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J. Mycopathol, Res, 57(1) : 1-11, 2019;
ISSN 0971-3719

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REVIEW

Harnessing beneficial microbial resources, analysis of their diversity and development of bioformulations for crop improvement

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Received : 18.03.2019

Accepted : 20.03.2019

Published : 29.04.2019

Microorganisms isolated from soil samples of forest, river basins and agricultural field of terai, dooars and hills of North Bengal were screened *in vitro* for their beneficial traits. Potential biocontrol agents (BCA), phosphate solubilising fungi (PSF) as well as phosphate solubilizing bacteria (PSB) were selected for molecular identification by amplification of their partial 18s rRNA and 16s rRNA gene sequence analysis respectively. All these sequences obtained for BCA, PSF and PSB isolates were further aligned separately with ex-type sequences obtained from NCBI genbank database. Phylogenetic inference was performed by the unweighted pair group method with arithmetic mean (UPGMA). The evolutionary distances were computed using maximum composite likelihood method. Phylogenetic analysis of BCA, PSF and PSB isolates were conducted in MEGA package version 4.1. The diversity of selective potential bioinoculants were analysed using RAPD-PCR methods. Dominant arbuscular mycorrhizal fungi (AMF) in the rhizosphere of cereal, pulses, horticultural and plantation crops were documented, identified using scanning electron microscopy as well as their root colonization were determined following indirect immunofluorescence test using polyclonal antibody raised against AMF. Talc based formulation of selective PSB strains showing plant growth promoting activities using mass mixture, whereas FYM based formulation of PSF and Tricho-compost for BCA were developed for value addition in vermicomposting. Suppression of fungal diseases of cereals, pulses, horticultural and plantation crops were evident either by single or joint inoculation by BCA, PGPR and AMF. In all cases, disease suppression was associated with enhanced activity of defence enzymes like chitinase, β -1,3 glucanase, phenylalanine ammonia lyase and peroxidase. Induction of PR-2 and PR-3 and their cellular localization in leaf and root tissues were determined by indirect immunofluorescence and their immunogold localizations were further confirmed by transmission electron microscopy using PABs of chitinase. Bioformulations with these bioinoculants can encourage entrepreneurship development in order to take future line of action in crop protection.

Key words: Agriculturally important microorganisms, biocontrol agent, plant growth promoting rhizobacteria, arbuscular mycorrhizal fungi, phosphate solubilizing fungi, bioformulation

INTRODUCTION

Microorganisms in soil are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in such key processes as soil structure formation, decomposition of organic matter, toxin removal and the cycling of carbon, nitrogen, phosphorus and sulphur. In addition,

microorganisms play key roles in suppressing soil borne plant diseases. Plants are not simply passive recipients of nutrients, but information from the environment affects their belowground allocations such as root proliferation, formation of symbiotic relationships e.g. mycorrhizal fungi or N₂-fixing bacteria, alteration in exudation rates, interactions with free living bacteria, or production of secondary defence compounds against herbivores (Chakraborty, 2013). Since root morphology is both genetically programmed and environmentally determined (Rolfe *et al.* 1997), there must be signal transduction pathways that interpret complex

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Review paper based on the 17th Professor S.N. Banerjee Memorial Lecture delivered by the author organized by Indian Mycological Society at the University of Calcutta

environmental conditions and activate genes to enter a particular symbiosis or to form a lateral root at a particular time and place. The exchange of signals between plants and microorganisms is reciprocal. Phillips and Strong (2003) have introduced the concept of “rhizosphere control points” to emphasize the importance of information exchange between plants and microorganisms. Microorganisms play key roles in suppressing soil borne plant diseases, in promoting plant growth, and changes in vegetation. In this connection agriculturally important microorganisms are used in a variety of agro-ecosystems both under natural conditions and artificial inoculation for diverse application such as nutrient supply, biocontrol, bioremediation and rehabilitation of degraded lands. Soil microorganisms also influence above-ground ecosystems by contributing to plant nutrition and plant health, soil structure and soil fertility. They also fix atmospheric N, decompose organic wastes and residues, detoxify pesticides, suppress plant diseases and soil-borne pathogens, enhance nutrient cycling and produce bioactive compounds such as vitamins, hormones and enzymes that stimulate plant growth. Biofertilizer and biopesticide containing efficient microorganisms, improve plant growth in many ways compared to synthetic fertilizers, insecticides and pesticides by way of enhancing crop growth and thus help in sustainability of environment and crop productivity. Sustainable agriculture is vital in today’s world as it offers the potential to meet our agricultural needs without affecting soil ecosystem(Chakraborty, 2016)

RHIZOSPHERE MICROBIAL COMMUNITIES

Plant species can be important in determining the structure of rhizosphere microbial communities, with both positive and negative effects on different microbial groups. Within plant species, microbial communities can be affected by plant genotype, plant nutrient status, pathogen infection, and mycorrhizal infection. Within root systems, microbial communities can even differ among root zones and at different distances from the root surface. The recent development and popularity of molecular techniques to identify soil organisms has allowed us to move beyond the small subset of culturable soil organisms and begin defining populations and communities of microbes belowground. It is increasingly common to characterize complex microbial communities

