophic community DNA from sediment samples (B1 to B5) were extracted using Power Soil DNA isolation kit (MoBio laboratories, Carlsbad) following manufacturers protocol. The integrity and concentration of purified DNA was determined on 0.8% agarose gel stained with ethidium bromide.
- The 16S rRNA gene was amplified from metagenomic DNA (B1 to B5) and then the sub region V3-V5 was amplified and product was run through the denaturing gradient gel electrophoresis (DGGE). Different bands were appeared and cut down to carry out the nested PCR with the primers having no GC clamps.The amplified products were purified and ready to sequence or purified bands were taken as insert for cloning.
- Quantification of *mxaF* gene was done in terms of copies using LightCycler software 3.5 based on 'second derivative maximum method' (Roche Diagnostics, Switzerland) in which exponential phase of amplification curve is linearly related to a starting concentration of template DNA molecules. Quantitative PCR was carried out using SYBR GreenI technology (Pfaffl 2001) with the primers *mxaF* and *mxaR* and environmental DNA samples (B1 to B5 and BN1 to BN5) were amplified from sediment, negative control and five plasmid DNA standards.
- The *mxa*F gene copy number from sediment of season1 was in the the range $8.8x10^2$ to $1.5x10^5$ and from season2 $6.56x10^3$ to $4.06x10^6$. Maximum abundance of the functional gene was observed at site B1 (Kanika).

Project 8 : Metagenomic approaches for exploring the biodiversity of antibiotic producing agriculturally important microorganisms (AIMs)

PI : Udai Bhan Singh
Co-PI : Dhananjaya Pratap Singh

Rationale

Microbial secondary metabolites are good source for the discovery of novel antimicrobial compounds. Microbial metabolite exhibit versatile chemical structure with diverse biological activities that exceed the scope of synthetic organic chemicals. As a result of increasing environmental concern and the development of resistance in pathogens to synthetic chemicals, exploitation of antibiotics from microbial metabolites is being considered as an approach to the identification of novel antibiotics which meets environmental requirements also. Metagenomics is a new field combining molecular biology and genetics in an attempt to identify, and characterize the genetic material from environmental samples and apply that knowledge. The genetic diversity is assessed by isolation of DNA followed by direct cloning of functional genes from the environmental sample. It is well known that less than 1% of the microbial world can be accessed using classical culturing approaches. Concerning biotechnological and pharmaceutical applications the genomes of the non-cultured microbes represent a shear unlimited and very valuable resource for novel biocatalysts and genes encoding for antibiotics or other drug molecules. Metagenomics will now unlock this vast potential for biotechnological and pharmaceutical applications.

Rhizosphere ecosystem is unique in nature and harbours unique microbial diversity. Rhizosphere ecosystem is rich in organic matter and macro and micro nutrient. Rhizosphere provide a unique ecological environment for divers microbial communities like antibiotic producing, nitrogen fixing, nutrient mobilizing microorganisms. Many of the communities are involved in various activities such as antibiotic production, nutrient mobilization, bioremidiation, nutrient cycling and decomposition etc. Metagenomic approach exploited to access the whole microbial community those having 2,4-DAPG, Type I PKS and Type II PKS gene which is responsible for the production of antibiotic. The gene(s) 2, 4-DAP, Type I PKS and Type II PKS play a key role in the synthesis of a number of antibiotic such as streptomycin, tetracycline, oxy- tetracycline, chlor-tetracycline etc. which are not only agriculturally important, they have wider applicability in human and animal health. Keeping this in mind, the present project is designed by taking metagenomic approach to dig out the culturable as well as non- culturable microbial genomic diversity of antibiotic producing agriculturally important microorganisms.

Objectives

- Evaluation of genetic diversity of antibiotic producing AIMs in rice-wheat cropping system of Indo-Gangetic plains of Uttar Pradesh.
- Detection, prediction and diversity of anti-microbial genes (2,4-DAPG, Type-I PKS and Type-II PKS) by using metagenomic approaches.
- Screening and expression of antibiotic producing genes (2,4-DAPG, Type-I PKS and Type-II PKS) and its possible application in agriculture.

Significant Achievements

- During the year 2011- 12, rhizospheric and non-rhizospheric soil samples were collected from Rice- wheat growing areas of Allahabad, Varanasi and adjoining areas of Uttar Pradesh (Fig. 1). Physico-chemical properties of these soil samples were tested using HiMedia soil analysis kit.
- A total of 56 different morphotypes belonging to bacteria and actinomycetes were isolated using different media. Further characterization of antibiotic producing AIMs were done using duel plate technique for preliminary screening. A number of actinomycetes are found to be good secondary metabolite producer which are of antimicrobial in nature. All the isolates were screened for the antimicrobial potential against potent fungal and bacterial phyto-pathogens.
- A total of 20 actinobacteria exhibiting distinct colony characteristic were isolated, purified and subjected to further molecular characterization. PCR amplification followed by restriction analysis of 16S rDNA gene, clustered the isolates into 12 groups.
- A total of 5 isolates were chosen as representatives based on PCR amplification of 16S rDNA and RFLP clustering using three different restriction endonucleases i.e. *Taq* 1, *Hae* III *alu* 1. Further phylogenetic analysis of 5 representatives was

Fig. Map depicting the wheat growing areas of Allahabad and Varanasi chosen for the survey and diversity analysis of antibiotic producing AIMs.

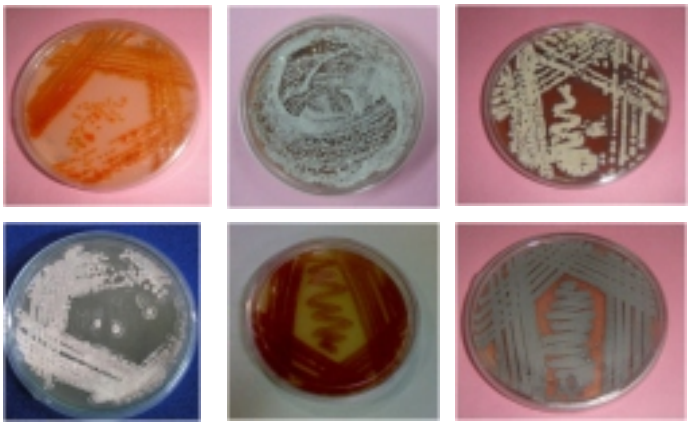


Fig. Representative morphotypes of actinomycetes isolated from wheat rhizosphere from Allahabad and Varanasi soil sample.

carried out for their similarity to known *Pseudomonades* aligned together with the sequences available in public database (Genbank, NCBI).

- The gene employed in this study 2, 4-DAPG, Type I PKS and Type II PKS were used to antibiotic producing microbial community analysis in wheat rhizosphere. The gene(s) 2, 4-DAPG, Type I PKS and Type II PKS are the key gene which is responsible for the synthesis of a number of antibiotic such as streptomycin, tetracycline, oxytetracycline, chlor-tetracycline urdamycin A, urdamycin B, urdamycin F, kinamycin D, medermycin, actinorhodin etc. The presence of 2, 4-DAPG, Type I PKS and Type II PKS gene in the isolate and soil metagenome detected by the partial amplification of the gene using specific primers i.e. 20-mer primers for antibiotics 2,4-diacetylphloroglucinol (Phl)-Phl2a(F) GAGGACGTCGAAGACCACCA, Phl2b(R) ACCGCAGCATCGTGTATGAG, Oligonucleotides primers for type I polyketide synthase F' TSAAGTCSAACATCGGBCA, R' CGCAGGTTSCSGTACCAGTA, Oligonucleotides primers for type II polyketide synthase U1 F' GCCGGAATTCATGATCCC GGTCGCGGTCA, U1 R' GCCAATGCATAA GCTTCACCGCCCGGCACGCACCGC (work under progress).
- Soil organic matter plays an important role in the presence and richness of microbial species in the rhizosphere ecosystem. Result revealed the positive correlation were observed for the microbial count (log cfu) and amount of organic matter present in the soil for all the groups of microorganisms i.e. bacteria, actinomycetes and fungi.

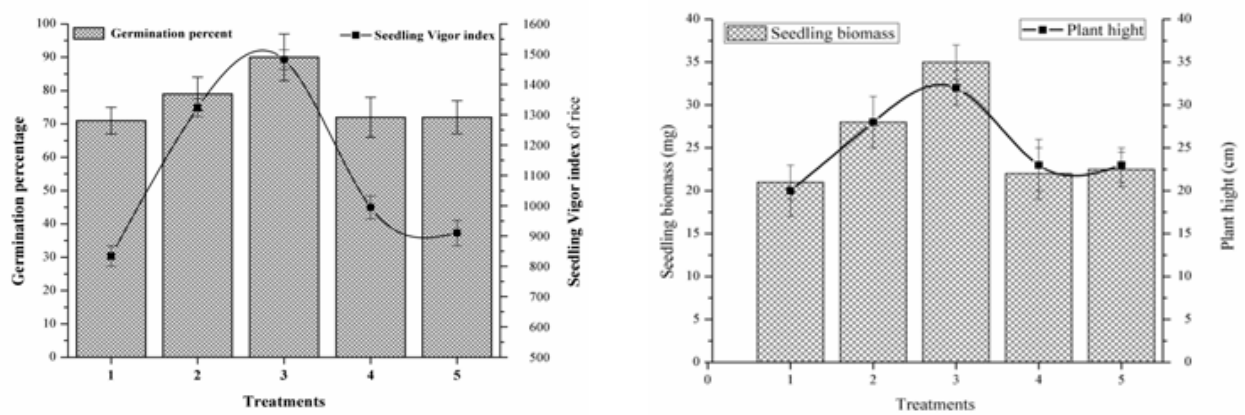


Fig. Effect of *Pseudomonas fluorescens* Pf-08 and Pf-10 on germination percent, seedling vigor, seedling biomass and plant height of rice. Treatments were: 1. Control, *2. P. fluorescens Pf-08, 3. P. fluorescens Pf-10, 4.* Streptomycin sulphate, *5.*Tilt (25 EC).

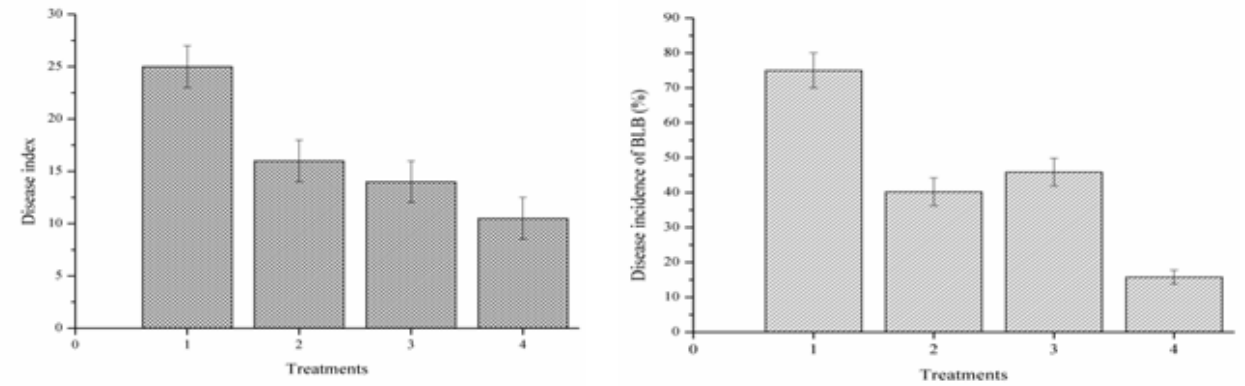


Fig. Effect of *Pseudomonas fluorescens* on disease dynamics in rice plants: 1. index of sheath blight in rice seedlings, 2. disease incidence of bacterial leaf blight disease in rice seedlings after 45 days of inoculation. Treatments: 1. control (only pathogen inoculated), 2. *Pseudomonas fluorescens* Pf-08, 3. *Pseudomonas fluorescens* Pf-10, 4. Chemical pesticide (Tilt 25 EC for sheath blight and streptomycin sulphate for BLB)

- Two *Pseudomonas fluorescens* strain Pf-08 and Pf-10 were selected for the *In vitro* studies. *In vitro* study showed an increase in the germination percent (10-18%) in seeds treated with these two strains as compared to control. In pot experiment, application of *Pseudomonas fluorescens* strain Pf-08 and Pf-10 (seed treatment) significantly increase the seedling vigor, seedling biomass and plant height as compared to control and pesticides treated plants.
- Application of *Pseudomonas fluorescens* strain Pf-08 and Pf-10 as seed treatment reduced the percentage of infected tillers, lesion length and disease index in plants infected with *R. solani*. It also reduced the bacterial leaf blight incidence in rice plants inoculated with either of the *Pseudomonas fluorescens* strain as compared to control.

Project 9 : Exploration of pathogenecity gene(s) of *Magnaporthe grisea* responsible for rice blast epidemic in hot spot regions of India

PI : Prem Lal Kashyap
Co-PI : Sudheer Kumar

Rationales

Rice blast caused by *Magnaporthe grisea* Barr (*Pyricularia grisea* anamorph Cav.) isone of the most destructive diseases of rice. The fungus is distributed world-wide and causes losses of up to 100% of the yield depending on cultivar susceptibility, environmental conditions and management system. Almost in each year, the fungus destroys rice enough to feed an estimated 60 million people. The pathogen infects most sections of the plant, but infections of the node or the panicle are the most damaging phases of the disease. Since the pathogen is highly variable, breeding for durable resistance to blast become a major challenge. Emergence of new pathotypes has been reported in different regions, causing the breakdown of cultivars developed with single major resistant genes. However, population evolution and virulence diversity of *M. grisea* in the field was still unexplored. Moreover, the detailed investigations on the exploration of diversity of pathogenicity genes of *M. grisea* have been fuelled by the necessity to develop cost-effective, eco-friendly and durable and novel strategies for the control *M. grisea*, in spite of ongoing strong efforts to develop and introduce new fungicides and resistant plant varieties. An essential cue in this ongoing battle is the identification and search for diseases controlling targets via the identification of pathogenicity determinants, encoded by virulence genes. Relatively little information exists on these aspects as fragmentary and preliminary efforts were made globally by various researchers to explore the significance of virulence genes in epidemiology and management of rice blast disease. In india, so far, no such type of study based on the diversity of virulence gene(s) on large scale was done. It is believed that the information generated from this research project will provide answer why rice blast epidemic occurs at a particular location at particular time-points and what gene(s) of *M. grisea* act as a master switch for the occurrence of rice blast epidemic. The identification and further exploration of these genes will help in proper identification of novel target sites to restrict the menace of the disease. The information generated through this project act as a model to devise eco-friendly, cost effective and integrated approach for the effective management of *M. grisea* under field conditions.

Objectives

- Isolation, identification and characterization of *M. grisea* isolates prevalent in hot spot region of India region
- Detection, prediction and diversity analysis of pathogenicity gene(s) by using molecular tools.
- Expression profiling of pathogenicity gene(s) to identify virulence pattern of *M. grisea*.

Salient Achievements

During 2010-11, a survey of rice blast infecting field of six different sites *viz.* Srinagar, Jammu, Almora, Ludhiana, Cuttack and Pattambi was conducted. The data presented in Table 1 depicts the per cent level of disease severity in different cultivars grown in these regions. The disease data was recorded on 0-9 scale. Maximum disease severity of leaf blast infection was recorded in Srinagar (41.3%) followed by Pattambi, Cuttack and Ludhiana. Similarly, the maximum infection and severity of node blast was recorded from Cuttack (19.52%) followed by Pattambi and Ludhiana. In case of neck blast, the highest severity level was obtained in Cuttack and Ludhiana samples. In general, the level of neck blast ranges between 16.49- 32.15% (Table 1).

To design the specific primers to detect and predicted virulence genes of *Magnaporthe grisea*, Board Institute *Magnaporthe grisea* database was surveyed. Thirteen different primers ((*CHM1*, *MPLC1*, *LpMOD*, *ABC1*, *CAMGEN*, *CALMOD*, *OSM1*, *TRE1*, *UEP1*, *MPS*1, *MAC1*, *TPAGEN* and MAGNA) targeted virulence were designed and validated in *in silico* using various bioinformatics tools (Table 2). Table 2 provides the relevant information pertaining to the genes and designed forward and reverse primers and the amplicon size.

Under *in vitro* conditions, the PCR conditions for the amplification of *M. grisea* specific primers were standardized to obtain the desired amplicon size. Table 3 depicts the amplification profile of different virulence in the rice blast infected samples collected from six different sites. Maximum number of virulence genes were obtained in the samples collected from Srinagar and Jammu (*CHM1, MPLC 1, LpMOD, ABC1, CAMGEN CALMOD* and*TPAGEN*)followed by Cuttack and pattambi (Fig. 1). Only five genes *CHM1, MPLC 1, LpMOD, ABC1* and *CAMGEN*) were detected in the samples collected

Table Disease severity of Rice blast in different hot spot regions of India (2010-11)

Location	Cultivars (s)	Severity (%)		
		Leaf blast	Node blast	Neck blast
Srinagar	Shamilar-1	41.3 ± 0.75	11.25 ± 1.2	23.11± 1.3
Jammu	Basmati-370, Saket-4	22.41± 0.64	10.40 ± 0.66	16.49 ± 0.52
Almora	VL Dhan 86, WL 227	25.42 ± 0.52	9.83 ± 0.72	28.34 ± 0.99
Ludhiana	Pusa basmati-1	26.14 ± 0.48	11.62 ± 0.58	30.84 ± 0.66
Cuttack	HR12, CSR-30	28.45 ± 0.68	19.52 ± 0.32	32.15 ± 0.39
Pattambi	Jyothi, Swarnaprabha	34.32 ± 0.72	12.50 ± 0.52	22.37 ± 0.59

Table *In silico* designing of pathogenecity genes of blast pathogen using NCBI and Board Institute *Magnaporthe grisea* database

Gene(s)	Forward primer	Reverse primer	Amplicon size(bp)
CHM1	ATTGGCACCGCAGGCTATG	GCAGCTCGACTCAGTGGTAGTG	400
MPLC 1	CCACTTTGACACCGAGACAG	GGATGTGGTAGAGGAAGTCATC	402
LpMOD	AATCCTCTTGGTGTTTCAG	ATAAATGGATCTTCAACGTG	424
ABC1	GCTTTGATTCTTAGTTGATACC	TTGGCTTAAGTTGTATTGTC	400
CAMGEN	GCGGACAAGGATGAGAATGAG	ACCGCCGCATCAAATTCG	442
CALMOD	TGCGGACAAGGATGAGAATGAG	CGCCGCATCAAATTCGCTG	442
OSM1	CCTACCTGTCATAACCATAC	TGATTATCTGATTGCGATG	200
TRE1	GACCCATACAATCAACGC	ACATTAGCTAGGTTTACGG	398
UEP1	CCAAGATTCAGGACAAGG	ACTTGTCAAATGTCCCTG	412
MPS 1	TTCCTCATCCAACTTTCTTCC	CCACACGATACCGTAGGCTC	401
MAC 1	GCCGATAGAGCAACATACAC	GCGTTTGTGCTGCGTTG	400
TPAGEN	CAAGGCACACAGGACTCAAAG	AACGTGTTCCCGAGGAAC	350
MAGNA	ACCCTCCCATCAGCAAAG	CTTCGTCACAAATCTGCTATC	400

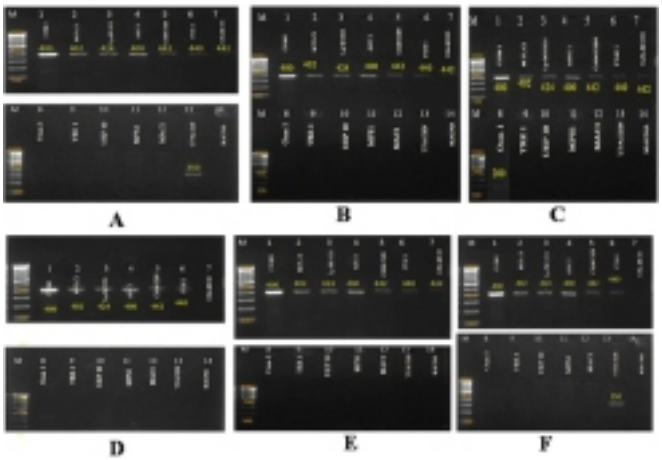


Fig. Detection of pathogenecity gene(s) in RB samples collected from(top to bottom) A) Srinagar, B) Jammu, C) Almora, D) Ludhiana E) cuttack, F) Pattambi

from Ludhiana (Fig 1).

Conclusion:

In nutshell, different primer pairs were designed to target thirteen different virulence genes of *M. grisea* using NCBI and Board Institute Magnaporthe grisea database. Seven genes (*CHM1, MPLC1, LpMOD, ABC1, CAMGEN, CALMOD and TPAGEN*) were detected in the samples collected from Srinagar and Jammu. *TPAGEN* is detected only in Srinagar, Jammu and Pattambi samples. *CHM1, MPLC1, LpMOD, ABC1, and CAMGEN* genes are universally present in the sample collected from all the locations. Thus, it appears that in most of Indian cultivars, these genes regulating the behaviors of virulence of rice blast.

Project 10 : Diversity analysis and microbial management of salt stress in solanaceous crops in UP

PI : Sanjay Goswami
Co-PI : Prem Lal Kashyap

Rationale

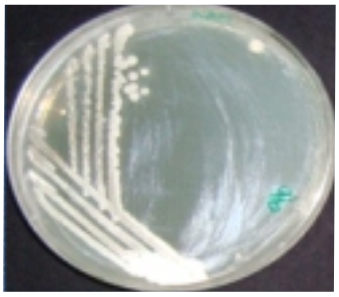
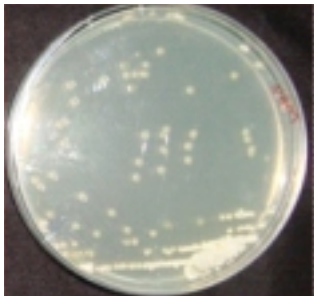
Plants are affected by different type of abiotic stresses. Soil salinity is one of the important abiotic stresses in plants. This is a major constraint to food production in the world. The United Nations environment programme estimate that approximately 20% of agricultural land and 50% crop land in the world is salt stressed. In India about 10 million ha of cultivated land is salt affected. Microbes have been implicated in alleviation of effects of abiotic stresses by various mechanisms like production of osmolytes, sugars, sugar alcohols, exopolysaccharides etc. Such microorganisms not only alter the environment around the rhizosphere of crops but also maintain the ratio of various nutrients. Saline habitats are frequently inhabited by an abundance of microbial communities adapted to these ecosystems. Among the microorganisms, the bacteria play a major role as important and dominant inhabitants of saline and hypersaline environments. The bacteria that live in saline environments may be assigned to two categories: archaebacteria and eubacteria. Archaebacteria are extremely halophilic micro-organisms which grow optionally at salt saturation (up to 30% NaCl).

Objective

- Isolation of microorganisms from rhizotic zones of solanaceous crops (potato, tomato, brinjal and chilli) grown under salt stress.
- Selection of salt tolerant micro-organisms
- Diversity analysis and identification
- Biochemical and molecular characterization of selected micro-organisms.
- Evaluation of selected micro-organisms in the rhizosphere of solanaceous crops (Green house studies) and plant-microbe interaction studies in the rhizosphere.

