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## Review:

# Mycorrhizoremediation—an enhanced form of phytoremediation\*

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**Abstract:** Study of plant roots and the diversity of soil micro biota, such as bacteria, fungi and microfauna associated with them, is important for understanding the ecological complexities between diverse plants, microbes, soil and climates and their role in phytoremediation of contaminated soils. The arbuscular mycorrhizal fungi (AMF) are universal and ubiquitous rhizosphere microflora forming symbiosis with plant roots and acting as biofertilizers, bioprotectants, and biodegraders. In addition to AMF, soils also contain various antagonistic and beneficial bacteria such as root pathogens, plant growth promoting rhizobacteria including free-living and symbiotic N-fixers, and mycorrhiza helping bacteria. Their potential role in phytoremediation of heavy metal (HM) contaminated soils and water is becoming evident although there is need to completely understand the ecological complexities of the plant-microbe-soil interactions and their better exploitation as consortia in remediation strategies employed for contaminated soils. These multitrophic root microbial associations deserve multi-disciplinary investigations using molecular, biochemical, and physiological techniques. Ecosystem restoration of heavy metal contaminated soils practices need to incorporate microbial biotechnology research and development. This review highlights the ecological complexity and diversity of plant-microbe-soil combinations, particularly AM and provides an overview on the recent developments in this area. It also discusses the role AMF play in phytoremediation of HM contaminated soils, i.e. mycorrhizoremediation.

**Key words:** Phytoremediation, Plant growth promoting rhizobacteria, Arbuscular mycorrhizae (AM), Mycorrhizoremediation, Mycorrhiza helping bacteria, Ecosystem restoration, Phytoremediation, Heavy metal (HM)

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## INTRODUCTION

Biosphere pollution by heavy metals and nucleotides was accelerated dramatically during the last few decades due to mining, smelting, manufacturing, treatment of agricultural soils with agro-chemicals and soil sludge, etc. Problems associated with the contamination of soil and water such as animal welfare, health, fatalities and disruptions of natural ecosystems are well documented (He *et al.*, 2005). Heavy metals such as Pb, Cr, As, Cu, Cd, and Hg, being added to our soils through industrial, agricultural and domestic effluents, persist in soils and can either be adsorbed in soil particles or leached into ground water. Human exposure to these metals through ingestion of

contaminated food or uptake of drinking water can lead to their accumulation in humans, plants and animals. Lead, Copper, Zinc and Cadmium are also found naturally in soils and they can cause significant damage to environment and human health as a result of their mobility and solubilities. They can occur in soil and water in several forms and their speciation in soils is determined by sequential extraction using specific extractants, which solubilize different phases of metals (Shuman, 1985). The physical and chemical characteristics of soil determine the speciation and mobility of heavy metals (Kabata-Pendias and Pendias, 1992).

## SOIL AND RHIZOSPHERE

Many soil microbes originate in the soil or are

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closely associated with the soil environment and have substantial impact on mankind (Doyle and Lee, 1986). We know very little about the enormous diversity of soil microbes, their properties, and behaviour in the soil environment. Soil microorganisms inhabiting the rhizosphere environment interact with plant roots and mediate nutrient availability, e.g. those forming useful symbiotic associations with the roots and contribute to plant nutrition. Implications of plants and their symbionts like mycorrhizal fungi, N-fixing rhizobia, and free living rhizosphere population of bacteria which promote plant growth need to be fully exploited and encouraged by inoculating nutrient poor agricultural soils with appropriate microbes (Khan, 2002a).

### Rhizosphere and microorganisms

#### 1. Glomalian fungi

Glomales comprise one of the oldest group of fungi, older than land plants. The first land plants, Bryophytes, appeared in the Mid Silurian era (476~430 My old). The oldest fossil evidence of Bryophyte-like land plants, 100 My ago in the Early Devonian, had AM like infections even before roots evolved (Phipps and Taylor, 1996). The first land plants were most likely evolved from algae but no fossil records are available for the rootless freshwater Charophycean algae, which are the probable ancestors of land plants, to show if they were mycorrhizal. Mosses, liverworts, and hornworts often contain structures like hyphae, vesicles, and arbuscules, characteristics of AM fungi (Schüßler, 2000). *Sphenophytes*, *Lycopodophytes*, and *Pteridophytes* are among the first land plants with roots which originated in the mid Devonian era, and AM associations are reported in these plants (Brundrett, 2002). Both living and Triassic fossil Cycades had AMF in their roots. AM associations are ubiquitous in the living angiosperms, which probably arose in the early Cretaceous era (Taylor and Taylor, 1993).