genotypically using the small subunit 18S ribosomal DNA gene (18S rDNA), a region that is very highly conserved, essential, subject to low homologous gene transfer and a good reflection of overall phylogenetic relatedness. A collection of 18S genes can be analyzed partially as with the fingerprinting methods T-RFLP and DGGE, or in detail by sequencing entire populations or communities in clone libraries. Using these methods, they have begun to understand how population and community ecology concepts apply to rhizosphere microbes. The microflora associated with a plant rhizosphere with both pathogenic and non-pathogenic community is generally influenced by the soil type, pH and temperature. Most population studies have focused on organisms that can be manipulated in agricultural settings either for biocontrol or for increased plant growth, including species of symbiotic nitrogen fixers (Carelli *et al.* 2000), plant growth promoting fungi, plant growth promoting rhizobacteria (Chakraborty *et al.* 2014c) and arbuscular mycorrhizal fungi (Chakraborty and Chakraborty, 2012; Chakraborty *et al.* 2014b). Rhizosphere colonization is one of the first steps in the pathogenesis of soil borne microorganisms. It can also be crucial for the action of microbial inoculants used as biofertilizers, biopesticides, phytostimulators and bioremediators. The physical, chemical and biological properties of any soil are the indicators of the quality of that soil as well as decides the nature of microbial populations it holds (Drinkwater *et al.* 2002). The fungal population on the other hand is thought to dominate over other microbial population in certain soil types due to their wide distribution and capability to adopt in all type of environments (Zervakis *et al.* 2002). Himalayan region represents a unique combination of plant and soil types that change drastically with altitude (Kumar *et al.* 2011). Keeping in view the recent developments made so far in the field of studying soil microflora for utilization in crop improvement in eco-friendly farming practices, microbial diversity (fungi and bacteria) of Darjeeling Hill region (Fig.1) which includes- forest, agricultural lands and riverine soil have been explored, analysed their microbial diversity, characterized them for agriculturally important traits viz. plant growth promoting fungus (PGPF), Biocontrol agents (BCA) and Plant growth promoting Rhizobacteria (PGPR) and finally bioformulations have been developed for their possible utilization

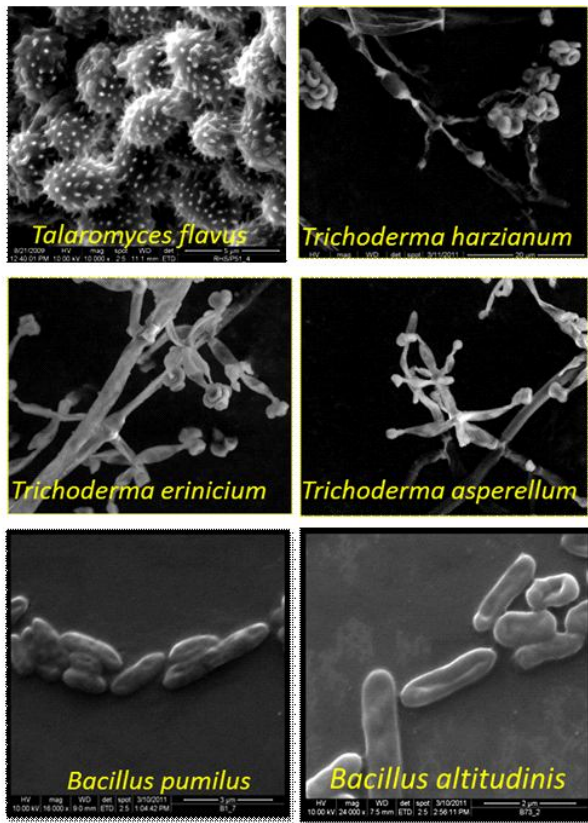


Fig. 1 : Scanning electron microscopic observation of Phosphate solubilizing fungi (*Talaromyces flavus*), Biocontrol agent (*Trichoderma harzianum*, *T. eriniceum*, *T. asperellum*) and plant growth promoting rhizobacteria (*Bacillus pumilus*, *B. altitudinis*)

for improving health status of cereals, legumes, plantation as well as horticultural crops.

BENEFICIAL MICROORGANISMS

The specificity of the legume and rhizobia association has been exploited by farmers and

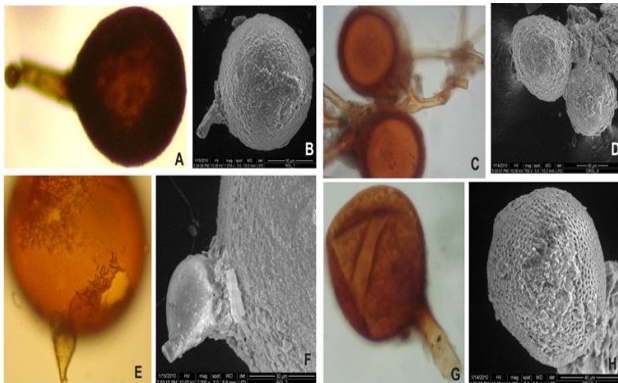


Fig. 2 : Arbuscular mycorrhizal fungi, (A,C,E,G) Brightfield and (B,D,F,H) scanning electron microscopic observation of (A,B) *Glomus mosseae*; (C,D) *Glomus intraradices*; (E,F) *Gigasporamargarita* and (G,H) *Glomus badium*