Significant Achievements

- Survey was conducted on different salt affected areas of UP i.e. Faizabad, Mau, Varanasi, Ballia, Gajipur, Aligarh, Kanpur, Etawah, Manpuri, Aurayai, Aligarh and Fatehpur. 90 Soil samples were collected from the rhizosphere of potato, chilli, tomato and brinjal crops.
- 4 salt tolerant bacteria (10-12% NaCl) viz; *Bacillus pumilus*, *B. safensis*, *Strteptomyces humidus* and *Streptomyces acrimycine* were identified.
- Nutrient agar, Soil extracr agar, Trypticase soy

Salt tolerant bacteria

Bacteria treated

Bacteria+salt treated (10%)

Salt treated (10%)

agar, King's B, Psuedomonas isolation agar, Jensen's N free media, Munaeir and Kenknight agar and Potato dextrose agar media were used for isolation of different salt tolerant bacteria. Many bacteria were found which were different morphologically as well as biochemically. Bacterial populations which were tolerant up to or more than 8% NaCl were selected and tested under pot conditions. Potato plants were observed for their growth enhancement under salt stress conditions in the presence of salt tolerant bacteria. It was found that there was increase in germination, shoot length, root length and yield over control plants.

Conclusion

Salt stress in plants can be manage by the proper and timely utilizatioin of the salt tolerant promising strains of bacteria. These strain has been used for alleviating the effect of salt stress in different solanaceous crops. Salt tolerant bacteria have great potential in salt stress management in plants.

Project 12 : Isolation and characterization of bacterial communities and their metabolites in rhizospheric rice ecosystem.

PI : Lalan Sharma
Co-PI : Sanjay Goswami

Rationale

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, 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. 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.

Significant achievements

Surveyed and collected the rhizospheric soil samples from different regions (Gorakhpur, Lucknow, Kanpur, Varanasi, Meerut and Mau) of Indogangetic plains U.P. Isolation of rhizobacterial populations were made by the different inoculation techniques (soil plate and serial dilution) on various culture media (Nutient agar, Jenson agar, Pikovshaya agar, Burk,s medium, NFB medium, Malate Medium and YEMA medium). A total of 143 rhizobacterial isolates have been isolated from rhizospheric soil samples that were visually characterized for their different morphotypes. Rhizospheric soil is determined for their pH (ranges 7.0-8.4), EC-value (ranges 1.3-1.9) and organic carbon in soil (ranges medium to medium high). As the morphotypes have been tested for the HCN production, Siderophore production and Phosphate solubilization, in which some of the isolates showed significant results to concern. Isolated prominent secondary metabolite producing

rhizosphere bacteria from rice rhizosphere their samples were prepared in methanol and ethyl acetate, and secondary metabolite profiling for the phenylpropanoids was done to characterize potent molecules using HPLC. Standardization of LC-MS conditions for identification of prominent molecules in the rhizosphere produced by these bacteria is underway.

Project 13 : Diversity analysis of archaea from different ecological niches and their characterization

PI : Dipak T. Nagrale
CO-PI : Renu

Rationale

Archaea typically thrive in extreme environmental condition. Domain archaea show an increased resistance to extreme conditions like cold desert, hot springs, hypersalinity and sulphate rich niche underlying their importance in studies of other possible habitable regions. Hypersaline habitats are common throughout the world, but extremely hypersaline habitats are rare and having unique niche. Most such environments are in hot, dry areas of the world. Salt lakes, saltern ponds and hypersaline niche can vary considerably in ionic composition. The predominant ions in a hyper saline lake depend to a major extent on the surrounding topography, geology and general climatic conditions. Many hypersaline environments have originated by evaporation of sea water e.g. Great Salt Lake Sambar lake, India . Their salt composition is similar to that of sea water. However, sodium and chloride are the dominating ions, and the pH is near neutral to slightly alkaline. Due to the evaporation changes occur in the ionic composition as there is the precipitation of gypsum ($CaSO_4.2H_2O$) and other minerals due to their increased solubility. Various salts of chlorides saturated brines as like found in saltern crystallizer ponds oftenly displays a bright colouration due to the large numbers of pigmented microorganisms living in these niches as they releases extracellular enzymes and/or metabolites. These hypersaline niche like crystallizer ponds, salt lakes in which the concentration of divalent cations is more than that of monovalent cations with relatively low pH(6.0-6.5).and in which the pH is relatively low (around 6.0).These extremophile microorganisms has adapted to environments combine high salt concentrations with very high pH values. Alkaline salt lakes are known to found in Africa, India, China and other parts of the world with pH values more than11 and higher and salt. Archaea host a new class of potentially useful antibiotics. Archaea can provide novel insights into possible early life formation since they are known for their longevity and their ability to survive for several million years.

Objective

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

Salient achievements

The distinct archaeal colonies were developed on haloarchaea media which are able to grow at 25% salt concentrations. Seven and three different haloarchaea isolates were isolated and differentiated on the basis morphotypes and pigmentation from Bhayender and Meera road Mumbai suburban salt crystallizer pond, respectively. The morphological, physiological, biochemical and molecular characterization of these isolates are in progress to study the diversity and community analysis from these niches.

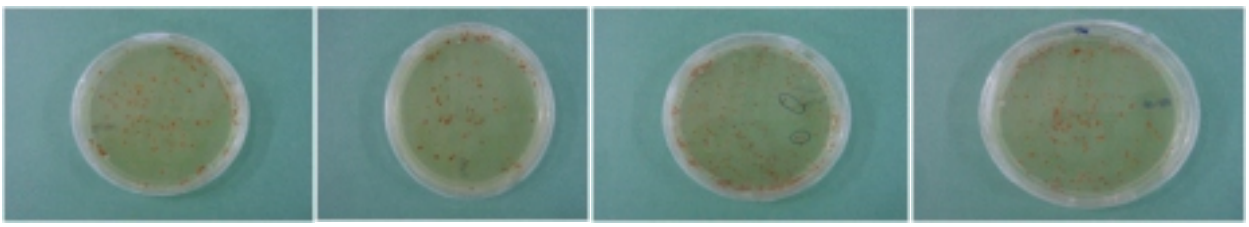


Fig. Haloarchaeal isolates growth on haloarchaea medium on 25% NaCl at 37°C

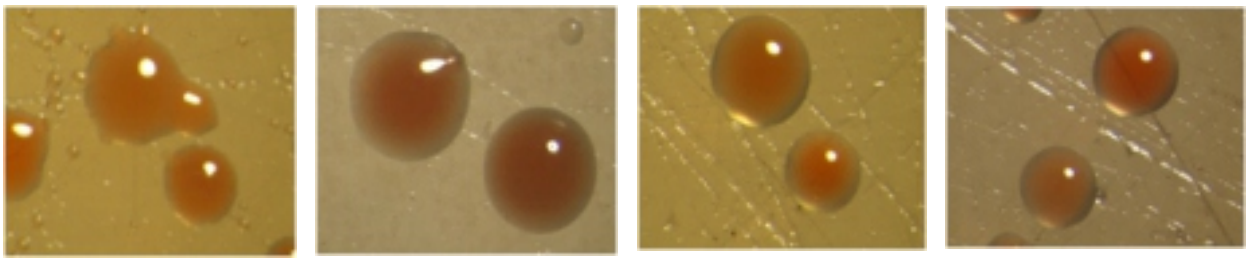


Fig. Stereomicroscopic view of haloarchaeal isolates showing distinct morphotypes and pigmentation on haloarchaea medium after 8-15 days at incubation at 37C°

Conclusion

Very few workers have done research on archaeal community structure and diversity in India and abroad. Also, a meager work has been carried on archaea from hypersaline and alkaline conditions. The project will be helpful in identifying the haloarchaeal communities and their diversity from different ecological niche especially hypersaline niches. Their characterization will give the valuable information about the archaea(s).Valuable information about archaeal diversity and communities arising from the project will add to the existing extremophile halophilic microbial diversity studies carried out at the bureau and in turn fulfill the mandate of the Bureau.

Rationale

India has been considered as a diversity hot spot in the world because of its climatic conditions ranges from extreme cold to hot desert of Rajasthan. The majority of life on Earth is microscopic in size, only visible through the microscope. Microbes are ubiquitous and they inhabit any environment, in very cold (below 0°C) to very hot (above 100°C), from very acidic to very alkaline sites, or high saline concentration, high pressure, or any other environment that might not look normal to humans. Microbes thriving under extreme conditions are the extremophiles.This variable selection of systems is reflected by the huge diversity exiting in the microbial world. Currently, the level of microbial diversity on earth is a topic of debate. While many microorganisms can easily be dispersed across the planet, the so called extreme environments present a selective factor for the living beings to develop in them. Thus, extreme environments can be considered as ideal model systems for studying ecological properties of microorganisms, their physiology, adaptative properties and many other characteristics related to microbial communities and specific microbial cells. Due to the small size of microbes, working with them is not an easy task. Microscopic examination is required to see them. Classical microbiological studies required the ability to grow the microorganisms in the laboratory to be able to study their properties, such as growth conditions, nutrient sources, metabolic products, and so on. We now know that most microorganisms in the environment can not be brought into cultivation. So, we have to design novel strategies to investigate their diversity, function, and potential applications. In order to understand our Planet, we are interested in the who, what, when, where, why and how questions about microbial life. The way to do it brings up an exciting experience to discover step by step the diverse microbial world. It is too difficult to duplicate an organism's host environment in a laboratory. However, some microbial properties can be analyzed in the laboratory using isolated microorganisms. This apparent contradiction suggests a need for a variety of methodologies to be applied either in laboratory or in field. With the advent of modern molecular techniques, we can understand the microbial environment in much greater detail. It is possible to use the information hold in the acids of microorganisms to detect those present in an environment. Thus, molecular methods based on DNA and RNA represents a very useful set of techniques to analyze microbial communities *in situ*. But nucleic acids can be used to both detect a microorganism and to look at its functional genes, and we can do similar research on microbial communities through metagenomic approaches. Of course, the amount of data to be processed increases exponentially with the number of different microorganisms in a sample and bioinformatic tools become an essential part of the research process. Detection of microorganisms and their functional genes are also complemented with an evaluation and analysis of the processes being carried out by those microbial communities in the environment. Microorganisms are never alone. They work in communities, often composed by a large number of different types of cells. The study of microbial communities and their interaction with the environment are key aspects to understand the role and function of microorganisms in nature, and consequently the implications of microbial life at local

and global scales in our planet. Microorganisms thriving under extreme conditions generally present specific adaptations which allow them to develop in unique environments. The properties of their biomolecules are of interest in biotechnology due to their high stability which can be used in potential applications to industry or processes of commercial interest. The search for unique microorganisms and molecules also require the use of a variety of techniques.

Objective

- Microbial diversity analysis in extreme climates
- Identification of osmolytes production by extremophilic microorganisms
- To look for production of enzymes (amylase, cellulose, CMCase, FPase, Xylanase and protease) in extremophilic microorganisms
- Biochemical and molecular characterization of extremophilic microorganisms
- Development of genus and species specific diagonastic kit for identification of potent microorganisms like pseudomonas and flurocent pseudomonads

Significant achievements

I. Temporal and spatial shift of methylotrophic bacteria in Bhitarkanica mangroove

Significant achievements

- A total of 90 isolates were selected on the basis of salt tolerance, pigmentation and colonial structure. The isolates were subjected to molecular identification using 16SrRNA gene, representative isolates were selected on the basis RFLP pattern generated using three different restriction enzyme digestions (Fig. 2).
- The 16S rRNA gene was amplified from metagenomic DNA (B1 to B5) and then the sub

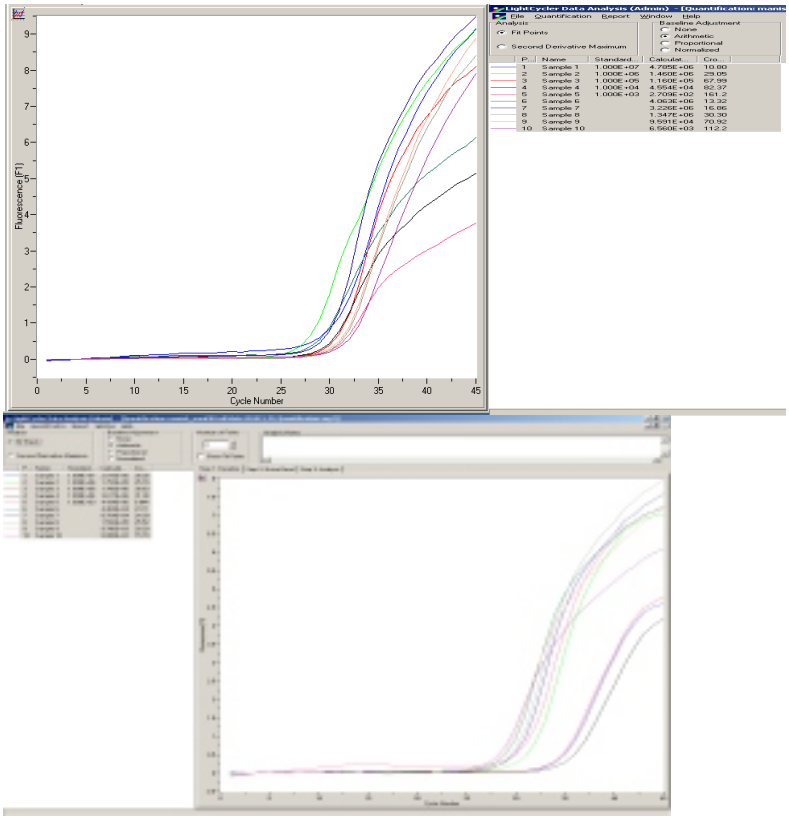


Fig. A &B B1 RT-PCR based mxaF gene quatification from environmental samples in different seasons

region V3-V5 was amplified and product was run through the denaturing gradient gel electrophoresis (DGGE). Different bands were appeared and cut down to carry out the nested PCR with the primers having no GC clamps.The amplified products were purified and ready to sequence or purified bands were taken as insert for cloning.

- Quantification of *mxaF* gene was done in terms of copies using LightCycler software 3.5 based on 'second derivative maximum method' (Roche Diagnostics, Switzerland) in which exponential phase of amplification curve is linearly related to a starting concentration of template DNA molecules. Quantitative PCR was carried out using SYBR GreenI technology with the primers *mxaF* and *mxaR* and environmental DNA samples (B1 to B5 and BN1 to BN5) were amplified from sediment, negative control and five plasmid DNA standards.
- The *mxaF* gene copy number from sediment of season1 was in the range $8.8x10^2$ to $1.5x10^5$ and from season 2 $6.56x10^3$ to $4.06x10^6$. Maximum abundance of the functional gene was observed at site B1 (Kanika).

II. Diversity of Actinomycetes from Extreme Environments of India

Rationale

Extreme environments represent a unique ecosystem and may harbor novel microbial flora. Extremophiles can be grouped according to the conditions in which they thrive. Thus, there are thermophiles and psychrophiles (which grow at the extremes of temperature ranges, barophiles or piezophiles-high pressures), halophiles (high salt), acidophiles and alkaliphiles (extremes of pH).

Objective

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

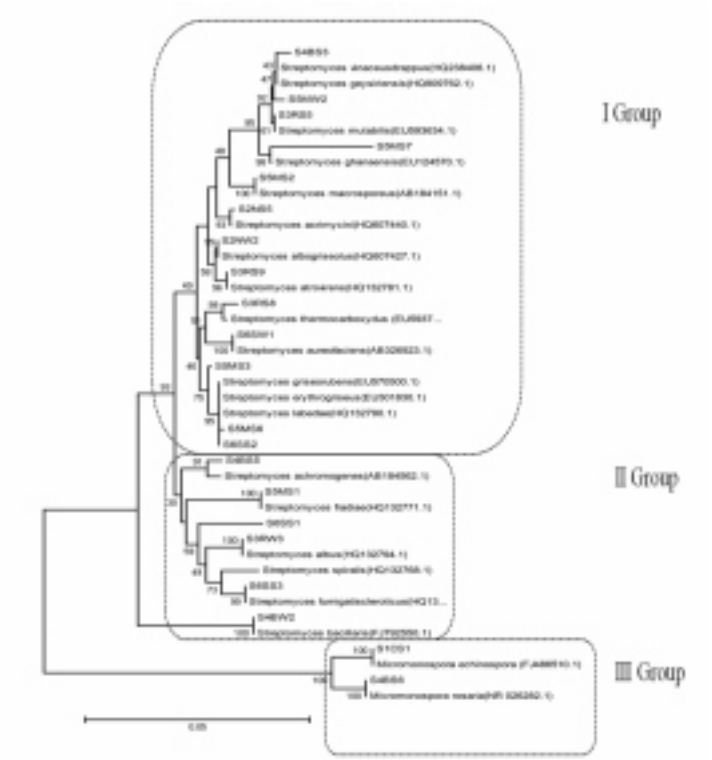


Fig. Phylogenetic tree based on the 16S rRNA gene sequences of alkali-halophilic actinomycetes isolates and their closest phylogenetic relatives. The tree was created by the neighbor-joining method. The boot strap values from 5000 pseudoreplications are shown at each of the branch points on the tree. bar indicates % similarity.

Significant achievements

- A total of 116 actinomycetes were procured from Chilika lake by employing various media and enrichment techniques and sorted out into 59 different morphospecies based on colour of aerial and substrate mycelium, pigmentation and microscopic examination.
- Further screening for alkali-halophilic nature of 59 morphospecies revealed that a total of 21 isolates possessed the ability to grow at pH 7.0 to 10.0 and 2.0 to 10% NaCl.
- The population frequency isolated per sample of streptomycetes in Chilika lake was shown to be different in all three sectors. Central sector harboured maximum population (Chadheiguha 28.4% and Nalabana 23.2%), followed by South sector (Rambha, 21.5%; Badakuda, 10.3%), while least population frequency was recorded in sea mouth sector (Manika patana, 10.3%; Sea mouth, 6.8%). Sediment samples had highest streptomycetes population frequencies than lake water while, population frequencies of alkali-halophilic streptomycetes (pH 9.0; NaCl 10%) shown to be decreased from marine habitat to fresh water lake.
- Characterization of 59 different morphospecies revealed that south sector harboured maximum percentage of siderophore producers (48.5%) while Central sector had highest IAA (45%), and extracellular protease enzyme (45.1%) producers. Sea mouth sector was enriched with nitrate reductase activity (42.3%) and had antimicrobial activity (38.8%) and chitinase enzyme (39%) production ability. Chitinase and protease enzyme producing isolates were procured from sediment samples while cellular siderophore, IAA, antimicrobial activity and nitrate reductase potential activity was found to be highest in water samples.
- Catabolic carbon assimilation pattern was analysed based on utilization/ non-utilization of 95 substrates studied by the BIOLOG™ system, and conversion of values into binary matrix (1/0) followed by clustering (dendrogram) for all isolates. At a 70% similarity level, all isolates were grouped into one major and eleven minor groups.

All actinomycetes isolates showed different types of carbon substrate utilization pattern ranging from 9-82 out of 95 substrates.

- Phylogenetic analysis representing three phyloclades with *S. albus*, *S. bacillaris*, *S. fradiae S. achromogenes, S. fumigatiscleroticus S. spiralis* falls under first group while, *S. albogriseolus*, *S. acrimycini*, *S. mutabilis*, *S. thermocarboxydus, S. geysiriensis, S. vinaceusdrappus, S. macrosporeus, S. griseorubens, S. labedae, S. aureofaciens, S. erythrogriseus, S. atrovirens, S. ghanaensis* represent second group and lastly *M. rosaria, M. echinospora* forms third group showing a complete out group among each other. Isolates S3RS8, S4BS3 and S3RW3 seems to be novel as they showed similarity with many *Streptomyces* species.

Conclusion

In conclusion, *Streptomyces* population in different sectors of Chilika lake were considerably varied with their physiological as well as biochemical profiles, which enable us to understand behavioural, ecological, as well as specific substrate requirement in a particular brackish niche. The marine environment is a good source of alkali-halophilic *Streptomyces* population having potentially new bioactive compounds which can be important in future bioprospecting in agricultural, industrial and pharmacological sectors. Further, emphasis should be made on research based on spatial and seasonal fluctuation of actinomycetes population as well as employing new methodologies for the isolation and diversity analysis of actinomycetes with inclusion of rare species and different genera.

Exploration, Collection and Characterization of some Agriculturally Important Biocontrol Agents Suitable for Disease Management

PI : Dilip K. Arora
Co PIs : Alok Kumar Srivastava, Sudheer Kumar

Rationale

In India, legumes are one of the sources of high quality protein for the rural poor and are also used as fodder, forages, and green manure. They are as well used for soil recovery and improvement through symbiosis with soil bacteria. Plants are constantly threatened by a number of pathogens. Fungal diseases are the most important biotic factors limiting crop production in chickpea. Nearly 172 pathogens attack chickpea, which includes 67 species of fungi. Early studies on PGPR focused more on biological control of plant diseases than on growth promotion, and involved bacteria like *fluorescent pseudomonads, Trichoderma* spp. and *Bacillus subtilis* that are antagonistic to soil-borne plant pathogens. A consortium of PGPR may often have more influence on biological control and plant growth than a single strain. The number of bacterial species identified as PGPR increased recently as a result of the numerous studies covering a wider range of plant species (wild, economically important and tree) and because of the advances made in bacterial taxonomy and the progress in our understanding of the different mechanisms of action of PGPR.

Objectives

- Selection of antagonists for pathogens (*Fusarium* spp.*).*
- Screening and selection of potential antagonistic isolates for important field crops.
- Characterization of active principle responsible for antagonisms.
- Dosage standardization and delivery system.
- Determination of shelf-life of formulations.
- Mass multiplication of antagonists.
- Field evaluation of potent bio control agents.

Significant Achievement

- A total 480 strains of *Bacillus* and *Bacillus* derived genera, 110 fungal were isolated from the rhizosphere of wheat, mustard, potato and chickpea crops. All the isolates were tested for *in vitro* screening against chickpea pathogens causes wilt disease (*Fusarium oxysporum* f. sp. *ciceri*, *FOC*), black root rot (*F. solani, FS*) and charcoal rot (*Macrophomina phaseolina, MP)* of chickpea are going to be commercialized.
- Out of these only four (B-7, A-90, B-11and T-8) isolates were screened *in vitro* activity for their plant growth promoting traits like production of indoleacetic acid, siderophore, phosphate solubilization and production of hydrolytic enzymes and in the test for antagonism they can also produced strong inhibition to chickpea

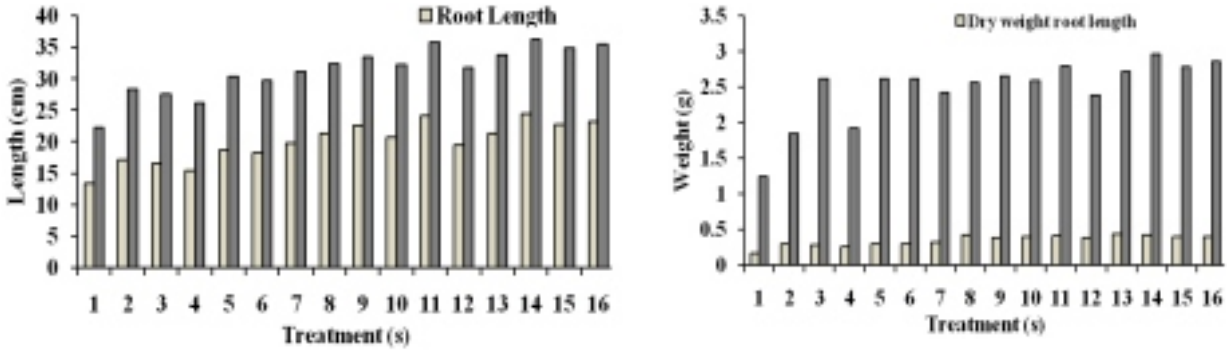


Fig. Evaluation of different combination strains inoculum for plant growth-promotion and protection against *Fusarium* wilt in chickpea (cv. BGD- 72) under green house conditions.