agricultural scientists for centuries. Application of Rhizobium inoculum to the seeds of leguminous species is the most widely practiced, conventional agricultural technology used to deliberately manipulate rhizosphere microorganisms. However, indigenous strains of Rhizobia are often more effective at colonizing nodules than inoculated strains, even if the seed is inundated with Rhizobia inoculum. Indigenous rhizosphere populations generally resist invasion by inoculated organisms in the absence of host-microorganism specificity. With the notable exception of the legume–Rhizobia association, inoculation techniques have not led to consistent or persistent effects on nutrient availability in conventional agriculture. A promising area of research is to examine the potential to manage these mutualisms in low-input and organic systems that provide an energetically and biologically favourable environment for displacing or augmenting indigenous micro-flora and fauna, compared to conventional agriculture. Plant growth promoting microorganisms (PGPM) and biological control agents (BCA) have been shown to possess secondary beneficial effects that would increase their usefulness as bio-inoculants, regardless of the need for their primary function (Chakraborty *et al.* 2011). Indeed, PGPM, as well as AMF can promote plant growth and productivity (primary effect) but have now been shown to also play a role in reducing disease (secondary effect). Conversely, BCA can control disease (primary effect) but have recently demonstrated stimulation of plant growth (secondary effect) in the absence of a pathogen. Further work shedding light onto the precise mode of action and ecophysiology of these microorganisms would assist with their timely and appropriate use and potentially unleash their full promise as beneficial rhizosphere bio-inoculants for improved growth and health of plants. The potential increased use of these microorganisms afforded by their multifaceted beneficial effects may further help in reducing problems associated with the use of synthetic chemicals in agriculture (Chakraborty *et al.* 2006, 2009, 2013).

Arbuscular mycorrhizal fungi (AMF) are symbiotic soil fungi that are intimately associated with the roots of the majority of land plants. They colonise the interior of the roots and the hyphae extend into the soil. It is well known that bacterial colonisation of the rhizosphere can be crucial for

many pathogenic as well as symbiotic plant–microbe interactions (Fig. 2). However, although bacteria colonising the extraradical AMF hyphae might be equally important for AMF symbiosis, little is known regarding which bacterial species would colonise AMF hyphae. Chakraborty *et al.* (2014b) investigated which bacterial communities might be associated with AMF hyphae. As bacterial-hyphal attachment is extremely difficult to study *in situ*, they designed a system to grow AMF hyphae of *Glomus intraradices* and *Glomus proliferum* and studied which bacteria separated from an agricultural soil specifically attach to the hyphae. The impressive diversity of the plant and fungal taxa involved in mycorrhizal symbiosis has demonstrated that colonization is a multistep, genetically regulated process. However, challenges are posed on their obligate biotrophic status, their multinuclear condition and an unexpected level of genetic variability. In addition to the well-known interactions between plants and fungi, mycorrhizal roots offer excellent ecological niches for other microbes, some rhizosphere bacteria adhere tightly to fungal hyphae, whereas others are directly associated with the root surfaces. Some species of bacterial genera (*Pseudomonas*, *Burkholderia*, *Bradyrhizobium*, *Bacillus*, *Paenibacillus*, *Rhodococcus* and *Streptomyces*) which help in mycorrhization and thus promote the symbiotic association between root and fungus are known as Mycorrhiza Helper Bacteria (MHB). Real-time quantitative PCR assays provide a method for directly detecting and quantifying MHB and mycorrhizal fungi in plant microcosms. MHB promote the establishment of symbionts by stimulating mycelial extension, increasing root-fungus contacts and colonization, and reducing the impact of adverse environmental conditions on the mycelium of mycorrhizal fungi. MHB may also reduce concentrations of antifungal metabolites in the mycorrhizosphere by direct antagonism against microbes that are harmful to mycorrhizal fungi. Their practical importance in plant protection and their potential applications in agriculture, horticulture and forestry centres around nutrient mobilization from soil minerals, fixation of atmospheric nitrogen and protection of plants against root pathogens. Growing concern about the pollution of soils, and the resulting trend towards reducing the input of chemicals in plant protection, should foster more environmentally friendly practices such as controlled mycorrhization or microbial bioremediation. This converging of

scientific and practical interests, supported by the development of genomics, may represent a unique opportunity to place MHB at the forefront of future mycorrhiza research and to boost the more general field of fungal-bacterial interactions in ecosystems. The significance and role of plant hormones in AM symbiosis as well as the role of Mycorrhizal fungi in control of various soil borne plant diseases has been reviewed (Adholeya, 2012; Chakraborty and Chakraborty, 2012)

Phosphate solubilizing microorganisms (PSM)

Phosphorus is one of major limiting factors for crop production on many tropical and subtropical soils as a result of high phosphorus fixation, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants. The concentration of soluble phosphorus (P) in tropical soil is usually very low. While most mineral nutrients in soil solution are present in millimolar amounts, phosphorus is only available in micromolar quantities or less. The majority of applied phosphorus is rapidly fixed in soil into fractions that are poorly available to plant roots. Inorganic phosphates are predominant form of inorganic phosphates in neutral or calcareous soils. Compared with the other major nutrients, phosphorus is by far the least mobile and available to plants in most soil conditions. Although phosphorus is abundant in soils in both organic and inorganic forms, it is frequently a major or even the prime limiting factor for plant growth. Phosphorus is added in the form of phosphatidic fertilizers, part of which is utilized by plants and the remainder converted into soluble fixed forms. To circumvent phosphorus deficiency, phosphate-solubilizing microorganisms (PSM) could play an important role in supplying phosphate to plants in a more environmentally-friendly and sustainable manner. PSMs solubilize insoluble phosphates into soluble form in soil by secreting formic, acetic, propionic, lactic, glycolic, fumaric and succinic acids. These acids lower the pH and bring about solubilization. Glucose, sucrose and galactose are the best carbon source for phosphate solubilization. Decrease pH in the medium during phosphate solubilization is due to the release of organic acid by isolates. Gluconic acid is the most commonly produced acid during phosphate solubilization other mechanism like CO₂ and H₂S production and chelation of other acids are also

responsible for phosphate solubilization. As we know that Phosphorus is one of the major nutrients limiting plant growth but most of the soils throughout the world are P deficient and therefore require P to replenish the P demand by crop plants. To circumvent the P deficiency in soils, P fertilizers are applied.

However, after application, a considerable amount of P is rapidly transformed into less available forms by forming a complex with Al or Fe in acid soils before plant roots have a chance to absorb it. Further, the use of rock phosphate as a phosphate fertilizer and its solubilization by microbes through the production of organic acids have become a valid alternative to chemical fertilizers. Rock phosphate is widely distributed throughout the world, both geographically and geologically in conjunction with phosphate solubilizing microorganism. Chakraborty *et al.* (2010) have conclusively shown that PSM solubilizes the fixed soil P and applied phosphates, resulting in higher crop yields. The alternative approach is to use these PSM along with other beneficial rhizospheric microflora to enhance crop productivity. In this context, the simultaneous application of *Rhizobium* and arbuscular mycorrhizal fungi has shown to stimulate plant growth when the soil is P Deficient (Zaidiet *al.* 2006). The effect of soluble phosphate in medium on the P-solubilizing activity of rhizosphere isolates were tested in liquid cultures with the addition of various concentration of soluble KH_2PO_4 . The medium was filtered after incubation and the remaining tricalcium phosphate was separated by filtrations. Filter papers with the remaining tricalcium phosphate were hydrolyzed with $2\text{NH}_2\text{SO}_4$. Phosphorus was determined spectrophotometrically. The P-solubilizing activity was expressed as a difference between the tricalcium phosphate added and its remainder after the incubation. PSM include fungi, which can grow on various phosphorous containing compounds such microbes not only accumulate P but a large portion of soluble phosphate is released in quantities in excess of their own requirement. Rhizosphere microorganisms also have the ability to assimilate different macro- and micro- nutrients and release them to the soils. These nutrients are utilized by the plants in a mutualistic way. *A. niger* were tested for their efficacy to solubilize rock phosphate (RP) and also to improve the growth of maize (*Zea mays*) in rock phosphate amended

soils. Both the species was able to grow and solubilize rock phosphate and solubilize rock phosphate and soluble P levels were significantly increased in the culture medium as the concentration of RP increased. The results of nursery experiment showed that the growth of maize plants and shoot were significantly increased by these fungi compared to control soil. Soil analysis results showed that the available P, organic carbon levels were significantly increased when compared to initial soil. The soil pH was also lowered compared to initial pH of the soil.

Five efficient phosphatase producing fungi (PPF) were isolated by Chakraborty *et al.* (2008) and identified as *Aspergillus niger*, *A. clavatus*, *A. melleus*, *Talaromyces flavus*, and *Trichoderma harzianum*. Their efficiency to hydrolyze different compounds of organic phosphorus (mono- and hexa) was examined. The fungi reduced the pH of the medium, which was maximum with *A. niger* and *T. flavus*. The maximum secretion of acid phosphatase by PPF was at 21 d and alkaline phosphatase at 14d. Acid phosphatase produced by PPF was three times higher than alkaline phosphatase. The intracellular phosphatase activity was significantly higher than extra cellular activity. The efficiency to hydrolyze mono-phosphate by phosphatases released from the PPF was 4-times higher than hexa phosphate. *T. harzianum* was found to be most efficient organic P mobilize as compared to the other fungi, tested. The efficiency per unit of enzyme produced by different fungi was different and that indicated the isoenzymes being of different types.

Molecular identification and diversity analysis

Variation within 18S rRNA gene sequences of different fungal species has significantly increased our understanding of the diversity and ecology of soil bacterial communities. In contrast to fungal, taxonomic identification of fungi based on sequences of the eukaryotic ribosomal small subunit, the 18S rRNA, is more problematic, with identification commonly limited to genus or family level. This is primarily due to the relative lack of variation within 18S rRNA genes between closely related fungal species as a result of the relatively short period of evolution of the kingdom fungi compared with bacteria. *Talaromyces flavus*, a potential phosphate solubilizer which was isolated

by Chakraborty *et al.* (2012a) from different rhizosphere soils collected from various locations of North Bengal region, India and their phylogenetic analysis along with other PSF isolates in relation

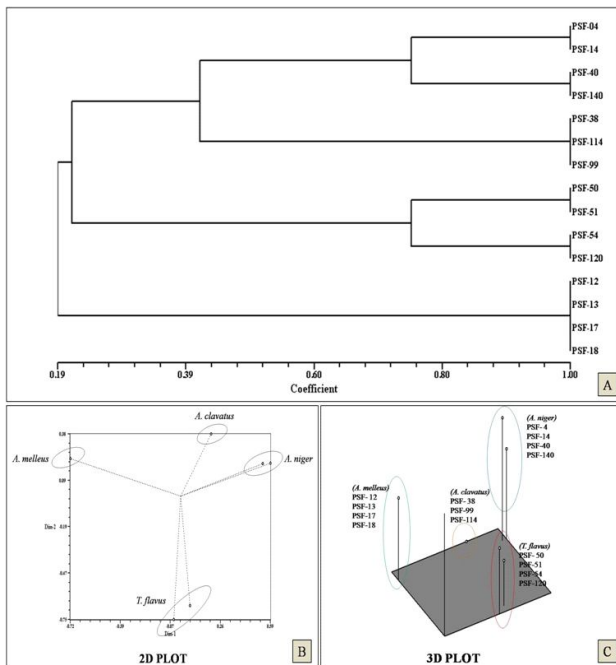


Fig. 1 : RAPD analysis by dendrogram of phosphate solubilizing fungi (*Aspergillusniger*, *A. melleus*, *A. clavatus* and *Talaromyces flavus*) and their 2D and 3D plots

to other ex types rDNA genes from NCBI database had been carried out (Fig. 3).