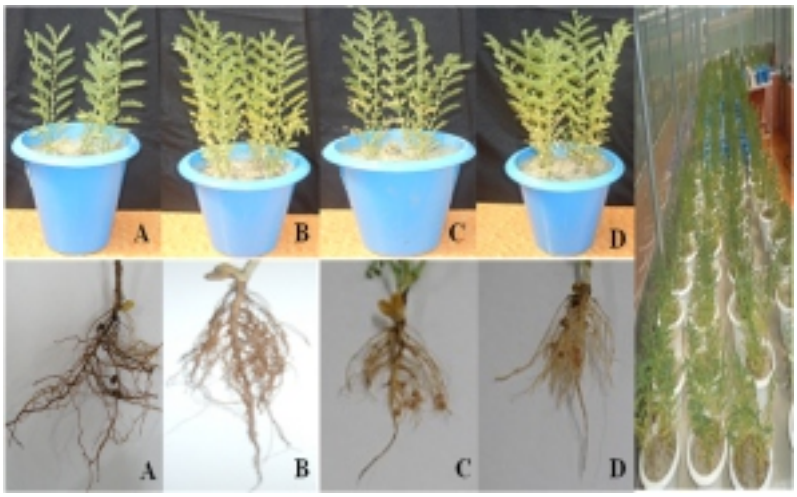


Fig. *In vivo* green house experiment three different combinations strain (B, C, and D) as compared to control (A) shows significant growth in root and shoots length.

fungal plant pathogens. All selected strains having several valuable functions were found to perform well under green house conditions in chickpea crops. These isolates for *in vivo* experiments in a greenhouse assay proved to be efficient in promoting a significantly increase in the length (root and shoot) and dry matter of chickpea plants.

- Plant growth-promoting rhizobacteria (PGPR) and Plant growth-promoting fungal (PGPF) strain *B. subtilis* (B-7), *F. pseudomonas* (A-90), Unknown bacterial sp. (B-11) and *T. asperullum* (T-8) were tested singly and in combinations for the different treatments is used: B-7, B-11, A-90, T-8, B-7 + B-11, B-7 + A-90, B-7 + T-8, B-11 + A-90, B-11 + T-8, A-90 + T-8, B-7 + B-11 + A-90, B-7 + A-90 + T8, B-7 + T-8 + B-11, B-11 + A-90 +T-8, and B-7 + B-11 + A-90 +T-8.
- Out of these combinations, three different combination strains for different treatment i.e., 11 (B-7 + B-11 + A-90), 13 (B-7 + T-8 + B-11) and 15 (B-7 + B-11 + A-90 +T-8) showed a significant results. For *in vivo* experiments in a greenhouse further these isolates were tested in large scale experiments in field conditions after we used these isolates in a formulation and consortium development.

Conclusion

Formulation and consortia development of 11 (B-7 + B-11 + A-90), 13 (B-7 + T-8 + B-11) and 15 (B-7 + B-11 + A-90 +T-8) selected strains based on their biocontrol potential having several valuable functions against *Fusarium* sp. causes wilt disease of chickpea are going to be commercialized.

Assessing structural and functional shifts in soil microbial communities of paper mill effluent contaminated soils and utilization of microflora for crop growth promotion in these soils

PI : Dilip K. Arora
Co PI : Kamlesh Kumar Meena

Introduction

Soil microbial communities are dynamic components of ecosystems, playing a major role in soil organic matter (SOM) decomposition as well as contributing to plant water and nutrient acquisition and mineral weathering. The size and structure of microbial communities affect soil nutrient cycling. Microbial communities are therefore key to understanding ecosystem-level processes including plant productivity. Shifts in microbial community structure and function occur due to changes in soil inputs over a period of time. The rapid shifts in native microbial community structure of arable lands due to discharge of pollutants in liquid or solid form affects the population dynamics of beneficial soil microflora. Thus, affecting the soil fertility in long run. The effluent from century pulp and paper mill, Lal Kuan, Uttrakhand is generated approximately at the rate of 72- 225 m^3/ton paper. This large amount of effluent is discharged in water bodies from which water is used for irrigating the crops. The addition of "mixed bag" of compounds, found in pulp and paper mill effluent, may induce changes in the physiochemical properties of soil and also create significant shifts in structure and function of the associated microbial community, which in turn may ultimately affect the soil viability for agriculture purposes.

Objectives

- To assess the functional and structural shift in culturable soil microbial population as a result of long term irrigation of pulp and paper mill effluent.
- Diversity analysis of unculturable microflora in pulp and paper mill effluent contaminated soils.
- Characterization and utilization of selected microbial isolates for plant growth promotion in effluent degraded soil.

Significant Achievements

Previously we reported changes observed in structure of microbial communities based on sequence analysis of 16S rDNA clones obtained from soil metagenome of effluent and fresh water irrigated soil. Variation was observed between phylas such as α, β, γ, and δ subdivisions of the *Proteobacteria, Acidobacteria, Firmicutes, Bacteroidetes, Planctomycetes, Cyanobacteria*, *Chloroflexi*, *Gemmatimonadates*, and *Aquificae*. Similarly, the dominant genera in which significant variation was observed between effluent and fresh water irrigated soil were *Bacillus*, *Brevibacillus*, *Amycolaptosis*, *Staphylococcus*, *Flavobacterium*, *Streptomyces*, *Arthrobacter*, *Rheinheimera*, *Sphingomonas*, *Azospirillum*, *Ochrmobacter* and *Pseudomonas*.In this year we carried out changes in functional aspects of microbial community as a result of long term effluent irrigation in soil

Bacterial isolates obtained from effluent and fresh water irrigated fields (71 from water irrigated fields (WIF) and 57 from effluent irrigated fields (EIF)) were selected for functional characterization.

- All the 128 isolates were screened for their functional attributes and extracellular enzyme production.
- Indolic compound production, ammonia production and P-solubilization activity and decreased in effluent irrigated soils as compared to fresh water irrigated. However, siderophore production was higher among effluent irrigated soils.
- Xylanse and cellulose activity was higher in effluent irrigated soil as compared to fresh water irrigated soils.
- *Bacillus, Brevibacillus, Amycolaptosis, Streptomyces, Arthrobacter,* and *Pseudomonas* showed significant xylanse activity among identified isolates.
- Based on C-substrate utilization pattern studied by ECO plates of BIOLOG it was observed that out of 57 isolates obtained from effluent irrigated soils (mainly belonging to group α - *Proteobacteria, Acidobacteria, and Bacteroidetes*) utilized 22-28 C sources out of 31 present in ECO plates. In contrast, out of 71 isolates from fresh water irrigated soils utilized only 11-21 C sources (mainly belonging to β-Proteobacteria and *Firmicutes*)

Conclusion

In conclusion, we found that microbial communities in pulp and paper mill effluent irrigated fields were functionally more diverse in terms of C source utilization. However, bacterial isolates obtained from effluent irrigated soils showed reduced plant growth promoting attributes.

Development of microbial consortium for alleviation of salt and drought stress for growth and yield of wheat.

PI : Dilip K. Arora
Co PI : Kamlesh Kumar Meena

Rationale

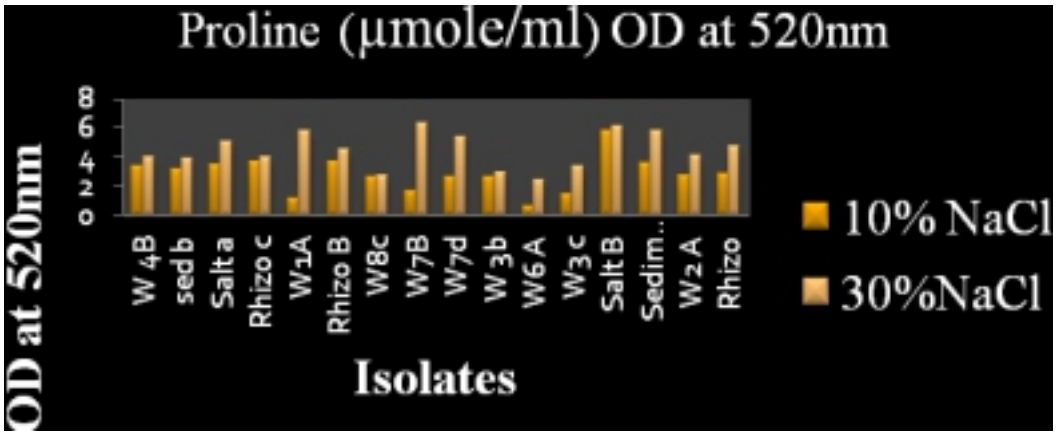
Environmental stresses represent the most limiting factors for agricultural productivity. Apart from biotic stress caused by plant pathogens, there are a number of abiotic stresses such as extremes in temperature, drought, salinity, heavy metals and radiation which all have detrimental effects on plant growth and yield. Drought and Salinity affects plant growth and development adversely and exerts negative impact on critical ecological balance in the agroecosystem to disturb biological stability (Yokoi *et al*. 2002).Metabolic imbalances caused by ion toxicity, osmotic stress and nutritional deficiency under saline conditions may also lead to oxidative stress (Zhu, 2002). It has been claimed by one study that abiotic stress causes the most crop loss of any other factor and that most major crops are reduced in their yield by more than 50% from their potential yield. Microorganisms surviving at extreme environmental conditions are suitable for use in different agricultural practices. Understanding the complexity of microbial adaptations into stressed rhizosphere environment and the effect of these microorganisms on biological, chemical, and physical properties of rhizosphere soil and the plants remains a significant challenge .The project therefore addresses the application of microbial consortium for the alleviation of salt and drought stress in wheat crop.

Objectives

- Survey of salt and drought affected area of India.
- Isolation of microorganisms from rhizotic zones of cereal crop grown under salt stress and drought stress.
- Screening of salt and drought tolerant bacteria at different NaCl and PEG concentration.
- Evaluation of selected micro-organisms in the rhizosphere of cereal crop on the basis of phytotron studies.
- Biochemical and molecular characterization of selected microorganisms.
- Development of consortium of microorganisms that can alleviate the effect of salinity and drought to improve the growth and yield of cereal crop (wheat).
- Field evaluation of consortium of microorganisms for improvement of wheat growth and yield.
- Osmoprotectant studies (proline, glycine betaine) on salt tolerant and drought tolerant bacteria.

Significant achievements

- Total 53 isolates from Rajasthan (Bikaner and Jaisalmer) , 110 isolates from drought affected region of Kutch region of Gujarat and 27 isolates were isolated from salt affected region of Sambhar salt lake Rajasthan .All isolates were screened at different concentration of PEG (5% (-0.5MPa) – 25% (-1.7MPa)) for drought and NaCl (25% to 30 %) for salt. Out of 163 isolates only 22



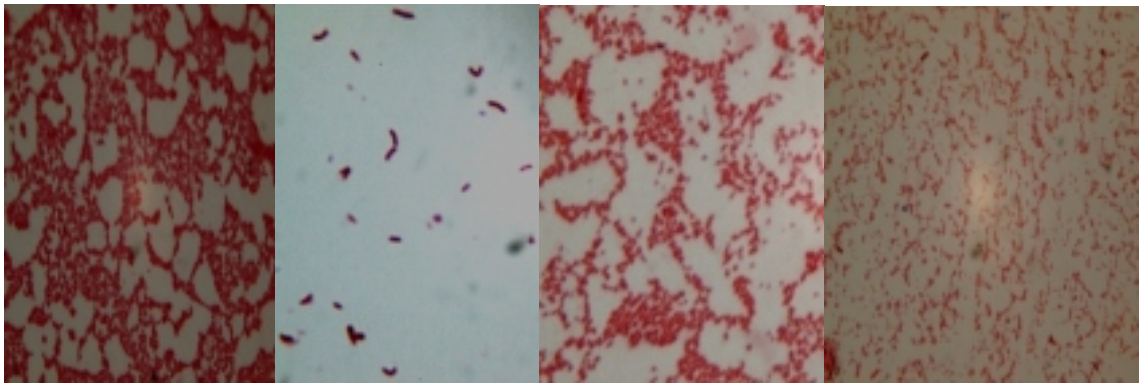
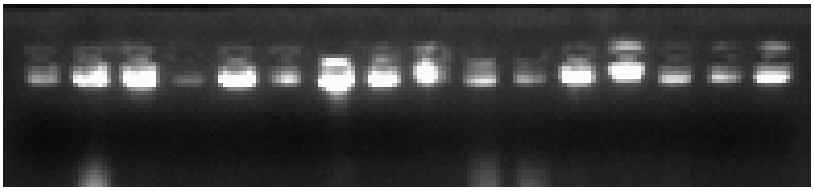


Fig: Microscopic pictures of bacterial isolates (Rajasthan soil samples)



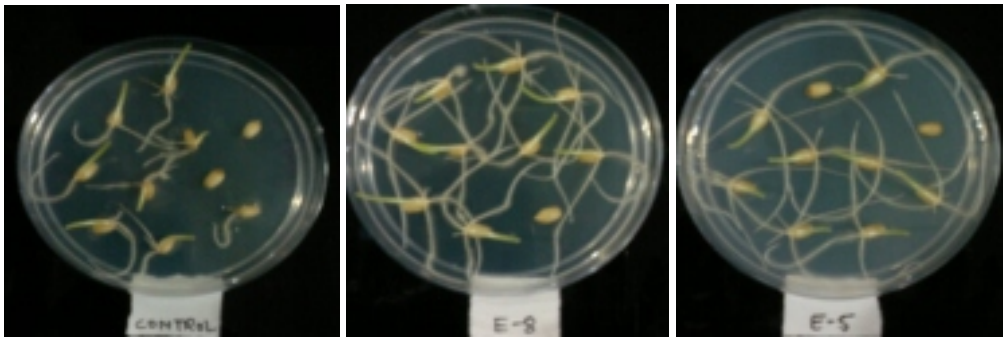


Fig: Seed germination test of wheat coated with drought tolerant bacterial strain .

isolates were grow up to 25% PEG concentration whereas out of 27 isolate from Sambhar salt lake only 16 isolates were able to grow up to 30%.

- The isolates which were able to grow at higher PEG conc. ,were screened for their different PGP traits like IAA, Siderophore, phosphate solubilization, ammonia production, ACC deaminase activity. And isolates grow on 30% NaCl conc. showed ammonia production, urease hydrolysis and gelatin hydrolysis only.
- Osmoprotectant like proline estimation of 16 salt tolerant isolates showed positive results.
- Isolates which were grow at higher PEG conc. were phenotypically as well as biochemically characterized by using 96 Carbone source utilization pattern
- 16S r DNA gene were amplified of Salt tolerant as well as drought tolerant. Restriction fragment analysis of all drought tolerant isolates has completed using restriction enzymes *HaeIII and MspI* for clustering the isolates and for further identification. Sequencing data were analyzed using different software's BIOEDIT, CLUSTRAL-W, BLAST which shows identification like *Bacillus pumilus, Acinetobacter baumanni, Micrococcus* sp., *Lysinibacillus sphaericus* etc.
- Seven potent isolates were inoculated in wheat individually and with combination to assess microbe mediated responses and plant growth promotion in drought affected soil through green house experiment and seed assay has carried out with different PEG concentration in petridishes. When inoculated with these isolates, plants showed enhanced root and shoot length, biomass, and biochemical levels such as

chlorophyll, carotenoids, and protein. Our results confirm other reports about plant growth promotion due to bacterial inoculation. These strains could be useful in stress management improving the wheat cropping systems into which they could be most beneficial.

Conclusion

In conclusion, present investigation revealed the microbes which showed drought tolerance up to 25% PEG concentration with innate potential of mineralizing phosphate, plant growth promoting traits and stress tolerance properties. Cultivable isolates of drought tolerant isolates further explored for consortia developmental studies as a bioinoculant under alleviated abiotic stress for growth and yield of wheat crop.

II. Utilization of Actinomycetes to Alleviate Salt and Drought Stress for Cereal Crops

Rationale

Abiotic stresses such as droughtis one of the major agricultural problems limiting crop productivity in most of the arid and semi-aridregions of the world. Over 68% of India is vulnerable to drought. Microbes have been implicated in alleviation of effects of abiotic stresses by various mechanisms like production of osmolytes, sugars, sugar alcohols, exopolysaccharides etc. Such microorganisms not only alter the environment around the rhizosphere of crops but also maintain the ratio of various nutrients. Actinomycetes constitute a significant proportion of the microbial population in most soils, their propagule and spore count being about 10^{-10}per gram. The temperate,well-drained soils constitute around 95% of the filamentous actinomycetes. The spores of most actinomycetes withstand desiccation and show slightly higher resistance to dry or wet heat than vegetative cells Most of the actinomycetes possess inherent capacity to tolerate drought (especially*Rhodococcus*spp. and *Streptomyces* spp.) by synthesis of the compatible solutes like alanine, proline, glycine betaine and -glutamine in response to stresses. It is also known that actinomycetes are known produce antibiotics and secondary metabolites of importance. They are known to inhibit many plant pathogens and some are known to produce plant growth promoting substances.Thus keeping these points in consideration, an attempt was made that they can be utilized to alleviate the drought stresses and increase the crop yields under drought affected soils.

Objectives

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

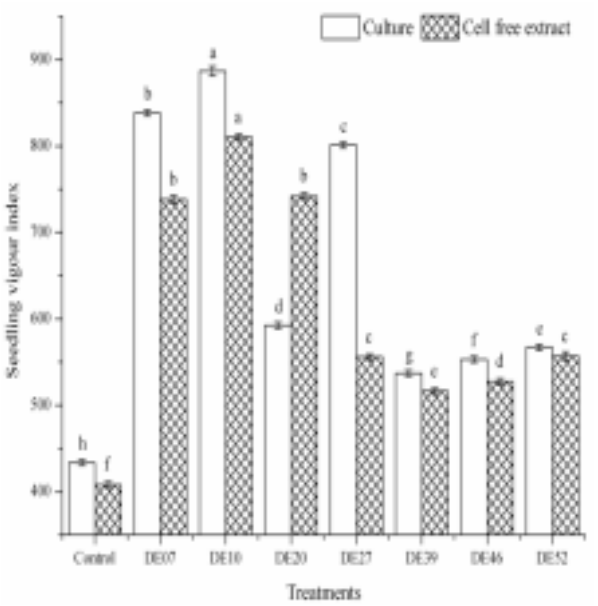


Fig. Seedling vigour indices of culture and cell free extracts of actinobacteria treated wheat seeds at ten days after inoculation. Control: seedlings without treatment of culture or cell free extracts of actinobacteria, DE07: *Streptomyces coelicolor* DE07, DE10: *S. olivaceus* DE10, DE27: *S. geysiriensis* DE27, DE20: *Streptomyces* sp. DE20, DE39: *Streptomyces* sp. DE39, DE46: *Streptomyces* sp. DE46, DE52: *Streptomyces* sp. DE52. Experiment was repeated three times and mean with different letters on the top of error bars indicate statistically different values at $P \leq 0.05$ using Duncan's Multiple Range Test (DMRT). Seedling vigour index = (mean root length + mean shoot length) × germination (%).

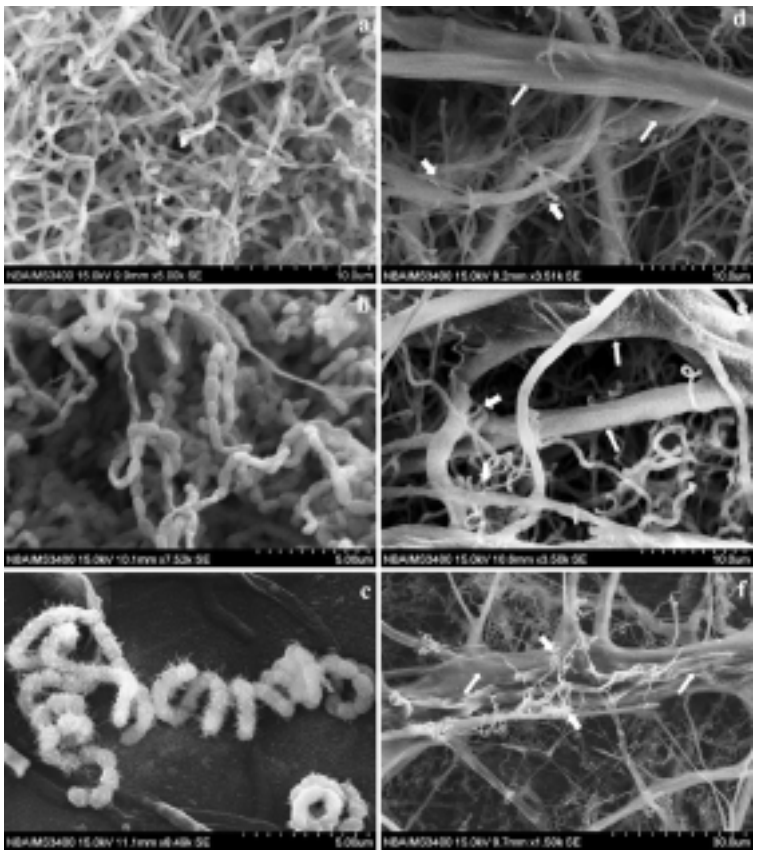


Fig. Scanning electron microscopy (SEM) of actinobacteria isolates, showing variations in spore chain morphology of a *Streptomyces coelicolor* DE07, b*S. olivaceus* DE10, c*S. geysiriensis* DE27 and wheat root colonization with d *Streptomyces coelicolor*DE07, b *S. olivaceus* DE10 and c *S. geysiriensis* after 10 days of inoculation. Thin arrows represent the wheat roots and thick arrows represent endophytic actinobacterial colonization on roots.

Significant achievements

- Forty six morphotypes of endophytic actinobacteria were isolated from drought tolerant plant roots. Nineteen morphotypes were able to show growth under -0.73Mpa (25% PEG) water stress condition. Among the endophytes 7 isolates DE07, DE10, DE20, DE27, DE39, DE46 and DE52 that showed significant growth and IAA production at -0.73Mpa of PEG6000 were chosen for seedling vigour assay and colonization studies. Isolate DE07, DE10 and DE27 showed higher seedling vigour with cell treatmentas compared to cell-free extracts.
- Maximum seedling vigour was recorded with isolate DE10, followed by DE07 and DE27 and showed significant vigour over the control. Scanning electron microscope studies also showed actinobacterial colonization on the root surface by the isolate DE07, DE10 and DE27, where actinobacteria were connected together by an extracellular polymeric matrix colony formation under water stress conditions.
- BLASTn homology search of NCBI for 16S rRNA gene sequences of all the three strains were confirmed as *Streptomyces* sp. The strain DE10 had 100% similarity with *Streptomyces olivaceus* whereas strains DE07 and DE27 showed 99% similarity with *Streptomyces coelicolor* and *S.geysiriensis*. The gene sequences of DE07, DE10 and DE27 were submitted to GenBank (NCBI) with accession numbers JN204723, JN204724 and JN204724, respectively.
- Quantitative IAA estimation at different time intervals resulted through growth kinetics and IAA synthesis in liquid culture demonstrated that actinobacterial isolates grew well in normal conditions and synthesized IAA significantly as compared to water stress conditions (Fig. 3). Isolate DE07 and DE10 synthesized and accumulated IAA in the logarithmic phase

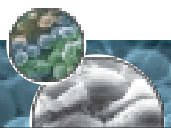
Table. Organisms and their habitats used in the study, accession numbers, PGP traits and water stress tolerance properties

Organisms	*Streptomyces coelicolor*DE07	*Streptomyces olivaceus* DE10	*Streptomyces geysiriensis* DE27
Habitat	Bikaner, Rajasthan, India (28.01°N,73.17°E)	Bikaner, Rajasthan, India (28.01°N,73.17°E)	Jaisalmer, Rajasthan, India (26.55°N,70.55°E)
GenBank Accession number	JN204723	JN204724	JN204725
Similarity %	99	100	99
PGP traits			
IAA (µg mg^{-1} protein)#	79.53	84.34	82.48
Siderophore production	ND	+	+
Ammonia production	+	+	+
Biochemical characterization			
Lysozyme sensitivity	+	+	+
Gelatin liquefaction	ND	ND	+
Hydrogen sulphide production	+	ND	ND
Casein hydrolysis	ND	+	ND
Urea hydrolysis	+	+	+
Water stress tolerance potential			
Water stress tolerancelimit (Mpa)*	-0.05 to -0.73	-0.05 to -0.73	-0.05 to -0.73

ND not detected, + positive; * polyethylene glycol (PEG6000) range on which the organisms were grown successfully; # IAA production estimated using HPLC

whereas isolate DE27 produced IAA after stationary phase of growth till to death phase under normal conditions. However, isolate DE07 and DE27 produced highest IAA under water stress condition till end of logarithmic phase of growth, whereas DE10 produced highest IAA in the midst of logarithmic phase. In general cultures showed increased IAA production under normal conditions, and decreased IAA production under water stress conditions after peak growth phase. DE27 showed minimum accumulation in normal and water stress condition in comparison to other isolates.