Biocontrol agents (BCAs)

The adverse effect to the environment due to indiscriminate use of chemical pesticides is of great concern and hence development of alternate control strategies such as biological control as substitute for chemicals or as a key component in integrated disease management system is gaining momentum (Harman *et al.* 2004; Mukherjee *et al.* 2013; Chakraborty and Chakraborty, 2017). Biological control has been developed as an academic discipline during 1970s and is now a mature science supported by both the public and private sectors which involves the use of beneficial organisms, their genes and or gene products, such as metabolites, that reduce the negative effects of plant pathogens and promote positive responses by the plant. Biological control is the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens. *Trichoderma* is

the most widely exploited fungal genus as biocontrol agent (BCA) in the field of agriculture for the management of crop diseases caused by a wide range of fungal pathogens(Singh *et al.* 2017).As a rule, *Trichoderma* species are fast

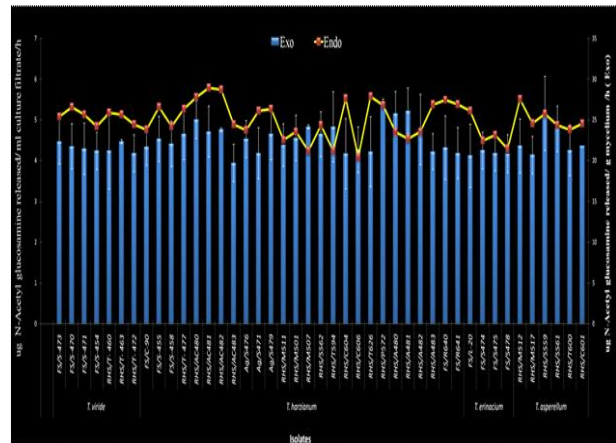


Fig. 2 : Screening of *Trichoderma* isolates for chitinase activity

growing fungi which are commonly found in a variety of soil types, such as, agricultural, forest, salt marsh and desert soils in all climatic zones. Many *Trichoderma* isolates are of great economic importance, producing hydrolytic enzymes (chitinases, cellulases and xylanases), biochemicals and antibiotics products which have been applied to fields such as food processing and pulp bleaching. In addition, some species produce heterologous proteins and others have been successfully used as biological control agents against a range of phytopathogens. *Trichoderma harzianum*, *T. asperellum*, *T. erinaceum* and *T. viride* species which were isolated by Chakraborty *et al.* (2010) from different rhizosphere soils collected from various locations of North Bengal region, India showed cellulase and chitinase activity (Fig.4) as well as antagonistic activity.

Identification and Phylogenetic studies of BCA Isolates

Phylogenetics is the study of relationships (or classification) based on closeness of evolutionary descent and usually involves the construction of a branch-like diagram known as a phylogenetic tree. Phylogenetic analysis can sometimes provide additional information about a group of isolates not immediately obvious from the raw data. For example, RAPD data generated from *T. viride* strains and 13 other strains of *Trichoderma* with three primers revealed that most of the isolates

could be differentiated from one another, and those that produced the same band pattern were identified as the same morphological species. However, when these data were subjected to parsimony analysis, it was further revealed that all isolates with biocontrol activity formed distinct clusters from the biocontrol-negative strains, thus, indicating a relationship between those isolates with biological control activity.

A polymerase chain reaction-amplified DNA containing the internal transcribed spacer (ITS)-1, 5.8S, and ITS-2 regions of the nuclear ribosomal DNA transcriptional unit was sequenced for 81 isolates of *Trichoderma* spp. used for biological control of plant pathogens. Phylogenetic analyses revealed that the biocontrol isolates were more closely related to an isolate of *T. harzianum* biotype 1 (Th1) than to the aggressive biotypes 2 and 4. Considering the wide range in sequence distribution of their samples, it was proposed that the consensus sequence found in their investigation be used as the reference sequence for further studies involving the identification and taxonomy of *T. harzianum*. RAPD analysis of *Trichoderma* isolates (*T. asprellum*, *T. erinaceum* and *T. harzianum*) by dendrogram using NTSYSpc software have been presented (Fig.5). Since *Trichoderma* BCAs use different mechanisms of

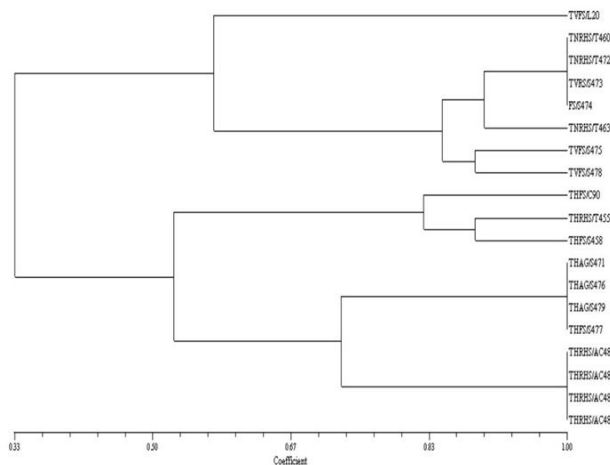


Fig. 5 : RAPD analysis by dendrogram of *Trichoderma* isolates (*T.asprellum*, *T. erinaceum* and *T. harzianum*) using NTSYSpc software.