- Plant growth under drought stress under field conditions with actinobacterial inoculation (single and combined) were significant ($P \leq 0.05$) in comparison to control for all the studied growth and yield parameters. Among all the treatments, *S. geysiriensis* DE10 culture treated plants recorded maximum shoot length, number of tillers and panicles, fresh shoot and root weight, dry shoot and root weight, and yield. In combined inoculations, *S. coelicolor* DE07 + *S.geysiriensis* DE27 resulted highest plant growth through root length, number of tillers and panicles, fresh shoot and root weight and dry shoot weight. Highest yield was recorded with combined inoculation of *S. olivaceus* DE10 + *S. geysiriensis* DE27 cultures and lowest yield with *S. coelicolor* DE07 + *S. geysiriensis* DE27 in comparison to control (242 kg ha^{-1}). Combined inoculations were better in yield as compared to single inoculations and cell culture treatments were better than cell-free extract treated seeds.
- Production of phytohormones, plant growth promotion traits combined with water stress tolerance potential in these endophyticactinobacteria played a cumulative synergistic role that supported enhanced plant growth promotion of wheat in the stressed soil.



Complete Genome Sequencing of *Mesorhizobium ciceri* Ca 181

PIs : Dilip K. Arora, N. K. Singh, Major Singh, K. V. Bhat
Co- PIs : Alok Kumar Srivastava, Kanika, A. B. Gaikwad, Rakesh Singh

Rationale

Mesorhizobium ciceri ca181 is a nodule forming soil bacterium of chickpea *Rhizobia* with very high specific qualities like, efficient nitrogen fixation and shows good nodulation competitiveness and performed well at different locations in different agro-climatic regions and soil types. In agriculture, *Rhizobium* sp. improves soil fertility in leguminous crops by biological nitrogen fixation. *Rhizobium* sp. strains are very sensitive to soil environmental abiotic factors such as desiccation, water stresses, high salt, pH, and temperature stresses that affect their nitrogen fixation capacity and hence the productivity of legumes. An understanding of the genetic potential for increased tolerance to these adverse environmental stresses could enhance production of food and forage legumes in semi-arid and arid regions of the world. Phosphorus (P) is one of the major essential macronutrients for plants and is applied to soil in the form of phosphate fertilizers. Microorganisms are involved in a range of processes that affect the transformation of soil P from phosphate and are thus an integral part of the soil P cycle. In particular, soil microorganisms are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization. Complete genome sequences give us a whole genomic blue print of an organism such as *M. c.* Ca181. This will dissect nitrogen fixation process at molecular level so as to enable us manipulate genes involved for increasing crop productivity. Simple sequence repeats (SSRs) or microsatellites are tandem short stretches of DNA with the repeat units of 1-6 bp in length for varying numbers of times, and the property of microsatellite is determined by their composition, motif length, and the distribution in the genome. Simple sequence repeats (SSRs) or microsatellites, as genetic markers, are ubiquitous in genomes of various organisms. The analysis of SSRs in various *Rhizobium* strains will provide useful information for a variety of applications in population genetics of *Rhizobia*.

Moreover, generating *M. c.* Ca181 mutants for important genes like gens involved in water stresses tolerance and phosphate solubilization will increase our understanding about these genes. Using the knowledge of different gene sequences and their function, it may by be possible to use them for increasing crop yield and development of transgenic with enhanced biological nitrogen fixation ability and enhanced phosphate solubilization under abiotic stresses.

Objective

- Complete Genome Sequencing of *Mesorhizobium ciceri* Ca 181

Significant Achievements

Genome Assembly and Annotation:

- Sequencing of the Genome was done by 454 Next Generation pyrosequencing as well as Solexa-illumina and Sanger technology. A total of 6461 genes have been predicted and annotated for the functions they perform in *Mesorhizobium ciceri* Ca181. Filtration of Annotated Genes according to their functional categories (Stress, Biosynthesis, Regulatory, and Signaling) is in the progress.
- *M. ciceri* Ca181 genomic sequencing has been completed. After analysis of genomic sequence of *M. ciceri* Ca181, we found 18 SSRs in the genome of *M. ciceri* Ca181 and the frequency of SSRs longer than 10 bp. Among the various class of microsatellite, trinucleotide and dinucleotide repeat was the most abundant class. The frequency of maximum mononucleotide SSRs are longer than 10 bp and one mononucleotide repeat was C motif longer than 22 bp. Dinucleotide repeats were all GC

(GC and CG were included) and GA motif. Within trinucleotide repeats CGG, GGC, GCC, TCG were the predominant repeat type. With the help of SSRs of *M. ciceri* Ca181, we designed 18 primers for making SSR markers profiling of *M. ciceri* Ca181 with other *Rhizobium* bacteria obtained from NBAIM, Mau. The 18 SSR primers presented here are capable of differentiating *Rhizobium* strains regardless of host origin. The amplification products in SSR were between 100 bp to 500 bp. However, despite the polymorphism observed with some primers, the diversity index was low. Data were collected based on the presence or absence binary scoring method. The binary data set was converted into a similarity matrix, Unweighted paired-group method using arithmetic averages (UPGMA) cluster analysis with simple matching coefficient of resemblance was performed with NTSYSpc, version 2.01 (Exeter Software, Setauket, NY). Cluster analysis of genetic distances divided the 20 isolates into four major clusters, whereas reference strain MSA-20 (*M. ciceri* Ca181) formed cluster with Group 1 (G1), and showed a similarity with MSA-1 and MSA-6. Thus all twenty *C. arietinum* isolates shared considerable homogeneity. We also identify these twenty isolates after 16S-rDNA sequencing and NCBI blast.

- There were two experiments executed for study of symbiotic competitiveness of *M. ciceri* Ca181.
- Random transposon mutagenesis was used for preparation of Tn5 mutants of *M. ciceri* Ca181. 4x96 Tn5 mutants of *M. ciceri* Ca181 has been prepared. 50 mutants were used for root nodulation activity of *Rhizobium*. 5 replicates of each mutant were used for pot experiments with chick pea variety PUSA 1053.

Summary: Genome Annotation
Genome size: 6.443939 Mb (Approx)
Genes: 6461 (Approx)
Unique genes: 600 (Approx)

Conclusion

After assembly, the genome was search for the similarity in genome database available at NCBI Genome browser and it found about 41%, 35%, 32% similar from its closest organism *Mesorhizobium ciceri* biovar biserrulae, *M. loti* and *M. opportunistum* respectively. These results show potential that after complete analysis of the genome, it will give some new and unique genes and process involved in the specificity of this organism. The study is incomplete to draw final conclusion. However the identification of few sequences with unknown function could be interesting to further work on because it does not have any match in the database

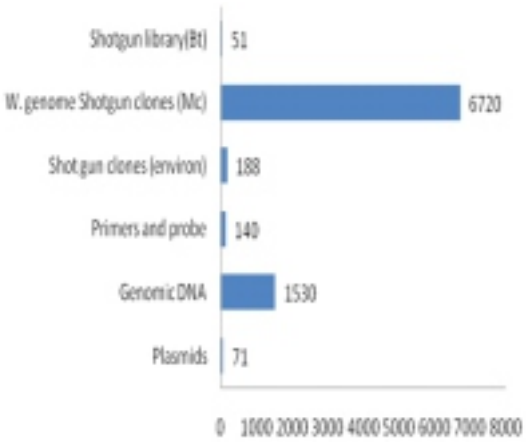
Microbial Genomic Resource Repository

PI : Dilip K. Arora
Co PIs : Alok Kumar Srivastava, Sudheer Kumar, Mahesh S. Yandigeri, Kamlesh Kumar Meena

Rationale

Microbial genetic materials (e.g. genomes, plasmids, vectors, cDNAs) are the 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 service repositories that provide reference materials and 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.

Indian Council of Agricultural Research has taken up an initiation to establish Microbial Genomic Resource Repository (MGRR) at National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau Nath Bhanjan. MGRR is a facility that preserves and conserves the genetic material of microorganisms, maintained in selected hosts or cloned and maintained in plasmids, accompanying the data details. This new organizational structure indicates the high importance and visibility that NBAIM places on our role as custodians of



microorganisms and its related genetic resources. The policies and procedures represent evaluation, maintenance, regeneration, distribution and documentation of genetic resources at MGRR. MGRR maintains genetic materials like whole genome shotgun and cDNA/EST libraries, PAC/BAC/YAC clone vectors, competent cells from sequencing projects, promoter DNA-fragments with reporter genes, RFLP probes specific for different microbes and expression vectors.

Genetic materials 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. It is equipped with improved infrastructure and techniques for conserving diverse genetic materials. To deposit the genetic material send the genetic material along with dully filled pass port data or MTA form. We can store your DNA samples at our secure, environmentally controlled facility for many years.

Objectives

- Nationwide survey and collection of information about the genetic resources/DNA.
- Development of linkages between research institutions/Universities, and researchers.
- Technology and Protocols development for isolation and long-term preservation of the Microbial Genetic Resources.
- Technology and Protocols development for collection/transportation of microbial samples.
- Development of infrastructure facilities for the preservation and maintenance of genetic recourses.
- Collection of environmental samples from different agro climatic regions and exploration of non-culturable microorganisms.
- Development of Databases/Information Bank for Microbial Genomic Resources.
- Documentation and electronic cataloguing of Microbial Genetic Resources.
- Exploration of non-culturable microorganisms and direct DNA isolations from environmental samples.
- Development and implementation of genome projects to explore non-culturable microorganisms.

Significant Achievements

- The center is enriched with genomic DNA of different groups of fungi which include Myxomycetes, Mastigomycetes, Zygomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes in addition to bacteria, actinomycetes and blue green algae (BGA).
- It is also enriched with total genomic shot gun library of *Mesorhizobium ciceri* ca 181 along with its genome sequences.
- Significant number of fluorescent marker like GFP (Green Flourescent Protein), YFP (Yellow Flourescent Protein), RFP (Red Flourescent Protein), and gene silencing vectors like pSilent-1 for characterization and identification of unknown fungal genes, His-tagged expression vectors for functional studies of a known or unknown gene, and various other gene constructs of *Bt* gene from *Bacillus thuringiensis* are preserved.
- Universal PCR primers from bacteria and fungi, species specific real time primers for various

organisms, functional gene primers like *Nif* are collected.

- MGRR has generated a well equipped world class laboratory with all modern equipments like Robotic DNA extractor, Pyrosequencer, Gel imaging system, Electroporator, High throughput gel electrophoresis system etc
- Each genetic material is preserved by at least two methods according to the type of genomic material, either under short term, or long term storage at 80°C. Apart from that an agriculturally important fungal microsatellite database has being developed.
- Apart from the preservation of microbial genetic resources, MGRR is developing databases like "Agriculturally important fungal microsatellite primer database" which consist of 50,000 microsatellite primers from various fungi.

Conclusion

In future, MGRR will collaborate with various institutions working in this field to collect and preserve the DNA materials and to provide necessary DNA samples for an active research study and will build a database library of anonymous microbial information which can provide clues for a research investigation. We hope that the MGRR will be efficiently utilized by the scientific community to facilitate innovative researches in both functional and applied genomics of microorganism for sustainable agriculture.

National Agricultural Innovation Project

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

Consortium Leader : Dilip K. Arora
Consortium PI : Sudheer Kuamr
Coordinating Scientist : Alok Kumar Srivastava

Rationale

Abiotic stress like high and low temperature, drought, salinity and acidity causes a serious problem of reduced agriculture production many countries. In India, there is a widespread saline soil problem, commonly known as the formation of usar land which results low income and poverty of farmers. In developing countries like India the problem could be more serious due to the increasing demand for food. High salt concentration leads to a decline in soil fertility by adversely affecting the soil microbial flora, and therefore further decreasing crop productivity. The most appropriate solution in such conditions is to use salt tolerant microbial inoculants that may prove useful in developing strategies to facilitate plant grow in saline soil. Microbes from the extreme environment have tremendous potential as they have developed many mechanisms for their growth and development under salt stress. Lesser attention has been paid to explore diversity of microbes in extreme environments like saline, acidic, and drought. Microorganism present in the rhizosphere reported to alleviate the salinity stress by different mechanisms. The rhizobacteria from saline soil crop rhizosphere often plays crucial roles in increasing crop productivity in the plants they colonize due to their close proximity with the roots they inhabit. For more than a decade, rhizobacteria have been investigated as possible replacements for chemical fertilizers due to the severe deterioration of the chemical, physical, and biological health of the cultivated land. Spore-forming bacteria, typically *Bacillus* species, are one of the major types of soil bacteria.

Bacillus and other predominant genera are focusing major attention in recent years primarily due to their nutritional versatility and survival under extreme condition. *Bacillus* sp. isolated from extreme condition and from rhizosphere has developed certain proteins allowing them to sustain life under extreme condition of salinity, drought high and low temperature and acidity. *Bacillus* and bacillus derived genera are employed in industries as sources of enzyme, in agriculture as inoculants (PGPR) and biocontrol agent. They are also implicated in bioremediation and insecticidal property of *B. thuringiensis* has been exploited largely. In India there is no base line information available on the species richness and thus its utilization is not understood. The use of *Bacillus* and bacillus derived genera as bio fertilizes, biocontrol agents and bioremediadiators will help Indian agriculture by reducing the dependence on chemical inputs and protecting the environment salinity and drought stress to attain optimum yield of field and horticultural crops.

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.

Significant Achievements:

- A total 55 *Bacillus* isolates and 72 predominant

genera which are growing on more than 4 % NaCl concentration were selected for diversity analysis of salt affected area of eastern Uttar Pradesh. The molecular characterization on the basis of 16S and 16-23S rDNA PCR-RFLP analysis with three restriction endonucleases *Alu* I, *Hae* III and *Taq* I revealed greater diversity among the isolates of saline soil of eastern U.P. Combined dendrogram based on 16s and 16-23s RFLP analysis revealed the existence of 37 and 59 clusters among the 55 bacillus isolates and 72 predominant genera respectively.

- Based on the 16s rDNA sequencing the *Bacillus* isolates were identified as : *Bacillus megaterium, B. subtilis, B. licheniformis, B. horikoshi, B. pumilus, Bacillus sp, B. cereus, B. simplex, B. flexus, B. arsenicus, B. thuriengiensis, Bacillus firmus, Lysinibacillus, B. marrisflavi* and 72 predominant genera identified as *B. licheniformis, B. niabensis, b. aryabhattai, B,. subtilis, B. thioparans, B. flexusm, B. marisflavi, B. endophyticus, B, cereus, B. Pumilus, Lycinibacillus xylanilyticus, Pseudomonas stutzeri, Pseudomonas* sp., *Staphylococcus, Enterobacter cloacae, Micrococcus sp, Cellulosimicrobium funki, Ochrobacterium sp., Acinetobacter* sp. etc. 16S rRNA gene sequences of *Bacillus* were submitted to NCBI and accession numbers were assigned for *Bacillus* isolates from JN215486 - JN215522.

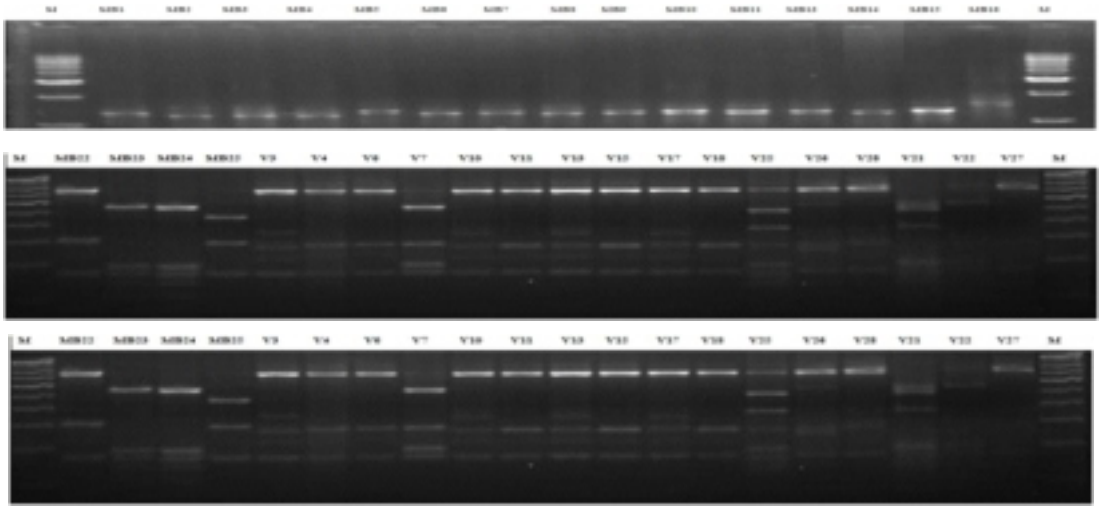


Fig. Amplified 16-23S rDNA restriction analysis of 55 isolates of Eastern Uttar Pradesh with *Alu*I *Hae*III &*Taq* I

Fig. Effect of bacterial inoculums on growth and yield of chickpea cultivars

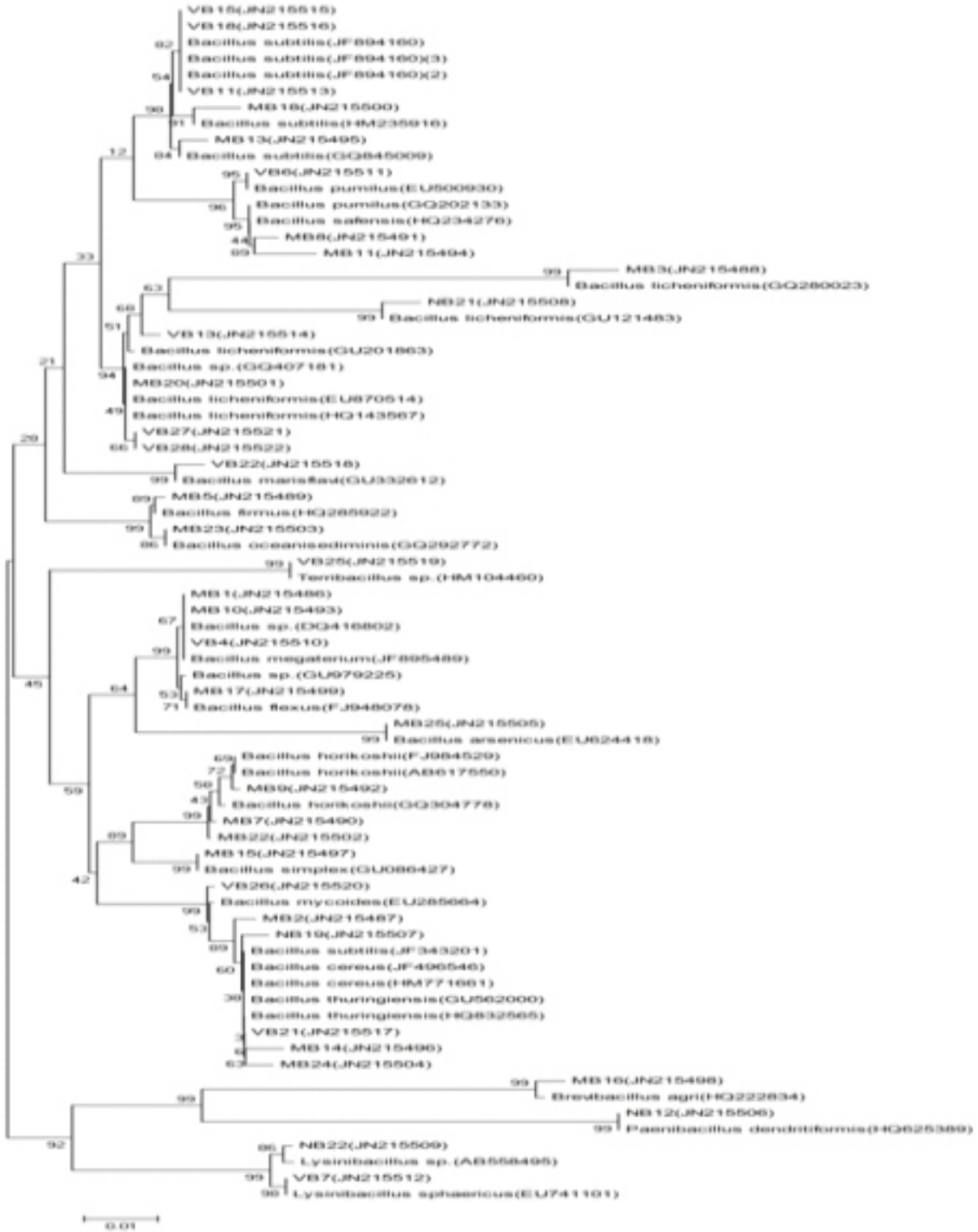


Fig. Phylogentic tree showing the position of the *Bacillus* isolates based on the partial 16S rRNA gene sequencing comparison obtained by the neighbour joining method.

- Out of 250 isolates recovered from chickpea rhizosphere, five isolates showed better growth promotion in chickpea under the glasshouse conditions were used for field experiment. On the basis of 16SrDNA sequencing these isolates were identified as *B. subtilis, P. fluorescence, P. putida, Pseudomonas* sp. and *Bacillus* sp.
- These bacterial isolates in 32 different combinations with recommended *Rhizobium* were applied on salt tolerant as well as salt susceptible chickpea cultivars for evaluating their performance for salt stress alleviation by using split plot method. Different growth and biochemical parameters were taken for assessing best bacterial consortium. A significant growth promotion has been recorded on bacterial fortification of chickpea seeds over the control.

Conclusion

The present study showed that *Bacillus* was the dominant genera in the saline soils and shows greater diversity among the all isolates recovered from saline soil of eastern U.P. Many of the isolates were able to tolerate up to 14-16% NaCl concentration. The isolates from chickpea rhizosphere possessed multiple plant growth promoting attributes were utilized to alleviate the effect of salt and enhancement in growth and yield of chickpea crop. There was significantly increase observed due to bacterial inoculation as compare to control on growth and yield of both salt tolerant as well as susceptible chickpea cultivars.

Allele Mining And Bioprospecting Of Gene For Abiotic Stress Tolerance

CCPI : Alok Kumar Srivastava
Co-PI : Kamlesh Kumar Meena

Rationale

The microbes are known to thrive well in abiotic stress environment and are good repertoire of the genes could contribute a lot in abiotic stress management in plants which can further be used for extensive activity screens and a number of the recent applications in the field of agricultural biotechnology. Bioprospecting under extreme conditions would provide a database that is currently unavailable and will enrich the Indian microbial culture collection. This project aims at preparing for meeting the challenges of abiotic stresses under the changing climate. The target traits are genetically very complex and thus problems posed are highly intractable. Relative simplicity of genomes and far greater genetic diversity of the microbial systems makes the gene discovery and allele mining at a faster pace than in case of plants and animals. The expected outputs of the subproject include information on spatial distribution of genetic diversity and structure of populations in relation to useful allelic variations

Overall Objectives of Sub-project

- Prospecting novel genes, promoters and alleles for economically important traits using indigenous bioresources with emphasis on less studied species.
- Functional validation of the new genes in model systems and different genetic backgrounds.
- Transfer of the validated genes and alleles to recipient species cutting across biological barriers.
- Development of highly competent groups of scientists drawn from various disciplines and institutions of international standard for undertaking research in genomics and its application for improvement of agricultural species.