biocontrol, it is very important to explore the synergistic effects expressed by different genotypes for their practical use in agriculture. A certain degree of polymorphism was detected in hybridizations using a probe of mitochondrial DNA. Sequencing of internal transcribed spacers

1 and 2 (ITS1 and ITS2) revealed three different ITS lengths and four different sequence types. Phylogenetic analysis based on ITS1 sequences, including type strains of different species, clustered the biocontrol strains into four groups: *T. harzianum*-*T. hamatum* complex, *T. longibrachiatum*, *T. asprellum*, and *T. atroviride*-*T. koningii* complex. ITS2 sequences were also useful for locating the biocontrol strains in *T. atroviride* within the complex *T. atroviride*, *T. koningii*. None of the biocontrol strains studied corresponded to biotypes Th2 or Th4 of *T. harzianum*, which cause mushroom green mold. Correlation between different genotypes and potential biocontrol activity was studied under dual culturing of BCAs (*Trichoderma harzianum*, *T.viride*, *Aspergillus niger*, *A. melleus*, *A.clavatus*) in the presence of the phytopathogenic fungi

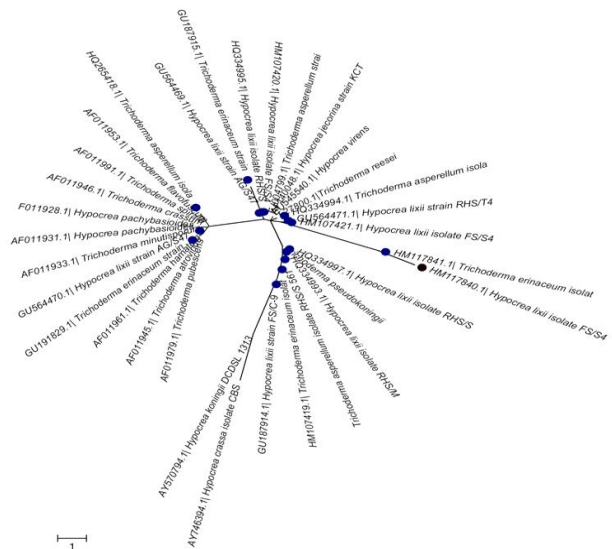


Fig. 6 : Evolutionary relationships among isolates of *Trichoderma* using MEGA4.1 version and Neighbor-joining method

Phoma betae, *Rosellinia necatrix*, *Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *dianthi* in three different media. Analysis of internal transcribed spacer -1 region of the rDNA can be used to detect species level of *Trichoderma harzianum*. Internal transcribed spacer- 1 region (ITS 1) of the ribosomal DNA was amplified by polymerase chain reaction (PCR). The PCR purification products were proved possible to amplify the ITS 1 region of all *Trichoderma* strains. The amplified DNA was sequenced and aligned against using ex-type strains sequencings from *TrichoBLAST/GenBank* and established *Trichoderma* taxonomy. Thirty-six isolates were positively identified as *Trichoderma harzianum*(32

strains) *Trichoderma virens*(3 strains) and *Trichoderma longibrachiatum*(1 strain) formed clearly defining phylogenetic analysis. *T. virens* and *T. longibrachiatum* which were used as an outgroup in these analyses. Amplification of ITS 1 region of the rDNA has showed potential as a rapid technique for identifying *Trichoderma harzianum* successfully fungi in all cases. Evolutionary relationship among isolates of *Trichoderma* sp. a potential biocontrol agent, using MEGA4.1 software and Neighbor-joining method has been carried out (Fig.6).

Activation of plant immune response

One of the greatest challenges now is to feed an increasing world population without exacerbating current environmental problems. Our approach should be to increase the utilization efficiency of scarce non-renewable fertilizers, which has the potential to simultaneously increase plant productivity and reduce pressures on environment. Soil microbes offer largely unexplored potential to increase agricultural yields and productivity in a low-input manner. Soil biota provide a number of key ecological services to natural and agricultural ecosystems. Increasingly, inoculation of soils with beneficial soil biota is being considered as a tool to enhance plant productivity and sustainability of agricultural ecosystems. In the development of sustainable crop production practices, the use of microbial inoculants as replacement for chemical fertilizers and pesticides is receiving attention. From the plant's perspective, biological control can be considered a net positive result arising from a variety of specific and non-specific interactions (Singh *et al.* 2011, 2017) .

Many of the microbes isolated and classified as biocontrol agents (BCAs) can be considered facultative mutualists, because survival rarely depends on any specific host and disease suppression will vary depending on the prevailing environmental conditions. Biocontrol can occur when non-pathogens compete with pathogens for nutrients in and around the host plant. Biological control can result in varying degrees from all of these types of interactions, depending on the environmental context within which they occur. Significant biological control, as defined above, most generally arises from manipulating mutualisms between microbes and their plant hosts or from manipulating antagonisms between microbes and pathogens.

Species of *Trichoderma* exhibiting good biological control activity have also proved to be particularly amenable to studies because, as a rule, they are ubiquitous, easy to isolate and culture and grow rapidly on many substrates (Harman *et al.* 2004; Singh *et al.* 2012). These species compete well for food and site, grow well on root surfaces, produce a wide range of antibiotics and act as mycoparasites utilizing an enzyme system capable of attacking a wide range of plant pathogenic fungi. Those *Trichoderma* isolates that have exhibited good biological control activity most frequently belong to one of four species-aggregates: *T. hamatum*, *T. harzianum*, *T. koningii* and *T. asperellum*. Biological control activity of isolates of *Trichoderma* and *Talaromyces flavus* has been demonstrated *in vitro* and glasshouse studies against sclerotial blight diseases of *Phaseolus vulgaris*, *Vigna radiata*, *Glycine max* and

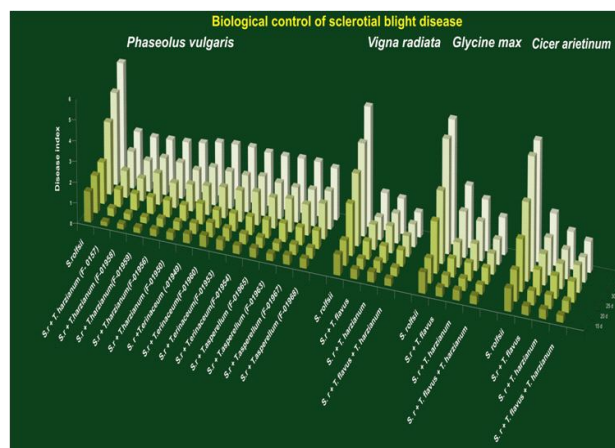


Fig. 7 : Biological control of sclerotial blight diseases of *Phaseolus vulgaris*, *Vigna radiata*, *Glycine max* and *Cicer arietinum* following application of isolates of *Trichoderma harzianum*, *T. erinaceum*, *T. asperellum* and *Talaromyces flavus* and challenge inoculation with *Sclerotium rolfsii*.

Cicer arietinum where the environment is controlled and in numerous field trials (Fig.7).

The arbuscular mycorrhiza is clearly a multifunctional symbiosis, an obligatory lifelong interaction involving close physical and biochemical contact, whose other services from plant host perspective include plant pathogen protection, phosphorus uptake and mediation of water status. Given the potential benefits to agricultural productivity, it is not surprising that manipulation of AMF communities either by inoculation with particular strains or through management of resident communities has been attempted at the field scale. Different hypotheses

have been proposed to explain bioprotection by AM fungi. These include (a) improvement of plant nutrition and root biomass in mycorrhizal plants, which could contribute to an increased plant tolerance and compensate for root damage caused by a pathogen, (b) changes in root system morphology, (c) modification of antagonistic microbial populations in the mycorrhizosphere, and (d) competition between AM fungi and pathogenic fungi to colonize root tissues, with the possible induction of resistance mechanisms

The role of Mycorrhizal fungi in control of various soil borne plant diseases has also been worked out with a positive impact in sustainable agriculture. During colonization, AMF can prevent root infections by reducing the access sites and stimulating host defence. Various mechanisms also allow AMF to increase a plant's stress tolerance. This includes the intricate network of fungal hyphae around the roots which block pathogen infections. Mycorrhizal colonization significantly reduced the percentage of disease severity and incidence in infected tea and mandarin plants. Stimulating the host roots to produce and accumulate sufficient concentrations of metabolites which impart resistance to the host tissue against pathogen invasion have also been reported. AM colonization (Fig.8) can reduce root diseases of horticultural crop (Chakraborty *et al.* 2014a) and plantation crop

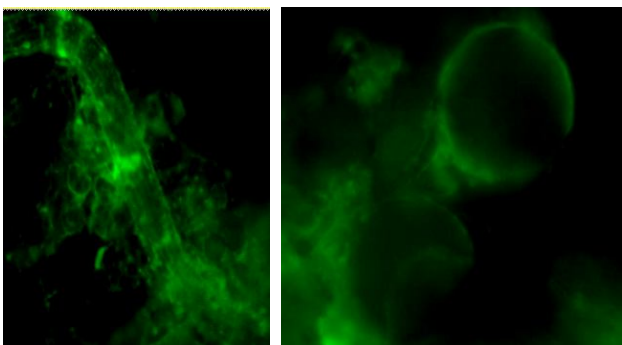


Fig. 8 : Indirect immunofluorescence of mandarin root colonized with *Glomus mosseae* labelled with FITC conjugates and reacted with PAb of AMF

(Chakraborty *et al.* 2012b) caused by fungal pathogens. Direct (via interference competition, including chemical interactions) and indirect (via exploitation competition) interactions have been suggested as mechanisms by which AM fungi can reduce the abundance of pathogenic fungi in roots. Presumably, pathogenic and AM fungi exploit common resources within the root, including infection sites, space and photosynthates within

the root. Interference competition may also arise if carbon availability within intercellular spaces and the rhizosphere or the number of infection loci within the root system is reduced as a result of AM fungal colonization. Moreover, increasing the richness of AM fungal taxa colonizing the root system may result in more intense competition with a pathogenic fungus. Extensive progress has been made in management of plant diseases using bioinoculants and its opportunity and challenges