Significant Achievements:

Analysis of Genetic Variability between Thermophillic and Mesophilic Fungi

The genetic variability among the thermophillic fungi and its nearest mesophilic species was evaluated by comparing the available transcripts sequences from various fungal species in public domain, which was further mined for microsatellite for the development of molecular method for routinely characterizing their population and to gain a better insight into the organization and evolution of their genomes as well as to evaluate the variability among the population. In order to understand the genetic variability between thermophilic and mesophilic fungi, frequency and distribution of microsatellites was studied in transcript sequences of two thermophilic (*Myceliophthora thermophila and Thielavia terrestris*) and two of its closest mesophilic neighbors (*Chaetomium globusum* and *Neurospoa crassa*). The annotated transcript sequences of *M. thermophila* and *T. terrestris* were downloaded from Department of Energy's Joint Genome Project (JGI) whereas transcript sequence of

Table 1: Number and distribution of SSRs in the transcript sequences of mesophilic and thermophilic fungi

	C. globusum	*N. crassa*	*M. thermophila*	*T. terrestris*
Genome size (Mb)	34.89	41.4	38.7	36.9
No. of transcripts	11124	9907	8806	9813
Size covered by transcripts (Mb)	16.4(47.0%)	14.9 (36.0%)	14.3 (36.9%)	16.5 (44.7%)
Total no. of SSR identified	4246	5090	6198	6073
Perfect SSR	3717(87.5%)	4439 (87.2%)	5378 (86.7%)	5257 (86.6%)
Compound	529 (12.5%)	651(12.8%)	820 (13.3%)	816 (13.4%)
Total length of SSRs (bp)	74162 (0.42%)	98046 (0.65%)	117483(0.76%)	106354(0.64%)
Relative abundance (SSR/Mb)	258.9	341.6	433.4	368.1
Relative density (bp/Mb)	4522.1	6580.2	8215.5	6445.6

Table 2: Percentage, relative abundance and relative density of SSRs in sequence sets of mesophilic and thermophilic fungi

	Motif length	**Count**	**Percentage**	**Relative abundance**	**Relative density**
Cg	di	22	0.45	1.3	16.2
	tri	3723	76.5	227.0	3263.7
	tetra	315	6.4	19.2	234.8
	penta	53	1.08	3.2	49.3
	hexa	753	15.4	45.9	957.8
Nc	Di	20	0.3	1.3	17.1
	Tri	4787	80.9	321.2	5166.4
	tetra	253	4.2	16.9	212.1
	Penta	31	0.5	2.0	32.8
	hexa	824	13.9	55.3	1148.0
Mt	Di	243	3.3	16.9	275.9
	Tri	5311	73.8	371.3	5882.5
	Tetra	562	7.8	38.4	522.7
	Penta	106	1.4	7.4	130.4
	hexa	969	13.4	67.7	1403.9
Tt	di	162	2.2	9.8	138.4
	Tri	5084	71.8	308.1	4454.9
	Tetra	719	10.1	43.5	557.1
	Penta	156	2.2	9.4	152.7
	hexa	955	13.4	57.8	1142.5

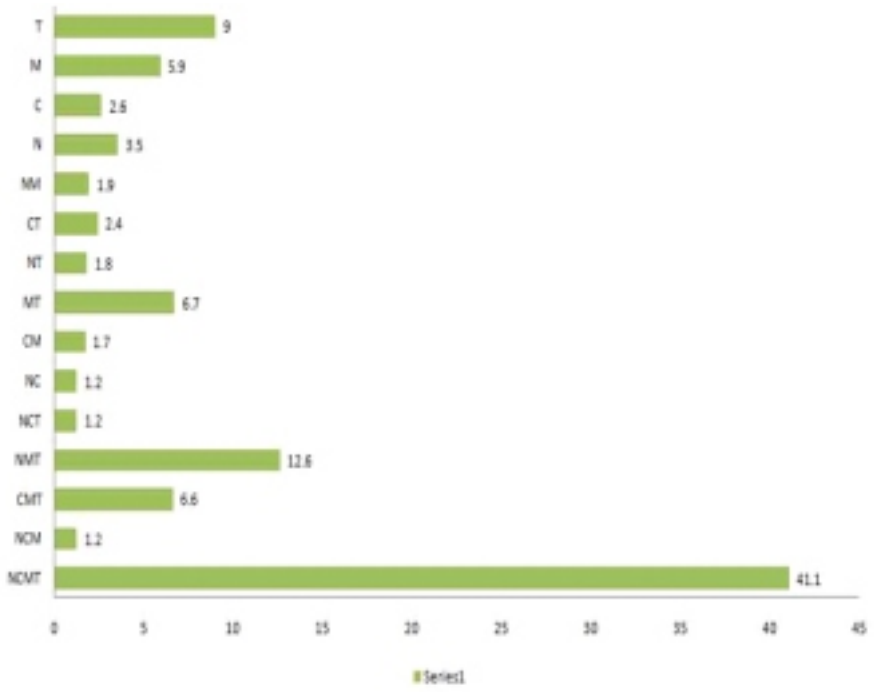


Fig. 1. Sharing of microsatellite among thermophilic and mesophilic fungi

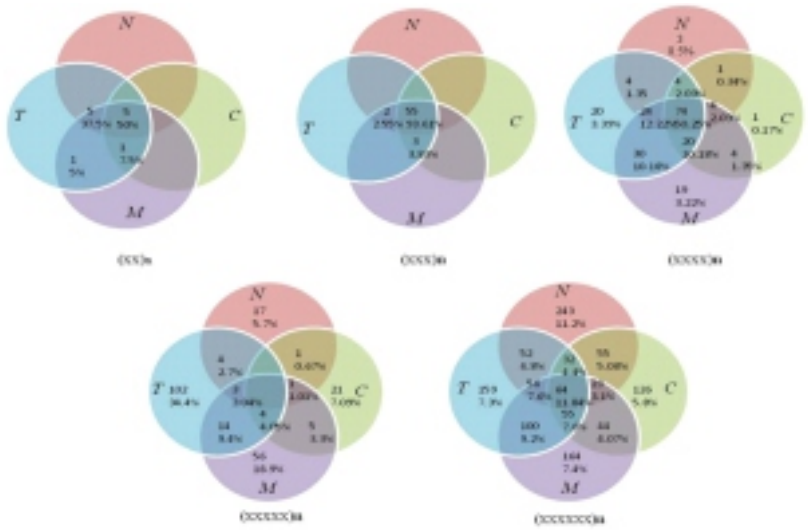


Fig. 2: Representation of sharing of motifs among the thermophillic and mesophilic fungi

mesophilic fungi were downloaded from Broad Institute. The SSRs were analyzed using WeSat online software.

Frequency and distribution of repeats

The frequency of repeat motifs in the annotated transcripts was assessed. Both perfect and compound SSRs were selected with a minimum acceptable length of 12 bp for di, tri and tetra-nucleotide motifs. Only SSRs with a minimum of three repeats were included in the analyses of penta- and hexa-nucleotide repeats. Maximum number of SSRs (6198) was identified in *M. thermophila* followed by *T. terrestris* (6073), *N. crassa* (5090) and *C. globusum* (4246). To compare the total SSR count between all four species more accurately, we have taken the total length of each set of sequences analyzed as a reference. Thus, total relative abundance (SSR/Mb) and total relative density (bp/Mb) were calculated (Table 1). It was found that relative abundance of SSRs in *M. thermophila* (433.4) was maximum when compared to *T. terrestris* (368.1), *N. crassa* (341.6) and *C. globusum* (258.9). The higher number of microsatellites in thermophillic fungi may have contributed to the temperature tolerant ability in these fungi. We have also designed primers to amplify all the motif. A total of 21606 primers were designed and their sequences were placed in the developing database.

All three sequence sets contained SSRs that were mainly trinucleotide repeats (75.6%), while the dinucleotide repeats represented less than 4.6%. Hexanucleotide repeats constituted the second most frequent motif (14.03%) which was followed by tetranucleotide (7.12%) and pentanucleotide (1.2%) repeat motifs. However, the percentage of di and pentanucleotide repeat was higher in *Fom*. This agrees with results from other eukaryotes, where trinucleotide repeats are overrepresented in coding region (Garnica *et al.* 2006). The total number of varying microsatellite motifs are shown in Table 2.

Sharing of repeats

In order to view the microsatellite variability more accurately, sharing of each motif was studied (Figure 1). A total of 41% of repeats were common in all four genome studied. It was interesting to note that the thermophilic fungi shared maximum number of repeats (6.7%) when two genomes were compared. Unique motifs were also found maximum in *T. terrestris* (9%) and *M. thermophila* (5.9).

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

CCPI : Alok Kumar Srivastava
CoPI : Kamlesh Kumar Meena

Rationale

Soil as natural bodies of mineral and organic constituents differentiated in horizons of variable depths which differ among themselves as well as from the under lying material in morphology, physical and chemical composition, biological characteristic and therefore act as medium for the plants growth. Different group of microorganisms perform special role in conversion, transformation and degradation in biogeochemical cycles. Many agriculture practices make adverse affect on microbial diversity of agriculture land. Urea can be hydrolyzed by both intracellular and extracellular ureases. The intracellular and soluble ureases are considered to be more labile than the stabilized extracellular urease. Enzyme activity assay has been developed for accurate biochemical method to determine microbiological activity. The preference of dehydrogenase activity over cultural methods in enumeration of microorganisms and monitoring of their activities is due to underestimation of numbers of viable cells as a result of difficulty in being readily desorbed from the substrate matrix or lack of homogeneity in distribution common to culture methods. The activity of the dehydrogenase (DHA) is considered an indicator of the oxidative metabolism in soils and thus of the microbiological activity because it is linked to viable cells.

Land quality is conceptualized as the major link between the strategies of conservation management practices and achievement of major goals of sustainable agriculture. Assessment of land quality is invaluable in determining sustainability of land management systems. Therefore assessment of land quality and the direction of change with time is the primary indicator of sustainable land management. Currently complete data sets on bio-physical parameters are not available for the preparation of a soil information system under SOTER environment. Therefore the project was conceptualized under NAIP. NBAIM is one of the partners contributing in microbiological analysis of soil samples.

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*

Significant Achievements

- 13 soil sample received from NBSS&LUP. Sampling location are from the states Sikkim and eastern states of India.
- Samples were examined for cfu and enzymatic analysis.
- Statistical analysis of 234 soil sample involve Pearson's correlation coefficient was done for the estimation of relationship between microbial load and enzymatic activity of IGP soil series.
- Correlation, 0.840 & 0.839 was recorded in soil urease activity and soil dehydrogenase respectively in indo gangetic plain of 15 soil series.
- Shannon's Diversity index ranging from 2.261 to 1.312 in high management followed by 2.088 to 1.318 in low management. This interprets that microbial diversity decrease in down depth.
- Attended the CIC/CAC meeting held at NBSSLUP Kolkata at in December, 2012.

Conclusion:

Significantly lower bacterial and fungal count along

Table.1: Pearson's Correlation coefficient between different enzymatic activity and microbial load in different management.

Microbial load	Pearson's Correlation coefficient value	
	Urease Activity	Dehydrogenase Activity
In Low Management	0.838	0.836
In High Management	0.853	0.851
Overall managements	0.840	0.909

with low enzymatic activities of the soil enzymes in low horizons clearly indicates their low fertility status. Microorganisms have been responsible for the most profound forms of terrestrial change. Shannon diversity index is high compare low soil profiles and thus indicating high microbial population at upper profiles. These microorganisms are significant player of many geochemical cycles and thus maintaining the constant flow of essential nutrients of soil by releasing exo-enzyme, soil urease, that catalysis the transformation reaction. Microbial presence is maximum rhizospheric region than deep non rhizospheric zone.

National Agricultural Bioinformatics Grid (NABG)

Capacity building programs under NABG

CCPI : Dhananjaya Pratap Singh
CoPIs : Alok Kumar Srivastava, Mahesh Yandigeri, Kamlesh Kumar Meena

1 Sensitization Workshop i.e. Bioinformatics and Computational Biology in Microbiological Research (29th Jan- 04th Feb, 2011).

1 Bioinformatics Workshop i.e. Data Mining and Computational Methods in Bioinformatics for Microbial Research (04th Mar- 15th Mar, 2011).

1 National Training i.e. Bioinformatics in Multi-omics Era: A Microbial Genomics perspective (23rd Feb- 03rd Mar, 2012).

Design and development of Databases of respective domain

StressMicrobesInfo

Web link:

http://nabg.iasri.res.in:8080/Stressmicrobs/stressdb.html

Description:

- Designed and developed it using WAMP technology.
- Microbes responds to different kinds of stress conditions i.e. heat, cold, drought etc. and helps plants to sustain in adverse conditions.
- Presently, no such database is available.
- This database Contains information about more than 175 microbes which are from four different groups i.e. Bacteria, Cyanobacteria, Actinomycetes and fungi.

BioInfoKnowledgeBase

Weblink:

http://nabg.iasri.res.in:8080/BIKB/edb_home.html

Description:

- Designing of E-R diagram and development through WAMP technology.
- This database provide information about bioinformatics tools according to the categories they are used. It also includes information about databases.
- It contains information about >1000 bio-informatics tools and >110 databases.

Research Activities

3.1. Identification of issues on bioinformatics and related fields in the domain.

3.2. Analysis of Codon selection pattern within and across microbial genomes.

3.3. Study of Thermophilic adaptations of microbes using various –omics approaches.

3.4. Evolutionary Genomic analysis.

3.5. Structure prediction and binding site analysis of Microcystin-LR degradation protein using comparative modeling.

3.6. Genomics approach for the identification of drug targets in Xanthomonas oryzae pv. oryzae PXO99A

Outreach Programmes

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

Conservation, characterization and documentation of different species of *Fusarium*

PI : Sudheer Kumar
Co-PI : Alok Kumar Srivastava

Rationale

Fusarium is a group of soil inhabiting filamentous fungi which cause mainly the vascular wilt and rot in wide range of cultivated plant species. The important ones are crown and root rots, stalk rots, head and grain blights and vascular wilt diseases. Among these, wilt and cortical rots caused in several agricultural, vegetable, fruit and ornamental crops are most predominant the word over. The genus *Fusarium* currently contains over 20 species. *Fusarium* species has no known sexual stage, but produces three types of asexual spores: microconidia, macroconidia, and chlamydospores. They are round thick walled spores produced within or terminally on older mycelium or in macroconidia. Chlamydospores unlike the other spores can survive in the soil for a long period of time. However, on the basis of conventional techniques the identification and mapping the diversity is very difficult.

Molecular markers are powerful tools for genetic mapping, molecular fingerprinting, population structure, and genetic diversity studies. Several molecular markers viz. Restriction fragment length Polymorphism (RFLP), Random amplified polymorphic DNA (RAPD), Inter-simple sequence repeats (ISSR), Sequence related amplified polymorphism (SRAP), Amplified fragment length polymorphism (AFLP), Simple sequence repeats (SSR) and Single nucleotide polymorphisms (SNPs) are presently available to assess the variability and diversity at molecular level. Among different classes of molecular markers, microsatellite markers are the most favored. Microsatellite sequences are characterized by their codominant nature, abundance, reproducibility, multiallelic nature and transferability across species. Various studies has been undertaken to identify microsatellites in different crop species. However, despite this wide spread use, little is known about SSRs in fungi. In fact, there are only a limited number of studies on these seemingly important and intruding sets of sequences in fungal species. The homology of flanking regions of SSRs allows the transferability of microsatellite loci between closely related species, besides the possibility of comparative map construction among them. However, most of these are genomic SSRs whose development is highly laborious, cumbersome and cost-intensive. Use of bioinformatics tools helps to maximize the identification of genome sequences and consequently, the efficiency in the number of generated markers. Although *Fusarium* expressed sequence tags (ESTs) or transcript databases are publicly available, no formal analysis of SSRs in these sequences has been reported. The information on abundance and distribution of SSRs may also help in understanding their relevance in gene function or genome evolution.

Objectives:

- Conservation, characterization and documentation of different species of *Fusarium*
- Development of data base for Indian isolates of *Fusarium*

Significant Achievements:

- Total 106 Fusarium isolates were collected and conserved for long term storage. For the diversity mapping the genomic DNA was isolated and

used for amplification of ITS region of different species of *Fusarium* using primers ITS1and ITS4. The PCR amplification yielded a fragment of approximately 550-650 bp.

- Variability among the isolates of Fusarium species collected from different parts of India was determined based on the random amplified polymorphic DNA (RAPD) techniques. finally, 4 primers that, including 3 primer
- Fungal primer-half set (10 primers) were screened and it was observed that RAPD primers RFu-3, RFu-7 and RFu-10 having good discrimination power in F. udum and primers RFu-1, RFu-3 and RFu-7 were able to discriminate different isolated of Fusarium oxysporum f. sp. ciceri. The similarity coefficients were used to construct a dendrogram depicting genetic relationships among the isolates by employing the Unweighted Paired Group Method of Arithmetic Averages (UPGMA) algorithm and SAHN clustering. Finally a combined Dendogram was constructed using amplified band pattern of Fusarium udum and Fusarium oxysporum f. sp. ciceri
- ERIC primers were used for PCR amplification of the all strains *Fusarium oxysporum* f. sp. *ciceri* and *F. solani*, number and intensity of amplified bands varied with ERIC consistently revealing more intense and more polymorphic bands. The dendrogram clearly shows the variability among these isolates.
- To study the genetic diversity analysis, cross species transferability and polymorphism through microsatellite markers, 20 isolates of *Fusarium udum*, 3 isolates of *Fusarium oxysporum* f. sp. *lycopesici*, 3 isolates of *Fusarium oxysporum* f. sp. *melonis* and 3 isolates *Fusarium oxysporum* f. sp. *cucumerium* were collected from different agro climatic zone under the network. Out of 30 SSR markers, 15 SSR markers showed transferability and polymorphism which includes 7 primer of Fom, 2 primer of Foc and 6 primer of Fol. These resulted in easily score-able bands ranged from 120 to 600 bp in all the isolates. Among all the markers, 7 markers (46.67%) were polymorphic, whereas rest 8 markers (53.33%) were monomorphic. A total of 29 alleles were amplified by 15 markers.
- Use of 30 SSR primers for the study of transferability and polymorphism in 29 isolates of *Fusarium* and combined dendogram was constructed on the basis of presence or absence of band and show a genetic relationship among the

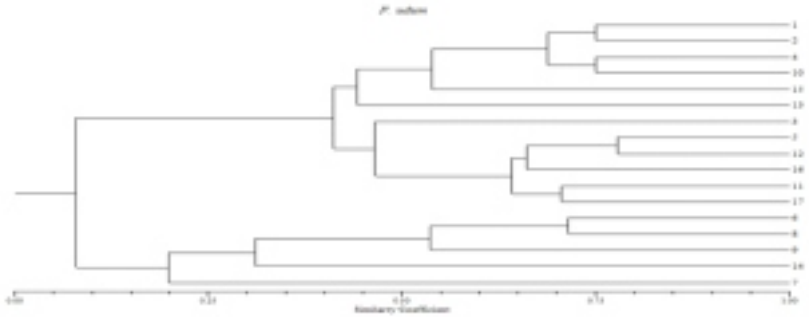


Fig. Genetic relationships among F. udum species based on Jaccard's similarity coefficients from RAPD data.

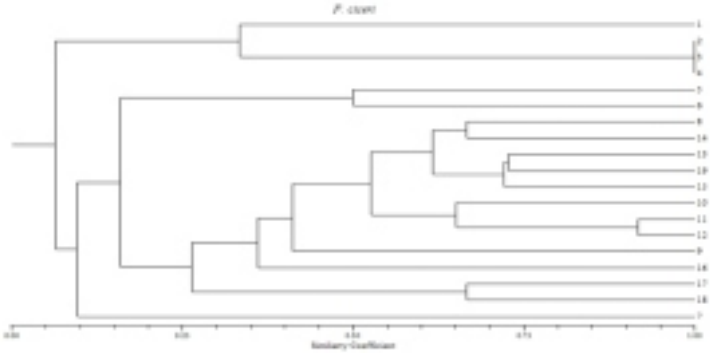


Fig. Genetic relationships among F. o. ciceri species based on Jaccard's similarity coefficients from RAPD data.

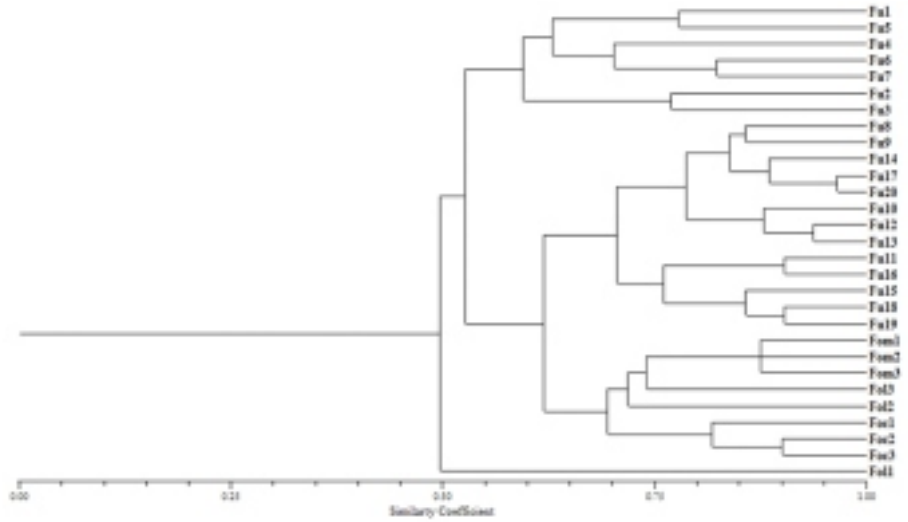


Fig. Genetic relationships among Fusarium species based on Jaccard's similarity coefficients from SSR data.

Fusarium isolates based on 17 microsatellites markers along with Jaccard's coefficient of similarity depicted.

Conclusion

The collection and conservation of wilt pathogen with digitization of all the related information will provide a user friendly bioinformatics platform. Molecular methods, such as RAPD, ERIC and PCR-RFLP and SSR, have been very useful for phylogenic studies of the pathogen. Assays of cross-amplification in taxa with some of the SSR markers used in this study indicated a moderate level of transferability with genetically distant organisms. The low rate of transferability observed in this study could be evidence of mutations in the flanking regions of the SSR, the possibility of interruptions within the repeat motif or the presence of introns in the amplified region.

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

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

PI : Sudheer Kumar
Co-PIs : Alok Kumar Srivastava, Kamlesh Kumar Meena

Rationale:

Leaf spot pathogens causing the economically important disease in a wide range of hosts including cereals, legumes, vegetables, perennial crops worldwide. Leaf spot occurs in warm and humid weather conditions hence very prominent in India. Leaf spot is a common descriptive term applied to a number of diseases affecting the foliage of crops and ornamentals. The majority of the leaf spots are caused by a variety of fungal pathogens such as *Alternaria*, *Colletotrichum* and *Cercospora* etc.

On the basis of symptoms, the identification of the causal agent of leaf spot diseases is very difficult as the symptoms may largely vary with the host variety, crop growth stage, agronomic practices and prevailing weather conditions. Accurate species identification is critical to understand disease development or epidemiology and also to develop effective control measures. The rapid and accurate detection of leaf spot pathogen through molecular techniques may also provide an opportunity for timely application of control measures. Being a foliar pathogen these are greatly influenced by weather parameters and spread rapidly under favorable conditions where timely application of management practice is directly correlated with economic return.