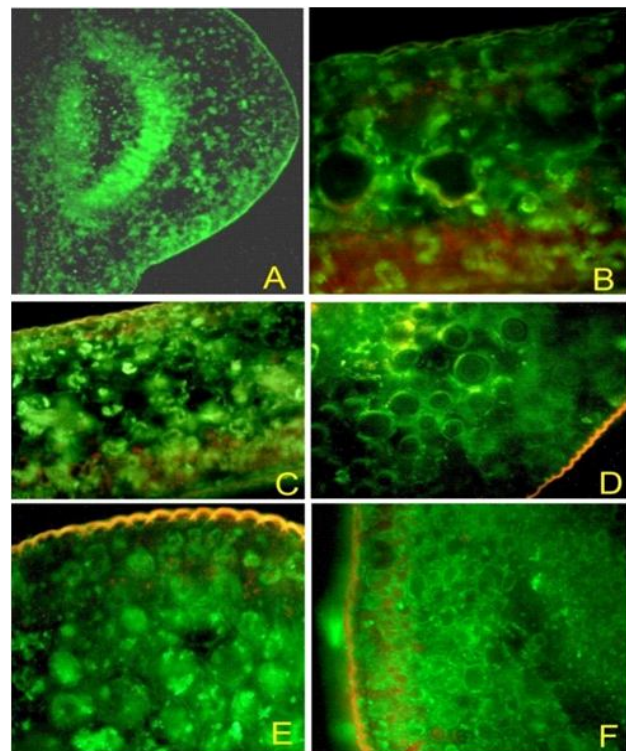


Fig. 8 : Indirect immunofluorescence of mandarin leaf tissue following induction of immunity labelled with FITC conjugates and reacted with PAb of chitinase. (A,C,D) Following application of bioinoculants (PGPR,BCA and AMF) and (E,F) after challenge inoculation with *Fusarium oxysporum*, (B) Untreated Uninoculated

in subsistence farming system of India has been highlighted.

The potential use of beneficial microorganisms (BCA, PGPR, PGPF, AMF) afforded by their effects towards promoting the induced systemic resistance (ISR) which can further help in minimizing problems associated with the use of synthetic chemicals in agriculture (Chakraborty *et al.* 2014b,2016b). Induction of resistance in *Camellia sinensis* against *Sclerotium rolfsii* by dual application of *Rhizophagus fasciculatus* and *Bacillus pumilus* has been documented (Chakraborty *et al.* 2016a). Application of PGPR (*Pseudomonas poae*), BCA (*Trichoderma asperellum*) and AMF (*Glomus mosseae*) on mandarin plants induced defence response against

Fusarium oxysporum (Chakraborty et al. 2019) causing wilt root rot complex disease. Immunogold localization of chitinase in mandarin leaf tissue following application of bioinoculants and challenge inoculation with *Fusarium oxysporum* (Figure 9 A-F) was confirmed by transmission electron microscopy using PAb of chitinase and gold labelled conjugate which showed distribution of intense gold particles throughout the cell (Figure 10). Increased activities of defense enzymes also observed during induction of resistance by

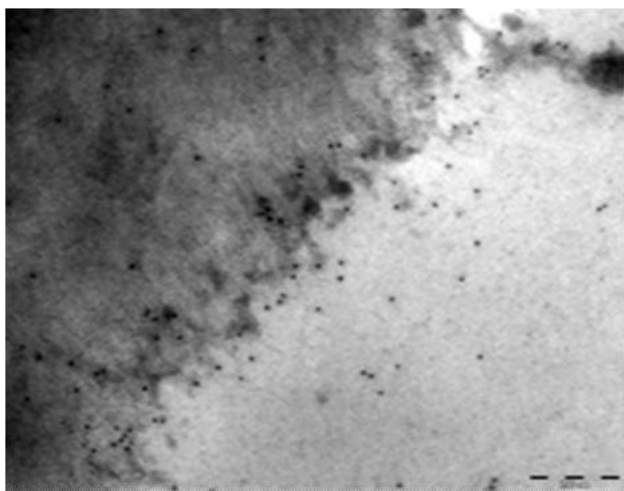


Fig. 10 : Immunogold localization of chitinase in mandarin root tissue following induced resistance against *Fusarium oxysporum* using bioinoculants

bioinoculants against spot blotch (Chakraborty et al, 2016b). TERI (The Energy and Resources Institute) has developed an *in vitro* based mass production technology which successfully produces pure inoculum in large quantities and overcomes the limitations of the soil based system. This inoculum has a long shelf life and is very concentrated with a small quantity itself carrying a very large number of propagules. This facilitates the application of desired amounts as it can be easily transported and mixed with a substrate of choice and applied as required. They have been able to develop modern biotechnologies concerning mycorrhization with proven impact. This technology coupled with bacterization would be more meaningful to 'biotize' *in vitro* propagated plantlets for transient transplant shock during acclimatization. The modern 'hairy-root technology' has played a significant role in establishment of AMF with transgenic host plants. The use of dual technology is promising as micro propagated plantlets are suitable platforms for understanding the mystery of host-endophyte interaction, excessive production of secondary

metabolites, heavy metal tolerance, bio protection, bioremediation and growth promoting activity. This will also help to unravel impediments for the growth and development of AMF in culture. Besides, this technology offers an opportunity to apply molecular approaches to understand host-symbiont interactions, secretion of flavonoids and signal transduction pathways. This may result in a more efficient and at the same time agriculturally and environmentally sustainable use of soil microorganisms for crop production. The ability of microorganisms to withstand biotic and abiotic stresses (Chakraborty *et al.* 2015; 2018) successfully in various climatic conditions and to support plant growth and development has made them an excellent source for valuable bio-products and genes that can be used to develop transgenic crop plants. The enormous functional diversity across the country needs to be deciphered and utilized to interweave microbes in agriculture sector.

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