Moreover understanding the variability in the pathogen is essential for resistance breeding programme. For the purpose a large number of collections of pathogenic isolates across agro-climatic regions from all over the country were generated. The molecular characterization for phynotypic and pathogenic variability is necessary for identification of race specific resistance genes in the host to develop resistant pure line and multilines. The project is aimed to develop a repository of the foliar pathogens like *Alternaria, Colletotrichum* and *Cercospora* and to study their molecular diversity.

Objectives:

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

Significant Achievements:

All the eighty four cultures of *Alternaria* species, including *Alternaria brassicae* (26) from mustard, *Alternaria solani* (28) from tomato, *Alternaria brassicicola* (10), *Alternaria porri* (10) *Alternaria sesame* (10) from different crop plant and ninety nine cultures of *Colletotrichum* species including *Colletotrichum gloeosporioides* (59) isolated from mango, *Colletotrichum falcatum*(15) from Sugarcane and other *Colletotrichum* spp. (25) isolated from grapes, received from different cooperating centers were morphologically characterized on the basis of pigmentation, growth pattern, colony color, mycelia color, shape, and size of conidia and preserved for short and long term conservation. For short term conservation, all the isolates were conserved in mineral oil and a copy of all is also conserved in 20% glycerol at -80^{0}C. For long term conservation, all the isolates were lyophilized.

Molecular Characterization

Molecular variability among the isolates of *Alternaria* species, collected from different parts of India was determined based on the random amplified polymorphic DNA (RAPD) techniques. RAPD analysis was conducted with 40 isolates (*Alternaria brassicae* (12), *Alternaria porri* (9), *Alternaria sesame* (9), *Alternaria brassicicola* (10). Five primers (RFu-C1, RFu-C2, RFu-C4, RFu-C9 and RFu-C10) were selected to perform RAPD fingerprints, which resulted in greatest number of polymorphic and reproducible fragments.

RAPD analysis was conducted at least twice to confirm reproducibility of amplified products. Statistical analysis of the data was performed using the NTSYS-pc program. The degree of genetic relatedness or similarity was estimated using the Jaccard coefficient. Each isolate was scored for the presence or absence of a given fragment, and a binary matrix was constructed. The UPGMA tree obtained from the data matrix was constructed with the five

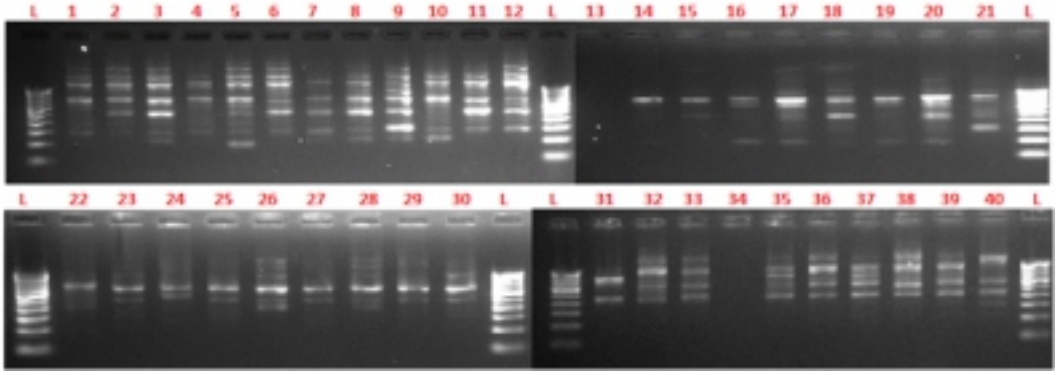


Fig. RAPD patterns for 40 selected isolates of *Alternaria* species using primer RFu-C1 RAPD patterns for selected cultures of *Alternaria brassicae* (1-12), *Alternaria brassicicola* (13-21), *Alternaria sesame* (22-30) and *Alternaria porri* (31-40). Lanes L: 100 bp markers

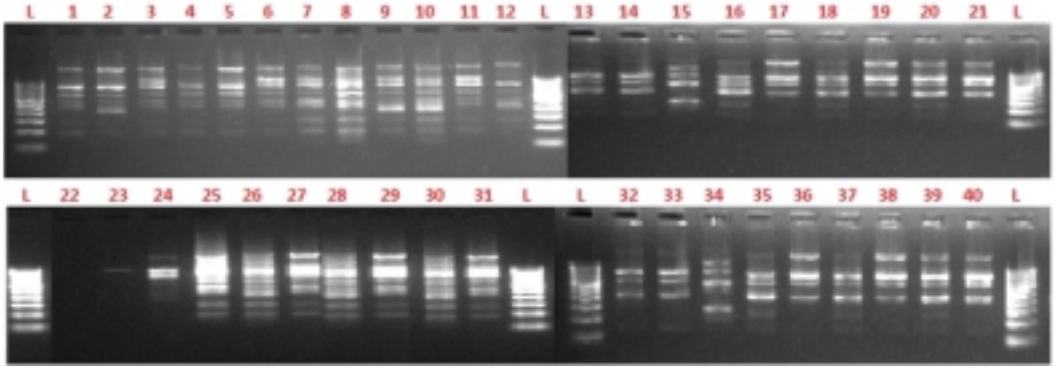


Fig. RAPD patterns for 40 selected isolates of *Alternaria species* using primer RFu-C9 RAPD patterns for selected cultures of *Alternaria brassicae* (1-12), *Alternaria brassicicola* (13-21), *Alternaria sesame* (22-30) and *Alternaria porri* (31-40). Lanes L: 100 bp markers

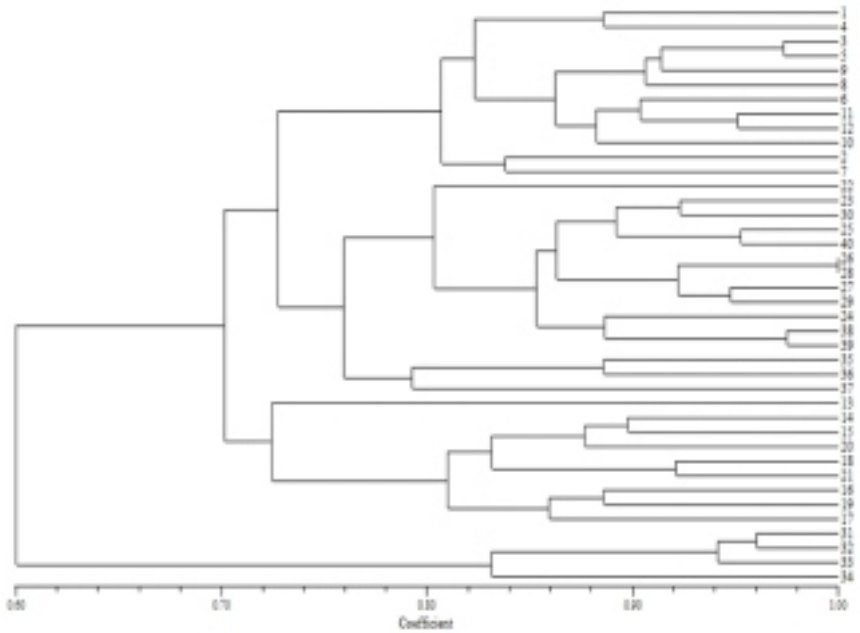


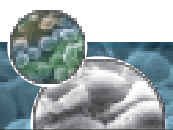
Fig. UPGMA dendrogram showing genetic relationships among *Alternaria species* based on Jaccard's similarity coefficients from RAPD data. Branches are labelled by isolate numbers. The line below represents the similarity index

combined RAPD primers. RAPD-PCR generated very distinct fingerprinting pattern showing considerable variability among the different isolates of *Alternaria*. The number of amplified bands was variable depending on the primers or the isolates used.

The analysis of RAPD profiles showed that there was high level of genetic variability among the isolates of different *Alternaria* species as well as the isolates of the same species collected from different geographical regions. *Alternaria brassicae* isolates produced 12, 6, 7, 7 and 8 fragments when amplified with primer RFu-C1, RFu-C2, RFu-C4, RFu-C9, and RFu-C10, respectively. The variations in size of the RAPD products were also detected among the isolates belonging to different host as well as within the isolates of a particular host.

Conclusion:

The collection and conservation of leaf spot pathogens with digitization of all the related information will provide a user-friendly bio-informatics platform that will facilitate the users to rapid excess of information on major foliar pathogens. Polymerase chain reaction (PCR) based molecular markers such as RAPD PCR, RFLP-PCR are useful tools for detecting genetic variation within populations of the pathogens.



Application of Microorganisms in Agriculture and Allied Sectors (AMAAS)

Significant Achievements

Theme 1: Microbial Diversity and Identification

- A total of 116 actinomycetes were procured from Chilika Lake by employing various media and enrichment techniques and sorted out into 59 different "morphospecies" based on colour of aerial and substrate mycelium, pigmentation and microscopic examination.
- Sediment samples had highest streptomycetes population frequencies than lake water while, population frequencies of alkali-halophilic streptomycetes shown to be gradual decreased from marine habitat to fresh water lake.
- Sea mouth sector was enriched with nitrate reductase activity (42.3%) and had biocontrol attributes as well (antimicrobial activity (38.8%) and chitinase enzyme (39%) production).
- Phylogenetic analysis representing three phyloclades with *S. albus*, *S. bacillaris*, *S. fradiae S. achromogenes, S. fumigatiscleroticusS. spiralis* falls under first groupwhile, *S. albogriseolus*, *S. acrimycini*, *S. mutabilis*, *S. thermocarboxydus, S. geysiriensis, S. vinaceusdrappus, S. macrosporeus, S. griseorubens, S. labedae, S. aureofaciens, S. erythrogriseus, S. atrovirens, S. ghanaensis* represent second group and lastly *M. rosaria, M. echinospora* forms third group showing a complete out group among each other. Isolates S3RS8, S4BS3 and S3RW3 seems to be novel as they showed similarity with many *Streptomyces* species.
- A total of 116 samples collected and used for isolation of AIM's include, 19 composite soil samples, 58 root samples of 21 plant species, 19 leaf samples, 01 leaf litter samples, 04 decaying wood samples, 07 mushroom samples, and 08 termite mound samples 19 grids (12.5 km x 12.5 km, each grid) in the central Western Ghats from19 grids (12.5 km x 12.5 km, each grid). The total number of isolates obtained from these samples is 368 including 103 *Azotobacter*, 19 *Azospirillum*, 105 *Beijerinckia*, 59 phospahte solubilizers, 16 lignin degraders, 55 fluorescent pseudomonads and 11 pink pigmented facultative methylotrophs (PPFM).
- Morphological, biochemical and physiological characterization tentatively identified *Pseudomonas fluorescens*, *Pseudomonas cichori, Pseudomonas putida* and *Pseudomonas aeruginosa.* Similarly, PSB isolates belonged to *Pseudomonas*, *Aminobacter* and *Flavomonas*, *Acidomonas*, *Rhizobacter*, *Acenitobacter* and *Phenylobacterium, Gluconobacter, Erythrobacterium* and *Flavobacterium, Acetobacter, Agrobacterium* and *Xanthomonas, Azomonas, Alcaligens, Azorhizobium, Bacillus, Rhizomonas* and *Staphylococcus, Methylobacterium.*
- Using ITS sequencing, *Hypocrea lixii, Aspergillus niger, Penicillium citrinum, Trichoderma longibrachiatum, Hypocrea* sp. *Trichoderma* sp. *Penicillium* sp. *Trichoderma aureoviride, Trichoderma sp., Rhizomucor variabilis, Rhizopus oryzae* and *Lichtheimia ramose* were identified.All the 58 sequences were deposited to NCBI Accession numbers issued were JF513187 to JF513194, JN043473 to JN043499 and JN695883 to JN695915.
- The inhibitory activity of 62 fluorescent pseudomonads and 43 PPFM isolates against four fungal plant pathogens viz., *Alternaria carthami*, *Fusarium oxysporum f. sp. carthami*, *Sclerotium rolfsii* and *Rhizoctonia bataticola* was tested under *in vitro* conditions. Out of 62 FP isolates, all the isolates possessed inhibitory activity against more than one pathogen; 3 isolates inhibited the 3 pathogens; 2 isolates inhibited 2 pathogens. Similarly, out of 43 PPFM isolates, 4 inhibited all the pathogens, 19 inhibited 3 pathogens and 14 inhibited other 2 pathogens.
- Fluorescent pseudomonad isolates were also tested for their antibacterial activity against 4

bacterial pathogens viz., *Xanthomonas campestris* pv. *malvacearum*, *X. axonopodis* pv. *punicae, X. axonopodis* pv. *citri*and *Ralstonia solanacearum*. Out of 62 FP isolates, 58 possessed inhibitory activity against one or the other pathgens, 2 inhibited all the pathogens, 4 inhibited 3 pathogens, 2 inhibited other 2 pathogens.

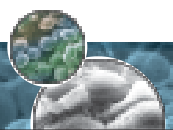
- Endophytic and phylloplane isolates exhibited very good antagonistic and other beneficial activities like N fixation, IAA and GA production. Two efficient cellulose and lignin degrading bacteria (*B. subtilis*&*B. niabensis*) exhibited very good lignin, cellulose, starch and lipid degrading activities. These are now under field evaluation for commercialization.
- Lab studies showed that B. *subtilis and B. niabensis* degraded vegetable waste completely within 19 days in conical flask.The aerobic composting unit inoculated with *B. subtillis* recorded shortest time for degradation, as compared to the one containing cow dung as the inoculum.
- The six most potent ACC deaminase producing strains (AR-ACC1, AR-ACC2, ARACC3, ANR-ACC1, ANR-ACC2 and ANR-ACC3) were identified as *Bacillus* sp., *Microbacterium* sp., *Arthrobacter* sp., *Bacillus* sp., *Microbacterium* sp. and *Paenibacillus* sp. respectively.
- The organisms induced drought tolerance in rice and increased seed germination by about 10% under anaerobic condition.The cellular proteins of 20 *Bacillus* and 23 *Pseudomonas* strains were checked and observed differences in some isolates.
- A limited number of Bt and Ps showed one or more PGPR functions, and more Bt isolates had PGP functions than the Ps isolates.Only 2 Bt isolates fixed nitrogen and 3 produced IAA, whereas, most of the potent Bt strains solubilized P, produced NH3 and cellulose.
- Metagenome of 123 coastal saline soils of Orissa isolated and analyzed by 16S rDNA and RE analysis. The metagenome sizes were mostly comparable, produced similar rDNA amplicons and had multiple restriction sites of the DNA. The electrophorogram of DGGE profile of 51 coastal soils showed wide variations indicating that the microbial guilds of all soils were not comparable.
- A total of 18 *Vibrio alginolyticus* were isolated from different brackish water system of east coast of India and identified using species specific 16S rRNA primers. ERIC-PCR was carried out to understand genotypic variation among these isolates using fingerprinting software.
- TNT is one of the most toxic explosives known to man, affecting plants, animals and most microorganisms. *Enterobacter cloacae* a Gram-negative bacterium, is able to utilize TNT as a sole source of nitrogen. The *nsfI* gene encodes the enzyme nitroreductase in *E. cloacae*. The nitroreductase gene was amplified from *Enterobacter cloacae* by using primers designed based on the sequences available in the NCBI database. The resulting 721bp PCR purified fragments were then digested with restriction enzymes and ligated in to pET32A vector system and transformed in to *E. coli DH5a*. The recombinant plasmid pET32A was isolated from *E. coli DH5a* and transformed into *E. coliBL21* expression host and was over expressed by IPTG induction. The over expressed proteins were confirmed by SDS- PAGE.
- A total number of 146 mushroom specimens were collected and tissue cultures of 116 specimens were obtained.
- Indigenous culture of *Lentinula edodes* was obtained from wild specimen. It will be attempted for its artificial cultivation.
- A new sp. of *Auricularia* has been cultivated on wheat straw and identified as *A. olivaceous* due to its olive colour.Two new species of *Cantharellus* and *Craterellus* were collected which could be interesting new records from the world
- Quantified plant growth promoting traits of important PGPR isolates. Quantification of phosphate solubilization and IAA production indicated that *Enterobacter* sp. R29 and *Pantoea dispersa* R5 were most potent in tri-calcium phosphate solubilization after 9th day of incubation and solubilized 29.97 and 34.22 mg of phosphorus/mg protein and there was decrease in pH of the medium from 7.2 in control to 4.99 and 4.33, respectively.
- To ascertain the presence of novel genus and species among the 23 different isolates of archaea of the tentative genera *Halorubrum*, *Haloferax*, *Haloarcula*, *Natrinema* and *Haloarcheon* studied so far, patterns of total intracellular proteins, polar lipids, GC content, electron microscopy and

minimum requirements of NaCl for initation of growth have been studied. The minimum NaCl requirement for growth of the present pool of archaea was 10%. The intracellular anions and cations accumulated during the growth of these archaea, from 10% NaCl to 35% NaCl were determined in Ion Chromatograph. It was found that with increase in NaCl concentration, there was gradual increase in accumulation of both NaCl and KCl with molar ratio of K:Na from 1:1 in lower concentration of osmolarity to 4:1 at the highest osmolarity.

- Identified 16 archaea on the basis of near complete 16S rDNA sequencing into the possible genus of *Haloferax*, *Natrinema*, *Halorubrum*, *Haloarcula* and unidentified *Haloarcheon* species and thus possibility of obtaining new genus and species was found as similarity with the existing 16S rDNA ranged between 95-99%. The phylogenetic relationship, studied on the basis of new 16S rDNA sequence data, divided the 16 isolates into 5 major clusters and 10 sub-clusters.
- The diversity of the archaea present at saturated NaCl concentration (35%) has been determined. A separate cluster has been identified for the present isolates among the existing database of genus and species of archaea at NCBI.
- DDRT-PCR conditions optimized for isolation of transcripts responsible for imparting tolerance to NaCl at different level of osmolarity in *Haloferax volcanii* H1.
- A unique segment consisting of 26 bases of conserved region of *Flavobacterium* species was selected for designing of probe.
- Genetic diversity of 20 *Azotobacter* isolates was done by 16SrDNA-RFLP PCR, RAPD PCR.
- Seventy more soil samples in addition to 146 earlier soil samples mainly covering 10 districts were collected and analyzed for pH, EC, organic C, ammonical N, nitrate N and total N. A total of **126** new morphotypes thus obtained from the above 70 different soil samples, out of which 35, 18, 26, 29 and 18 morphotypes belonged to Malate, Burk's, Doberiner, Nfb and LGI media, respectively and were added to already exiting **380** morphotypes.
- Genomic DNA of all the *nif*H +ve morphotypes was also amplified with 16S rDNA gene primers using 19F and 1378R primers. The amplified product was subjected to RFLP analysis with *Rsa*I, *Hae*III and *Msp*I restriction enzymes. The ARDRA analysis showed that the isolates growing on Malate, Burk's, Nfb and Doberiner media were distributed into various groups and the divergence among these started at 67, 46, 56 and 60 per cent similarity, respectively.
- Ten isolates (DB15(3), MDB10(3), MM10, MM35, MM80, MM15, NFB1, NFB4 and NFB28) isolated from arid and semi-arid zones of Haryana and showing better performance in pearl millet were belonged to *Bacillus pumilus, Bacillus firmus, Stenotrophomonas maltophilia, Bacillus, Paenibacillus* and *Agrobacterium* species on the basis of partial 16SrDNA gene**.**
- The five diazotrophs JPA2, SS2, DB76, DB14 and MDB4 which were isolated from arid and semi arid zones of Haryana and performed better in pearlmillet and wheat under pot house conditions were identified as *Gordonia* sp., *Bacillus subtilis, Psedomonas* sp., *Agrobacterium* sp. and *Brevundimonas* sp.
- Fourteen different diazotrophs isolated from salt affected areas of Haryana, having an EC of 1.04 to 21.00 dSm^{-1} andhaving 3 to 10% NaCl tolerance were tested for plant growth parameters in pearl millet *Var.* HHB146 (salinity tolerant) at EC of 0, 6, 8 and 10 dSm^{-1} under pot house condition vis-à-vis reference strain, *Azotobacter chroococcum* (HT 54).
- Ten isolates (*Rhizobium* species, *Pseudomonas stutzeri, Planomicrobium* species, *Paenibacillus polymyxa, Bacillus licheniformis, Pseudomonas* sp., *Bacillus sonorensis, Bacillus mycoides, Agrobacterium tumefaciens* and *Stenotrophomonas maltophilia*) isolated from salt affected soils of Haryana having 3-10% NaCl tolerance and showing better performance pearl millet at different levels of induced electrical conductivity (EC) were identified on.
- RFLP analysis of 16S rDNA of rhizobial isolates from *Vicia faba* nodules using *Msp*I and *Hae*III showed wide diversity among themselves. These were distributed into two major groups with different subgroups and the divergence among these started at 66 and 54 per cent similarity with *Msp*I and *Hae*III, respectively.
- Fifty fluorescent pseudomonads were isolated from rhizospheric soil of green gram from nearby

area of Kaziranga, Assam, India and assayed for their extracellular proteinase production. Out of these isolates 20 were found to be prominent in proteinase production. Genetic diversity of the 20 isolates were analyzed through BOX-PCR fingerprinting and 16S rDNA-RFLP along with three reference strains, *viz.,Pseudomonas fluorescens* (NCIM2099^T), *P.aureofaciens* (NCIM2026^T) and *P. aeruginosa* (MTCC2582^T).

- A total of 130 fluorescent pseudomonads from green gram rhizospheric soil of Jorhat district of Assam were isolated and characterized for multiple plant growth promoting traits, such as the production of indole acetic acid (IAA), nitrogen fixation, phosphorus (P) solubilization, siderophore production, production of ammonia (NH_3) and production of different enzymes *viz.*, protease, chitinase, pectinase and cellulase. On the basis of PGPR characteristics 8 isolates were tested for 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity.
- Twenty four bacteria were isolated in four different media. Based on the sequence similarity of 16S rDNA and phylogenetic analysis, isolates belonged to nine genera, *Providencia* sp., *Bacillus, Pseudomonas* sp., *Achromobacter* sp., *Brevibacillus* sp., *Sphingobacterium* sp., *Proteus* sp., *Caryophanon* sp. and *Acinetobacter* sp.
- Genetic diversity of 50 bacteria isolated from Tawang, Arunachal Pradesh was investigated for antifungal activity against *Fusarium oxysporum* and 5 isolates were found to be positive. 15% of the Streptomyces isolates produced bioactive metabolite found to active against the pathogens *viz*., *Fusarium oxysporum* f. sp. *ciceri* (Foc) and *Rhizoctonia solani.*
- Potential bacterial isolates which showed a positive response in PKV medium were further evaluated for their phosphate solubilizing ability using two types of inorganic phosphates-tricalcium and rock phosphate in liquid medium.
- Selected potential strains of *Bacillus amyloliquefaciens, Bacillus altitidinis*, *Bacillus pumilus* were tested against *Sclerotium rolfsii, Thanaetophorus cucumeris* and *Fusarium solani* in dual culture test.
- Two isolates of *Bacillus altitudinis*, three isolates of *B pumilus*, and one each isolates of *B amyloliquefaciens*, *B. cereus*, *B. methylotrophicus*, *Burkholderia symbiont* and *Serratia marcescence,* showed potential as phosphate solubilizer as well as antagonist against phytopathogens.
- PCR amplification rDNA gene of nineteen selected isolates of *Trichoderma* which showed antagonistic reactions against phytopathogens (*Fusarium graminearum, Fomes lamaoensis, Sphaerostilbe repens*, *Sclerotium rolfsii, Rhizoctonia solani* and *Macrophomina phaseolina*) obtained from rhizosphere and forest soil of North Bengal.
- Fifty (50) unialgal isolates have been screened for estimation of nitrogenase activity by Acetylene Reduction Assay (ARA) and 05 best strains were selected for large scale biomass production and algalization in terraced hill rice cultures.
- 32 isolates of *Trichoderma* sp. have been isolated from Assam and screening for antagonistic activities against bacterial and fungal plant pathogens is underway.
- Five potential *Trichoderma* five (MTB5, TKR1, TBB1, TNB4 and NKT2) species isolated from rhizosphere soil of crops of Andaman and Nicobar Islands showing antagonistic activities were selected for plant growth promotion properties as seed bio-priming in black gram and okra under greenhouse conditions.
- On the basis of the antagonistic, plant growth-promoting and salt tolerance properties, 12 bacterial isolates isolated from rhizosphere soil of Middle & North Andaman which showed multiple traits were selected for evaluation of PGP abilities under controlled conditions. Seed biopriming was carried out in chilli, brinjal and okra seedings. A significant enhancement of all plant growth parameters was recorded by *B. thuringiensis* (MPP5) over untreated controls of brinjal and chilli seedlings. *Pseudomonas tolasii* (NNB4) of Middle & North Andaman soils showed significant increase in all the parameters of okra seedling i.e., stem length (63.7%), root length (62.5%), secondary roots (75%), root and stem dry weight (22.86% and 96%).
- A total of 83 bacterial isolates were recovered from tropical soils of Little Andaman. Among the isolates 25.3% isolates showed IAA production, 33.7% isolates produced PO_4 solubilization and 53.01% isolates were siderophore producers. These isolates also exhibited cell wall degrading enzyme like amylase (65.06%), cellulase (65.06%),

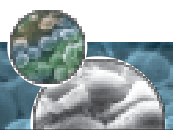


chitinase (9.63%), pectinase (14.45%) and proteolytic enzyme activity (84.33 %) were exhibited .All the isolates were tested for antagonistic activity against three major fungal plant pathogens, namely *S. rolfsii* (colar rot), *Macrophomina* sp. (charcoal rot) and *Colletotrichum gloeosporioides* (leaf spot). The results revealed that 8.43% and 10.84% isolates showed statistically significant inhibition of mycelial growth of *S. rolfsii* and *Macrophomina*sp., respectively.

- Seven potential PGPR isolate tested under *in vitro* condition showed that *Pseudomonas* strains enhanced the shoot growth in comparison to un-inoculated seedlings at 10 days. *Pseudomonas* strain BHK2 showed significant stimulation of root and shoot growth (54.23% and 52.94%, respectively) and lateral root formation (30.43%).
- Molecular characterization of Little Andaman isolates by using ARDRA analysis of 16SrRNA with two restriction enzymes viz., *Hae*III and *Bg*II showed RFLP studies also showed minor variations among the isolates.
- A total of 37 sequences were submitted to NCBI which includes 27 bacterial spp from *Tsunami* affected soil and 10 from tropical soils of Little Andaman whereas 22 bacterial microorganisms were submitted to NBAIM.
- Water and sediment samples collected from waste water and fish pond were inoculated in Giltay and Nitrite(GN)media for isolation of ammonia oxidising bacteria.
- 25 bacterial isolates were obtained and identified by 16Sr-DNA sequencing. The identified bacteria were *P aeruginosa, Citrobacter spp., Morganella morganii, P fluorescens, Alcaligens feacilis* and *Prividencia vermicola*
- Out of 25 bacteria, 5 were having terminal denitrification activity in addition of nitrification activity
- When glucose was used as a sole carbon source majority of bacteria oxidized ammonia completely within 24 hr with minimum accumulation of NO_3
- Beyond 48 hr of incubation, majority of bacteria showed the assimilatory effect i.e reformation of ammonia.
- Sodium acetate and succinate when used as sole carbon source, oxidation rate of ammonia was found to be less as compared to media using glucose as carbon source. No assimilatory effect was observed in any of the tasted isolates in sodium acetate media, and also same was observed in sodium succinate.
- Functional genes responsible for nitrification and denitrification (amoA-ammonia mono oxygenase, napA-periplasmic nitrate reducatse, nirk and nirs-nitrite reductase, norB- nitric oxide reductse and nosZ-nitrous oxide reducatse) were detected by PCR.
- Enzymes activities like Hydroxylamine reductase, nitrate reductase and nitrite reductase responsible for nitrification, denitrification respectively was estimated.
- Pathways for ammonia assimilation used by isolates differed. Some of them were showing denitrification from nitrate and some from nitrite as sole nitrogen source. In some isolates ammonia oxidation occurred via hydroxylamine or NO_3 as intermidaie products.
- This observation implies that although all isolates have the capacity to nitrify but their denitrification pathways are different.
- A few isolates were inoculated in 5 litres of wastewater in glassjar. Ammonia was completely removed by 72 hrs with accumulation of NO_3.
- Soil samples were collected from submontane undulating region of Punjab, the soil-sampling site was geo referenced and analyzed for physicochemical properties viz. soil texture, pH, EC, OC, ammoniacal and nitrate nitrogen. Soil texture varied from loam to sandy loam. The physicochemical properties of the soil samples were studied which were as : pH: 6.0 - 7.8, EC: 0.12 - 0.58 dSm-1, OC: 0.17 - 0.86 per cent, ammonical nitrogen: 21 - 123 ppm[1], nitrate nitrogen: 14 - 105 ppm. Punjab soils are deficient in organic carbon resulting in reduced total diazotrophic count. Soils with high ammonical-N and nitrate-N inhibited the diazotrophic population but some samples with high OC were found to have promising diazotrophic count. In addition to these factors pH and EC also play potent role in establishment of diazotrophic population.
- A total of 141 isolates were isolated from wheat based cropping system as wheat-rice, wheat-

maize and wheat-cotton from different agroclimatic regions of Punjab.Enormous diazotrophic diversity was observed on eight different nitrogen free media and population (cfu/g) ranging from 10^5 - 10^6cfu /g on different media: Jensen's : 57-76 x 10^6 , Burk's : 45-73 x $10^{6,}$ N deficient medium : LGI :23-47 x 10^5 , Dobereiner's medium: 26-48 x 10^5, Nitrogen free agar: 38-73 x 10^5, N deficient medium for : Derxia: 35-48 x 10^4 , *Beijerinckia*: 1-15 x 10^3 *Klebseilla&Enterobacter* : 29-55 x 10^5

- Cultural diversity of diazotrophs varied from transparent, translucent white, creamy, yellow, lemon yellow, peach, pink, orange and brown. Majority of bacterial colonies were mucoid and round with smooth margin whereas some of the colonies were non-mucoid.
- Most of the isolates were Gram negative, exhibiting variable shape as rods, cocci and spiral. Majority of isolates were non endospore formers, while some were positive for endospore formation. Majority of isolates were found to be negative for metachromatic staining. Most of the isolates were found to be motile, while some were found to be non motile. Majority of isolates were found to be positive for oxidase, catalase, citrate, urease, while variable reactions were obtained for MR, VP, indole, gelatin, starch hydrolysis, nitrate reduction and TSIA test. Majority of the isolates were negative for H_2S production.
- The isolates were characterized functionally for IAA production and eighty one isolates were found to produce IAA which ranged from 03 -40 g/ml.
- Thirty one diazotrophs were found to solubilize phosphorus on Pikovskaya's agar medium with distinct halo zones formation on the medium. Twenty three isolates were found to produce siderophore with the formation of orange colored zone on CAS agar. Sixty isolates were found to produce ammonia.
- The diazotrophic potential of the isolates at the molecular level was ascertained by performing *Nif* H analysis using two different *Nif* H primers (*Nif* H 1 and *Nif* H 2) from submontane undulating region of Punjab.
- The *Nif* H positive isolates were amplified for 16S rDNA. RFLP analysis of the 16s rDNA using three different enzymes viz. *Rsa I*, *HaeIII* and *Taq I* showed different DNA fingerprinting profiles for various diazotrophic isolates thereby deciphering their diversity at the molecular level.
- The data analysis was carried out using NTSYS software and dendrogram was prepared using UPGMA clustering.
- Cultures were identified on partial 16s rRNA gene sequencing on the basis of which phylogenetic tree was constructed using Mega 4based on multiple sequence alignment.
- *Azotobacter vinelandii, Brevibacillus brevis, Pseudomonas reactans, Enterobacter cloacae, Ochrobactrum anthropi, Klebsiella sp., Acinetobacter sp., Paenibacillus polymyxa* and *Bacillus amyloliquefaciens* were found to be predominant in Punjab soils, which were identified by 16s rDNA sequencing and were submitted to NBAIM, Mau.
- The ability of bacteria to grow on variable herbicide concentrations was tested using atrazine, pendimethalin and butachlor *Bacillus amyloliquefaciens, Klebsiella sp.* and *Azotobacter vinelandii* were found to tolerate up to 500ppm concentration of atrazine, pendimethalin and butachlor, respectively.
- Identified cultures were screened for potential cultures to study their effect on various growth parameters *viz.* root-shoot biomass and root-shoot length using maize crop as host under glass house conditions. Effect of inoculation of diazotrophs on nutrient uptake efficiency as nitrogen, phosphorus, potassium and micronutrient uptake was also studied to get potential diazotrophs. Inoculation with the diazotrophs showed significantly higher plant growth parameters and nutrient uptake as compared to uninoculated control.
- *Azotobacter vinelandii* was found to be thebest followed by *Pseudomonas reactans* and *Bacillus amyloliquefaciens* in promoting growth of the plants in terms of root-shoot length and root-shoot biomass, chlorophyll content and in enhancing phosphorus and nitrogen content of the plants. Inoculation with diazotrophs also improved micronutrient content in the plants.
- Phenotypic characterization for 146 bacterial strains isolated from 21 marine fishes of economic importance. Of these 31 bacterial strains, isolated from four marine fish species, Red-filament

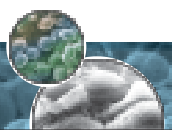


threadfin bream *Nemipterus mesoprion,* Flat-head grey mullet *Mugil cephalus,* Croaker fish *Johnius sp.* and Lutkei's half-beak *Hemiramphus lutkei* collected from Mangalore and 67 bacterial strains, isolated from ten fish species Spotted catfish *Arius maculatus,* Indian scad *Decapterus russeli,* Sin croaker *Johnius dussumieri,* Japanese threadfin bream *Nemipterus japonicus,* Indian mackerel *Rastrelliger kanagurta,* Fringescale sardinella *Sardinella fimbriata,* Goldstripe sardinella *Sardinella gibbosa,* Brushtooth lizardfish *Saurida undosquamis, Obtuse barracuda Sphyraena obtusata* and Goldband goatfish *Upeneus moluccensis* and for 6 bacterial strains from the gut live shrimps Coastal mud shrimp *Solenocera crassicornis* and Specked shrimp *Metapenaeus monoceros,* collected live from Visakhapatnam. Phenotypic characterization has also been completed for 48 bacterial strains isolated from seven marine fishes Bullet tuna *Auxis rochei,* Pompano dolphin fish *Coryphaena equiselis,* Japanese threadfin bream *Nemipterus japonicus,* Dusky finned bullseye *Priacanthus hamrur,* Indian Mackerel *Rastrelliger kanagurta,* Needle-scaled queen fish *Scomberoides tol* and tongue sole *Symphurus* spp. collected live from Colachel.

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

- Cross nodulation studies showed that soybean was nodulated by both soybean and chickpea rhizobial strains but chickpea was specific and nodulated only by its own strains.
- Inoculation of *Rhizobium* (R33) of soybean in field gave 19.3 % increase in seed yield; PGPR strain P10 (*Bacillus subtilis*) gave 28.6 % increase and the combinations of both gave 29.9% increase in vertisols.
- In chickpea, the rhizobial strain R40 gave 11.2 % increase in seed yield; PGPR P10 gave 13.2% increase and the combinations of both gave 17.2 % increase in seed yield.
- In wheat, the PGPR strain P10 gave 13.1 % increase; P10 + P25 (*Lysinibacillus fusiformis*) gave 18.2% and P3 (*Bacillus megaterium*) + P10 +P25 gave 26.0 % increase when inoculated as consortium.
- Inoculation of 5 effective strains of PGPR on wheat in the field resulted in average increase of total DM by 9.2%. There was significant improvement in activity of acid phosphatase (with 2 strains), alkaline phosphatase (3 strains), associative nitrogen fixers (1 strain), ammonium oxidizers (2 strains) and nitrite oxidizers (all strains). Overall the results point to improvement in soil health as a result of PGPR inoculation in wheat.
- All the fourteen cold tolerant P solubilizing bacterial strains were screened for the production of organic acids (Gluconic acid, 2-keto gluconic acid, fumaric acid, maelic acid, iso-cirtic acid and formic acid) in the culture supernatant by HPLC at 28°C. All the cold tolerant P solubilizing bacterial strains produced gluconic acid in the range of 84.0 to 1195.5 µg/ ml followed by the 2KGA 75.0 to 702.0 µg/ ml (except *Pseudomonas fragi* CS11RH4 and *Pseudomonas* sp. PB2RP2). However, *Pseudomonas poae* RT5RP(2) produced all the six organic acid.
- Eight bacterial consortium developed from five elite cold tolerant P solubilizing strains were evaluated under pot condition and significantly enhanced the pea (VL 47) root length, shoot length by 43.3 and 30.9%, respectively & root and shoot dry weight by 2.2 fold at 60DAS.
- Field experiments conducted during 2011-12 indicated that *Bacillusamyloliquifaciens* (GRB 35) and *Serratia marcescens* (GRB68) were effective for disease control and plant growth promotion of ginger. These strains were found to enhance the sprouting of rhizomes besides reducing the soft rot in ginger and enhancing the yield.
- Talc based formulations of efficient PGPRs were prepared and shelf life studies carried out at monthly intervals. Standardization of liquid formulation medium for efficient PGPR is also being done.
- Bioassay of *Metarhizium anisopliae, Lecanicillium lecanii, Paecilomyces* spp. *and Beauveria bassiana* isolates against *Aphis craccivora,Myzus persicae andBemisia tabaci*: Promising entomofungal isolates like, NBAII-Bb-77, Ma-44 & Ma-45 for *Aphis craccivora,* and Ma-44, Vl-32 & Vl-33 for *Myzus persicae,* were identified based on laboratory bioassay studies which caused 76-85% of mycosis on these pests.

- Development of Oil formulation of *B. bassiana* and *M. anisopliae*: In order to develop oil formulations of promising isolates of *B. bassiana* and *M. anisopliae*, twelve combinations of oil formulations were prepared with eight vegetable oils, four emulsifiers and one stabilizer. The oil formulations were prepared in October, 2011 and stored at room temperature (21-32ºC temperature and 43-78% RH). The cfu counts of *B. bassiana* and *M. anisopliae* were found to be as per the CIB standard of 1x 10^8/ml even after 5 months of storage in the Formulation No. 6 and 9. The Experiment is in progress for further shelf life studies
- The viability of bacteria and cyanobacteria consortia were tested in different carriers (compost, vermiculite, multani mitti, charcoal) in individually as well as in combination of carriers in a period of three month at 15 days time intervals. Multani mitti based formulations performed better as carrier maintaining 10^{-9} cfu g^{-1} population of bacteria and retaining the chlorophyll in cyanobacterial cultures. A liquid and gel formulation was also prepared and this experiment is under progress for testing the viability of bacterial and cyanobacterial consortia.
- Arbuscular mycorrhizal fungal (AMF) colonization significantly increased the soil available Fe (M- 1.9; M+ 2.1 mg kg^{-1}) and Zn (M- 4.16; M+ 4.50 mg kg^{-1})
- The increased availability of Zn is closely associated with siderophore production in M+ plants (51.4 µmol cm^{-3} hr) were higher than M- plants (39.5 µmol cm^{-3} hr)
- Front Line Demonstrations (FLDs) were conducted in farmers' field at Aanaimalai, Pollachi and Madathukulam, to study the Zn nutrition in maize using mycorrhizal symbiosis during 2011-12. Application of $ZnSO_4$ (37.5 kg ha^{-1}) recorded comparable grain yield of 5876 kg/ ha as that of mycorrhiza along with $ZnSO_4$ (@12.5 kg ha^{-1}) with a higher B:C ratio (2:09) when compared to control (1:91).
- Pretreatment of tomato plants with *B. subtilis* BS2 induced significantly more amount of defense related enzymes *viz*., peroxidase, polyphenol oxidase, chitinase and phenylalanine ammonia lyase and phenolics when challenged with the pathogen (*F. oxysporum* fsp. *Lycopersici* FOL). However the induction of defense enzymes PAL and PPO was significantly at high level in BS2 than BA1.
- The survival ability of *Trichoderma asperellum,* (oneamong the potent *Trichoderma* strains) was evaluated in cassava thippy, a cheaper as well as a waste material of cassava starch factory. Granular pellet formulation of thippy based *Trichoderma* developed using a pelletizer with 20% moisture and the viability as colony forming unit (cfu/g) of the stored formulated bio-agents were recorded monthly. It is observed that it could maintain the survival ability with no significant reduction (10^7cfu/g) all over the storage period up to eight months.
- Field evaluation of PGPR strains in chickpea: Field inoculation trial conducted with chickpea variety DCP 92-3 confirmed the growth response and increase in grain yield due to inoculation with different PGPR strains. There was differential response to inoculation ranging from 4 to 27 per cent increase over un-inoculated control level of 16.25 q/ha of chickpea grain. Highest increase in grain yield about 27% over un-inoculated control was obtained with PGPR strains J 7 over the last two years of field experimentations.
- Scanning electron microscopy of the cuticle of combination formulation treated larvae revealed mycelial growth of *B. bassiana* on the inner surface of the cuticle within 48 h after exposure while this was absent in larvae treated with *B. bassiana* singly, where as Transmission electron microscopic studies of the mid gut of combination formulation treated larvae revealed complete vacuolation of the columnar epithelial cells (lining the mid gut) within three hours after treatment while it took 12-24 h in larvae treated with Bt singly.
- In papaya, immature seeds cultured in vitro with a view to attain somatic embrogenesis gave rise to embryogenic callus in more than 50% cultures. Culture indexing at the time of initiation through the culturing of seed-covering on NA and TSA did not indicate any culturable bacteria while using seeds from surface sterilized fruits.
- The colony growth from the index-positive cultures on nutrient agar and trypticasein soya



agar were taken through bacterial culture purification. All the cultures showed the association of same bacterium. This organism was identified as *Brachybacterium rhamnosum* based on 16S rRNA gene sequence homology analysis.

- Presence of non-culturable bacteria in seed-derived embryogenic cultures of papaya was further conformed through phase-contrast microscopy on the tissue homogenate under high magnification revealing viable but non-culturable bacteria in considerable numbers.

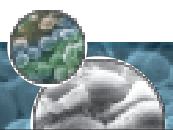
Theme 3: Microbial Management of Agro waste, Bioremediation, Microbes in Post Harvest and Processing

- Potent PAHs degrading microbial consortium was developed which consist of a bacterium (*Serratiamarcescens* L-11), actinomycetes (*Streptomyces rochei* PAH-13) and a white rot fungus identified as *Phanerochaete chrysosporium* VV18.
- A total of 25 strains were isolated by enrichment method and were analyzed for HCH-degradation by gas chromatography. Majority of strains belong to sphingomonads group. Only 7 strains were found to degrade HCH isomers. Southern hybridization confirmed the presence of *lin* genes. Some of the isolated strains have been while some are still being characterized by using poltphasic approach for bacterial classification. This work has led to several publications in international journal.
- Variants of *linA* and *linB* were found by direct PCR and cloning from HCH contaminated soil.For *linA* nearly 300 clones were screened, out of which 43 clones showed positive result using High Throughput Assay. This result was confirmed using GLC Analysis. Out of these 43 clones, 20 clones showed better degradation potential than LinA of *Sphingobium indicum* B90A. Kinetic analysis of these clones is yet to be carried out. While, in case of *linB*, the active LinB clones (50) were further sequenced. None of the clones were 100 % similar to any of the reported *linB*. A nearly common difference in all the clones was observed at 147 position with Alanine replacing Aspartic acid. Time kinetic studies with few of the variants will reveal if these clones are more active than *Sphingobium indicum* B90A.
- Pot scale bioaugmentation experiment was conducted using a consortium of bacteria which indicated its further use in conducting bioremediation field trials. Bioaugmentation of HCH contaminated soils was attempted with *Sphingobium indicum* B90A, which was not very successful owing to low survival of the bacteria. Hence, it was decided to use HCH degrading bacteria isolated from HCH dumpsite in the form of consortium rather than using a single bacterial strain. The main reason behind it is the fact that these bacteria have been isolated from the HCH dumpsite itself and their survival in the field is expected to be better than that of *Sphingobium indicum* B90A. Thus, pot scale bioaugmentation study was carried out.
- Molecular characterization of twelve *H. insolens* isolates was done for taxonomic confirmation and it was found that all the isolates were *H. insolens*. Later RAPD was done by using five OP-primers of A and P series to see the intra specific variations and it was noted that out of twelve four strains showed variability
- Methodology for total indoor compost production using *S. thermophilu* and *H. insolens,* standardized. In this case, compounding mixture after its thorough wetting (3days) was directly filled in the Phase II tunnel, escaping Phase -I conditions altogether. Compost was subjected to pre- pasteurization conditioning, pasteurization and post pasteurization conditioning. Entire operation lasted for 10 days (3days mixing + 7days in tunnel).
- The most potent organism, *Methylobacterium* sp., was found to degrade solely β-hexachlorocyclohexane and it did not show any marked degradation for α, γ and δ isomers of hexachlorocyclohexane. Growth kinetics of revealed that the degradation of β–HCHstarted with onset of growth and optimum at stationary phase.
- Effects of the β-HCH on the degrade *Methylobacterium, Sphingomonas* and 2 *Bacillus* spp. and the chlorpyriphos on *Achromobacter, Xanthobacter, Bacillus* and *Sphingobium* spp. revealed expression of 3 additional proteins for HCH and 4 for chlorpyriphos treatments. The proteins would be some enzyme proteins but their identity could not be ascertained.

- Agrowaste materials like sludge, charcoal, pressmud, rice husk and rice straw were inoculated with microbial consortium of five fungi (*Aspergillus niger, A. terreus, T. longibrachiatum,T. fasciculatum, A. awamori*) and two bacteria (*B. cereus*, *B. sp.*) individually and in combination. Data indicated encouraging results for removal of heavy metals (Cu, Zn, Ni) from synthetic and industrial wastewater by pressmud, rice straw and pressmud in combination with rice husk inoculated with microbial consortium.
- Occurrence sulfur oxidizing bacteria, *Beggiatoa* sp. in shrimp culture ponds and estuaries was studied and five isolates were successfully enriched, characterized and identified. Investigations on *Beggiatoa* spp. Has been hindered by the inability to grow sufficient quantities of cells. There is no precise for mass cultivation of *Beggiatoa* and are extremely difficult to grow these cells *in vitro*. Hence, *Beggiatoa* filaments were purified out using CsCl gradient ultracentrifugation and subjected to further molecular characterization.
- Maximum nisin activity (3.8logAU/ml) and growth (9.48, 8.98 and 8.88 log CFU/ml) were obtained with MRS, GM17 and BHI broth, respectively followed by M17 broth having nisin activity and growth 3.5 log AU/ml and 8.82 log CFU/ml, respectively. Nutrient broth plus skim milk and skim milk broth plus glucose (2%) exhibited the lower but similar nisin activity (2.9 log AU/ml) with the growth of 8.46 and 8.35 log CFU/ml, respectively. Minimum nisin activity (2.6logAU/ml) was assayed with skim milk broth and nutrient broth plus glucose (2%). However, no nisin activity was obtained with nutrient broth with the poor lactococcal growth (7.44log CFU/ml).
- **Studies on characterization and scaling up of honey wine (mead)**: Honey samples *viz*., processed honey, water heated honey, reprocessed honey, Italian bee honey (sealed, unsealed), little bee honey, Indian bee honey, sting less bee honey, fermented honey from Department of Entomology, TNAU, rock bee raw honey samples from Visakhapatnam, East Godavari, Chittor, Mahaboobnagar, Nellore, Munar were collected in a sterile container and stored at 27°C for the isolation of fermentative yeast cultures. The yeast isolates were isolated from various honey samples collected from different places. These yeast isolates were named based on the type of honey samples. Fourty five yeast isolates were identified based on their colony morphological characters *viz*., colony size, shape, margin, elevation, texture, appearance, pigmentation and optical property. Among the 45 isolates, yeast isolate YR15 (rock bee raw honey, Nellore) was selected based on its tolerance to higher concentration to glucose, adaptability to higher temperatures (35°C) and pH (5.5).
- Ferric reducing antioxidant power activity (FRAP) and DPPH (2, 2- diphenyl-1-picrylhydrazyl radical scavenging activity: The FRAP assay was selected to be used as the first step in this investigation, because intrinsic antioxidant potential of honey could be measured, taking antioxidant constituents as reductants in a redox-linked colorimetric reaction. The antioxidant power of honey compared with ferrous sulphate as a reference standard. The FRAP results showed a greater difference in the antioxidant capacity of various honey samples. The observed range of FRAP value (16.12 µM Fe (II)/100g to 85.11 µM Fe (II)/100g). Among honey samples string less bee honey showed more ferric reducing antioxidant power (85.11 µM Fe (II)/100g) and DPPH radical scavenging activity (88.09%). This property suggested that honey may act as a free radical scavenger, capable of transforming reactive free radical species into stable non radical products.

Theme 4: Management of Abiotic Stress

- 12 salt-tolerant isolates (3 each of Associative N2 fixers, free-living N2 fixers, P-solubilizers and fluorescent pseudomonads) were subjected for cytokinin production under salt stress using HPLC. Irrespective of the functional group, significant reduction in the amount of cytokinin was recorded with increase in medium salt concentration. The amount of cytokinin produced by the isolates ranged from 20.7 to 48.8 and 1.3 to 20.4 µg per l in the presence of 5 and 10 per cent NaCl respectively, as compared to 39.31 to 53.66 µg per l in the control medium (without any added salts).
- Out of 65 salt tolerant isolates identified using

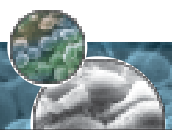


16SrDNA sequencing technique, 13 isolates showed closest affiliation to *Pseudomonas aeruginosa,* 10 to *Pseudomonas nitroreducens*, 9 to *Bacillus endophyticus,* 6 to *Bacillus megaterium*, 5 to *Enterobacter aerogenes,* 3 to *Staphylococcus* sp., 2 each to *Bacillus subtilis*, *Enterobacter cloacae*, *Staphylococcus sciuri*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Agrobacterium tumefaciens*, *Pseudomonas mendocina*, 1 to *Pseudomonas putida, Pseudomonas fluorescens*, *Pseudomonas monteilii*, *Enterobacter oryzae, Pseudomonas* sp.

- Field efficacy of four efficient salt-tolerant bacterial isolates and their consortia along with one salt-tolerant fungal isolate as against un-inoculated control was assessed in wheat and sorghum under graded soil salinity at ARS, Gangavati during *Rabi* 2011.
- Anti-oxidative enzyme (AOE) activity viz., catalase, peroxidase and SOD in wheat plants were analyzed at 15, 21 and 30 DAS. It was interesting to note that, inoculation of salt tolerant isolates gave significant reduction in the activity of anti-oxidative enzymes when compared to un-inoculated wheat plants in all the salinity levels tested. Activity of AOE was directly proportional to salinity.
- **Pot evaluation of new rhizobacterial isolates:** Two hundred new rhizobacteria were characterized for abiotic stress tolerance, plant growth promoting traits and biochemical characteristics were screened in the previous year. Among these, drought tolerant isolates possessing plant growth properties were screened in plastic cups with sorghum and pigeonpea under drought stress. Four selected drought tolerant isolates KB 142, KB 129, KB 133 and KB 122 from the preliminary screening were tested as seed inoculants in sorghum under drought stress conditions. Experiment conducted in two sets with one set maintained at 50% water holding capacity (WHC) throughout the experiment period (and second set maintained at 75% WHC followe by water withholding after 27 days of germinaton). Observation recorded after 32 days of germination indicated positive effect of bacterial inoculation on plant growth as well as biochemical parameters sugar, chlorophyll, relative water content, soil moisture levelof sorghum. Variation in terms of proline content were observed among different treatments with isolate KB122, KB142, KB133 showing 2-3 fold increase in proline content, whereas isolate KB129 showed more than two fold reduction in proline content under 50% continuous moisture condition. However under 75% moisture followed by no irrigation all the treatments showed reduction in proline content with isolate KB129 showing highest reduction. These results indicate that different mechanisms might be operating behind microbial induced abiotic stress tolerance Scanning electron micrograph confirmed bacterial colonization on root surface.
- The best four bacterial isolates possessing drought stress tolerance as well PGP traits were characterized based on microscopic, morphological and biochemical studies. Microscopic studies revealed that all the four isolates were Gram positive, rod shaped bacteria. On Petri plates, the bacterial isolates were observed as irregular margin, dry wrinkled and opaque colonies and none of the isolates produced pigment. Based on colony morphology, microscopic observations and biochemical properties, the isolates were identified as *Bacillus* spp. For species level identification, molecular characterization of the isolates was done on the basis of 16S rDNA gene sequence and the four sequences were submitted to Genbank (Accession no. JQ623487 - JQ623490). Among the four isolates, *Bacillus* spp. Strain KB 129 showed increased plant growth promotion under drought conditions and it could be exploited as potential drought tolerant isolate for commercial application.
- **Screening of low temperature N_2 fixing bacterial communities:** A total 70 psychrotolerant nitrogen fixing cultures were isolated from seven different locations of Western Indian Himalayan belt.
- On the basis of their growth profile 26 different cultures were selected for further study**.**
- Four isolates were found to be *nif*H positive and two of them were *nif*D positive too**.**
- One among the four *nifH* positive isolates and both the two *nifD* positive isolates were sequenced and submitted to NCBI GenBank (JN020960-B4, JN055437-B4 & JN055436-N26, respectively)**.**

Theme 5: Microbial Genomic

- *M. ciceri* Ca181 genomic sequencing has been completed.
- After analysis 18 SSRs in the genome of *M. ciceri* Ca181 and the frequency of SSRs longer than 10 bp. Among the various class of microsatellite, trinucleotide and dinucleotide repeat was the most abundant class.
- The frequency of maximum mononucleotide SSRs are longer than 10 bp and one mononucleotide repeat was C motif longer than 22 bp.
- An iron starvation tolerant (IST3) was isolated by screening a transposon mutant library of *Pseudomonas putida* S11 under iron-limiting condition.
- Genome walking PCR and nucleotide sequence analysis showed the ORF (*pstS*) into which transposon had inserted encodes sensor histidine kinase of a two-component signal transduction system.
- Amplification and sequence analysis of the two-component single transduction system (*pstSR*) showed it encodes an integral membrane sensor signal transduction histidine kinase (*pstS*) and its cognate transcriptional regulator (*pstR*).
- A pyoverdine - negative derivative of mutant (IST3) was constructed.
- Mutant IST3 showed significantly improved biofilm formation, seed adhesion and competitive root colonization than the parent strain.
- By inactivation of *sdi*A root colonization by *E. cloacae* GS1 has been significantly increased.
- The transcriptional level of chitosanase encoding gene in *A. fertilissima*(*cho*) measured using quantitative real time PCR (qRT-PCR) also indicated increased expression levels under same optimized conditions.
- The chitosanase activity measured using different substrates showed the highest activity against colloidal chitosan. HPLC profile of the products of enzyme activity with different chitosan oligosaccharides revealed in the production of dimer units $(GlcN)_2$ or more confirming the endo-type nature of the purified chitosanase.
- Cyanobacterial strains (*Anabaena laxa* RPAN8 and *Anabaena variabilis* RPAN59), along with negative control *Anabaenavariabilis* (RPAN16), grown under controlled and optimized environmental/nutritional conditions, were amended with formulation (compost-vermiculite as carrier) and evaluated against phyto-pathogenic fungi and their interactions with tomato plants. The strains RPAN8/59, grown under optimized conditions, had higher fungicidal activity, which caused enhanced microbial activity, nutrient content and plant growth in the same treatments. The induction of defense and pathogenesis (PR) related enzymes were also evaluated in roots of tomato seedlings, which were found to be enhanced and moreover highest in 14d old *F. oxysporum* f. sp. *lycopersici* challenged–cyanobacterium amended formulations treated tomato root tissues.
- Genes involved in lignin biosynthesis, phenylpropanoid biosynthesis, chorismate metabolic process, response to oxidative stress, oxidoreductase activity, chitinase activity, calcium ion binding, iron ion binding and genes known to be involved in response to biotic stimuli are overrepresented in the set of genes which are overexpressed upon LPS treatment.
- Treatment with the BXO1003 strain induces more number of genes than treatment with wild type Xoo (BXO43) but lesser number of genes than treatment with BXO1002. This might be due to the fact that, even though BXO1003 lacks EPS it also has a defective LPS. The lack of native LPS might make this strain a less potent inducer of host innate immunity.
- Around 70% of the genes which are induced by treatment with bacterial strains (wild type Xoo, BXO1002 and BXO 1003) were also induced by LPS treatment. This indicates that the repertoire of defense genes induced by LPS are also those that are upregulated upon bacterial infection of rice.
- Pathway analysis done using "Ricecyc" software showed that pathways for chorismate, Phenylalanine, Suberin, Salicylic acid, Phenylpropanoid, Ethylene and Indole alkaloid biosynthetic pathways are up regulated upon treatment with LPS, BX1002 and BXO1003 strains.
- Different pathogenesis Response (PR) genes like



PR1, PR2, PR3, PR4 and PR10 were also found to be upregulated upon treatment with LPS, BXO1002 and BXO1003.

Theme 6: Microbial Genomic Resource Repository

- The center is enriched with genomic DNA of different groups of fungi which include Myxomycetes, Mastigomycetes, Zygomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes in addition to bacteria, actinomycetes and blue green algae (BGA).
- It is also enriched with total genomic shot gun library of *Mesorhizobium ciceri* ca 181 along with its genome sequences.
- Significant number of fluorescent marker like GFP (Green Flourescent Protein), YFP (Yellow Flourescent Protein), RFP (Red Flourescent Protein), and gene silencing vectors like pSilent-1 for characterization and identification of unknown fungal genes, His-tagged expression vectors for functional studies of an known or unknown gene, and various other gene constructs of *Bt* gene from *Bacillus thurengensis* are preserved.
- Universal PCR primers from bacteria and fungi, species specific real time primers for various organisms, functional gene primers like *Nif* are collected.
- MGRR has generated a well equipped world class laboratory with all modern equipments like Robotic DNA extractor, pyrosequencer, Gel imaging system, Electroporator, High throughput gel electrophoresis system etc
- Each genetic material is preserved by at least two methods according to the type of genomic material, either under short term, or long term storage at 80°C. Apart from that an agriculturally important fungal microsatellite database has being developed.
- Apart from the preservation of microbial genetic resources, MGRR is developing databases like "Agriculturally important fungal microsatellite primer database" which consist of 50,000 microsatellite primers from various fungi.

Human Resource Development

One of the important activity of NBAIM is Human Resource Development (HRD) and training for trainers and scientific/technical personnel for evolving technologies in relation to research, management and application of microorganisms, and for addressing the issues of biosafety, management of intellectual property, facilitated access, and equitable benefit sharing. Following are the main area of trainings imparted at NBAIM:

- Provide training to researchers in the field of molecular identification of AIM's and tools for technology development and its implementation.
- Transfer of technology from the laboratory to land.
- HRD in the field of molecular taxonomy will be carried out.
- Identification of hitherto microbial diversity of the country and also strengthen the extensive training programs on molecular characterization of AIMs.

During 2011-12, following specialized trainings were organized:

- National training programme under NAIP on "Trends in Bioinformatics and Computation Systems: Exploring Interconnections for Molecular Biological Applications" from July 16-29, 2011 at NBAIM.
- National Training Programme on Metagenomics: A practical Approach to Molecular Taxonomic Profiling from November 22- December 1, 2011.
- National Training Programme on "Genome Mining: From Functional Readouts to Practical Applications from 06-17th December 2011.
- National Training Programme on "Development of Gene Chip for Microbial Identification using DNA Probes" has been scheduled from 28th January to 10th February, 2012.
- National Training Programme on "Bio-informataics in Multi Omics Era: A Microbial Genomics Perspective" from 23 February 2012 to 3rd March 2012
- Sensitization meeting on Intellectual Property Rights (IPR) in Biotechnological Research on 25th March, 2012.

"Development of gene Chip for Microbial Identification using DNA Probes"

"Data mining and computational methods in bioinformatics for microbial research"

"Trends in Bioinformatics and Computation Systems: Exploring Interconnections for Molecular Biological Applications"

"Genome Mining: From Functional Readouts to Practical Applications"

Meetings and Visits

- Annual Review meeting of AMAAS on May 14-15, 2011
- Visited NBPGR for discussion about cryopreservation facilities at NBPGR gene bank July 15, 2011
- Visited NRC Equine to explore the possibilities of establishment of alternate set of culture collection of NBAIM. August 6, 2011
- Attended the meeting to fix the performance indicators for bureaux at NBPGR, New Delhi August 29, 2011
- Participated in National Training Programme on "Allele Mining" at IISR Calicut from Sept 10-24, 2011.
- Participated in stakeholders meet on Platform Water at NBFGR Lucknow on October 18, 2001
- Participated in stakeholders meet on Platform Waste at NAAS on October 13, 2011
- Participated in Annual Review Meeting (Allele Mining) Nov1-3, 2011
- Participated in the Interactive Meet of Administration and Finance at NAAS on November 22, 2011
- Participated in Interactive meeting of the scientists trained abroad from Nov 28-30, 2011
- Participated in the Interactive Meet of Stake holders for nantechnology mission on March 11-12, 2012.
- International Conference on Microbes in waste water & waste treatment, bioremediation and energy production (MWT 2011) held at Goa, from January 24-27, 2011
- Panelist, in Agriculture Science Congress at NBFGR organized by National Academy of Agricultural Sciences from 07.02.11-11.02.2011
- National conference on Microbial diversity and its applications in agriculture, health and industry from 5-6 March, 2011 at ICAR Research Complex, Goa
- Panelist, Brain storming session on Microbes and sustainable agriculture on 13.09.2011 at INSA, New Delhi.
- 3rd Global Conference on "**Plant Pathology for Food Security"** held on January 10-13, 2012 at College of Agricultural Science, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan (India).
- International conference on "Environmentally Sustainable Urban Ecosystem (ENSURE 2012) held on February 24-26, 2012 at Indian Institute of Technology, Guwahati, Assam (India).
- International Conference on Perspective and Challenges in Chemical and Biological Sciences: Innovation crossroads from 28- 30 Jan, 2012 at IASST, Guwahati.
- 5th Asian Young Researchers Conference on Computational and Omics Biology (AYRCOB) at Daejeon, Korea with full travel grant from "Genome Big Bang" group (Global COE program), University of Tokyo.
- All India Essay Competition on "Algae BioFuel: India's opportunities" organized by Nandini Chemical Journals, Chennai.
- Lecture for farmers on "Mushroom Ki Kheti: Kam Samay Mein Jyada Labh" in Kisan Mela held at DSR-NBAIM campus on 25/2/2012.

Foreign Visit 2011-12

- Prof. Dilip K Arora was selected as National Expert International collaboration/ Bilateral Exchange Program at Prague Agrc. University, Cecz Republic.
- Prof. Dilip K Arora was Invited Visitor at Munich University, Germany in 2011

Honours, Awards & Recognition

- Mr. Anurag Chaurasia selected as Member of the Editorial Board of Biodiversity & Conservation, Springer; Biodegradation, Springer and Journal of Industrial Microbiology & Biotechnology. Springer.

Publications

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Library Information and Documentation

Books

Administration	60
Bacteriology	85
Biochemistry	33
Bioinformatics	60
Bioinstrumentation	01
Biotechnology	206
Botany	33
Environmental Sciences	14
Integrated Pest Management	16
Microbiology	449
Molecular Biology	203
Phycology	12
Plant Pathology	120
Plant Virus	14
Mycology	169
Genetics	52
Genomics	37
Intellectual Property Rights	57
Proteomics	05
Virology	37
Enzymology	07
Others	22
Biocontrol	04
Biostatistics	07
Biofertilizers	03
Soil Biology	14
Yeast	05
Total	**1725**

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Advanced Biotech

Hindi Books

Current Contents of Life Sciences

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1. Applied and Environmental Microbiology
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3. Biology and Fertility of Soils
4. Canadian Journal of Microbiology
5. Clinical Microbiology
6. Current contents
7. Current Science
8. FEMS Microbiology Reviews
9. Fungal Genetics and Biology
10. Indian Journal of Experimental Biology
11. Indian Journal of Microbiology
12. Indian Journal of Sugarcane Technology
13. Indian Phytopathology
14. Journal of Biochemistry and Biophysics
15. Journal of Biosciences
16. Journal of Biotechnology
17. Journal of Eco-friendly Agriculture
18. Journal of Indian Institute of Science
19. Journal of Mycology and Plant Pathology
20. Journal of Scientific and Industrial Research
21. Journal of the Indian Institute of Science
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23. Molecular Plant Pathology
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25. Mycologia
26. Mycological Research
27. Nature
28. Pestology
29. Plant Diseases
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32. Eukaryotic Cell
33. Indian Medicinal Plants
34. Journal of Bacteriology
35. Microbiological Research
36. Science

Linkages and Affiliations

Local Institute

The NBAIM has effective linkages with different ICAR research institutions and SAUs situated in U.P. The Bureau is actively participating networking with NRCs on microbial biodiversity along with other Institutes having expertise. These linkages provide library facility, characterization and fingerprinting of selected AIMs, including logistic support in R&D activities.

National Institute, Agricultural Universities and Organizations

The NBAIM has strong linkages with national institutes, agricultural and conventional universities, other Government and Non-Government organizations. We are strengthening the "Indian Microbial Genetic Resources Management System" by making up the national base for collection and deposition of AIMs meant for long term storage and evaluation. The Bureau is presently linking with different small and private organizations along with individual collector of AIMs and persuading them to deposit their collection at NBAIM.

NBAIM is participating in the research programmes (Outreach) of IIHR and IISR.

Under the World Bank aided National Agricultural Innovation Project, NBAIM is consortium leader and partner in several projects.

International Institutes

- The Bureau has linkages with International microbial resource centres covered under the umbrella of WFCC and OCDE. NBAIM has imported cultures from CABI, Bioscience, U.K., ATCC, USA, HUT, Hiroshima University, Japan, Fungal Genetic Stock Centre, USA, Agricultural Research Service Culture Collection, USA, Bacillus Genetic Stock Centre, USA.

RAC and IMC

Research Advisory Committee (RAC)

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University of Mysore, Mysore

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Dr. Appa Rao Podile
Head
Department of Plant Sciences
University of Hyderabad, Hyderabad

Shri Haribansh Dwivedi
Pakhaipur
Mau, Uttar Pradesh

Dr. V. N. Singh
Pratima Hospital
Mau, Uttar Pradesh

Prof. D. K. Arora
Director, NBAIM

Member Secretary
Dr. Alok K. Srivastava
Senior Scientist, NBAIM

Recommendations

- The members highly appreciated the progress of research in last one year. Further the RAC expressed their concern about the utilization of selected potential isolates and suggested that in 12th Plan period these isolates may be evaluated in field by various participating institutes as per their mandate crops and AICRP schemes. Under the theme diversity, it was suggested to focus on Agro ecological zones.
- The progress of NAIP projects and out-reach projects are rated as excellent and PIs are advised to stick with the approved plan and participate in national seminars and conferences in respective field of research.
- The thrust areas for the 12th Plan period has been presented and discussed. The members recommended to priorities the work under AMAAS in the area of :

i. Development of "National Microbial Map" : Exploration of the extreme environment microbiota using a combination of meta-omics approaches

ii. Deciphering rapid gene expression/signature profiling of certain microbial communities, predicting their function and detecting their relative abundance within extreme habitats

iii. Development of nano-bioformulations for biocontrol of phytopathogens, insect pest and weeds

iv. Whole genome sequencing (de novo/reference) of some elite agriculturally important microorganisms and bioprospecting for novel genes

v. Development of Microbial consortia for rapid degradation of agrowaste and their muti-location testing:

- The RAC expressed very serious concern regarding the culture collection of NBAIM and felt that: there is an urgent need to have units of NBAIM at a suitable locations near Delhi and another at Mukteshwar, to establish the alternate culture collection units. Therefore, the RAC reiterates its earlier recommendation that "SMD may deal with this issue on **"Top Priority"** and provide a suitable place to establish alternate culuture collection unit, so that a back up of Agriculturally Important Microorganisms may be created, which is in public interest".
- The RAC also recommended to apply for the approval from IDA
- The RAC has taken a serious note about the deployment of scientist as it is less than 50% of the sanctioned cadre strength and all the post of Principal Scientist's are lying vacant. Further it was suggested that the SMD may look in the matter and expedite the process of deployment at NBAIM
- RAC felt that in 12th plan post of more scientist may be given to Bureau looking in the magnitude of the mandate's of Bureau in the area of Agricultural Microbiology, bioinformatics, Virology, Molecular biology.

Institute Management Committee (IMC)

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Dharwad

Deputy Director (Agriulture)
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F&AO
IIPR, Kanpur

Shri Haribansh Dwivedi
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Mau, Uttar Pradesh

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Member Secretary
Sri S. N. Yadav
A.A.O., NBAIM

Recommendations

- The Action Taken Report was approved and accepted.
- IMC greatly appreciated the efforts made by NBAIM in the field of research and infrastructural development.
- IMC was satisfied and appreciated the budget utilization and revenue receipts.
- IMC was satisfied with replies of the para related to use of equipments and other issued related to audit.
- Taking into consideration the mandate and subsequently allocated allied schemes (AMAAS, externally funded projects, MGRR etc.), the committee felt that adequate and commensurate strengthening of the scientific and technical manpower is utmost necessary. Special efforts should be made to fill the vacant scientific positions.
- IMC agreed with the proposal for the construction of girl's hostel at NBAIM as it is located in a remote place and no rental accommodation is available outside the campus.
- Proposal for dedicated power supply for the Bureau was critically discussed in the meeting. IMC strongly recommends **a mini-power station** with high power transformer, be established at the centre very soon, before an irretrievable loss to the national microbial treasure occurs.
- IMC recommended replacement of the water supply systems which are old and has developed several leakage points of maintenance and minor replacements.
- IMC reiterated its last year recommendations and again strongly recommended that the creation of alternative culture collection and maintenance facilities in New Delhi, NBAIM culture collection is a national treasure of agriculturally important microbes, and its security is a national duty and a prime concern for NBAIM as well as ICAR; a second set is indispensable to take care of the unforeseen events.
- IMC recommended establishment of two regional stations is 12th Plan one at temperate North Himalayas (Mukteshwar) and another at Delhi (as above) for smooth functioning of different activities as per the mandate of NBAIM.

NBAIM Personnel

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Sudheer Kumar
Senior Scientist

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Senior Scientist

Mahesh Yandigeri
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Manoj Kumar
Bali Ram
Chetan Singh
Rekha Gupta
Ram Gopal
Ram Avadh Singh
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