It is hypothesized that AM fungi were instrumental in the colonization of land by ancient plants (Simon *et al.*, 1993). This hypothesis is supported by observation that AM can now be found worldwide in the angiosperms, gymnosperms as well as ferns, suggesting that the nature of the association is ancestral. Furthermore, the origin of AM fungi coincides with that of vascular plants suggesting the nature of

the association and supporting the hypothesis that AMF were instrumental in the colonization of land by ancient plants.

It is now established that universal and ubiquitous symbiotic arbuscular mycorrhizal (AM) fungi, belonging to Glomales, form symbiotic relationships with roots of 80%~90% land plants in natural and agricultural ecosystems (Brundrett, 2002), including halophytes, hydrophytes and xerophytes (Khan, 1974; Khan and Belik, 1995; Khan, 2003), and are known to benefit plant nutrition, growth and survival, due to their greater exploitation of soil for nutrients (Smith and Read, 1997). These associations represent a key factor in the below ground networks which influence diversity and plant community structure (van der Heijden *et al.*, 1998; Burrows and Pflieger, 2002; O'Connor *et al.*, 2002; Chaudhry *et al.*, 2005), but we know very little about the enormous AM fungal diversity in soils and their properties and behaviour in the soil (Khan, 2002a). The degree of benefit to each partner in any AMF-plant host interaction depends not only on the particular plant and AMF species involved but also on the rhizobacteria and soil abiotic factors.

#### 2. Glomalin and heavy metal sequestration

Heavy metals in soil are associated with a number of soil components which determine their behaviour in the soil and influence their bioavailability (Boruvka and Drabek, 2004). The cell wall components such as free amino, hydroxyl, carboxyl and other groups of soil fungi can bind to potentially toxic elements such as Cu, Pb, Cd, etc. (Kapoor and Viraraghavan, 1995). Many filamentous fungi can sorb these trace elements and are used in their commercial biosorbents (Morley and Gadd, 1995). The proteins in the cell walls of AM fungi appear to have similar ability to sorb potentially toxic elements by sequestering them. There is evidence that AMF can withstand potentially toxic elements. Gonzalez-Chavez *et al.* (2004) showed that glomalin produced on hyphae of AMF can sequester them. AMF play a significant ecological role in the phytostabilization of potentially toxic trace element polluted soils by sequestration and, in turn, help mycorrhizal plants survive in polluted soils.

One of these components is Glomalin, a glycoprotein produced by the hyphae of AMF fungi (Wright and Upadhyaya, 1998), which is released into

soil from AMF hyphae (Driver *et al.*, 2005). These authors, using an *in vitro* system of Ri T-DNA transformed roots infected with *Glomus intraradices*, an AMF, showed that glomalin is tightly bound in AMF hyphal and spore walls. Small amounts (<20%) of glomalin were found to be adhered to soil via release into liquid medium from hyphae and not through passive secretion and their function is physiological in the course of the life of the organism. It has been hypothesized that glomalin has a role in the immobilization ('filtering') of heavy metals at the soil-hypha interface, i.e. before entry into fungal-plant system.

The extra-radical mycelium of AMF, in addition to its crucial role in enhancing nutrition of host plant, also plays a role in soil particle aggregation and soil stability (Dodd *et al.*, 2000; Wright and Upadhyaya, 1998).

There has been few analytical studies of AM in polluted soils. While some workers observed that the external mycelium of AMF was the main site for trace element localization (Kaldorf *et al.*, 1999; Turnau, 1998), others reported selective exclusion of toxic and non-toxic elements by adsorption onto chitinous cell walls (Zhou, 1999), or onto extra-cellular glycoprotein, glomalin (Wright and Upadhyaya, 1998), or intra-cellular precipitation. All these mechanisms have implications in reducing a plant's exposure to potentially toxic elements, i.e. mycorrhizoremediation technology. Gonzalez-Chavez *et al.* (2002) studied the form and localization of Cu accumulation in the extra-radical mycelium of three AM fungi isolated from the same polluted soil contaminated with Cu and As. The authors reported differential capacity of AMF to sorb and accumulate Cu as determined by TEM and SEM. However, the nature of accumulation and mechanisms involved require further studies in order to better understand the participation of AMF in plant tolerance and its ecological significance in polluted soils.

The AMF can be screened for their ability to produce maximum levels of extra-radical mycelium in polluted soils (Joner *et al.*, 2000), and to utilize adapted AMF to help accumulate HM both within the plant roots (phytoaccumulation) and the extra metrical fungal mycelium.

Most of the information available in literature concerning fairly recently discovered soil compound,

glomalin, an iron-containing glycol-soil-proteinaceous substance produced by AMF, is in relation to its role in soil aggregation (Rillig *et al.*, 2003). Glomalin makes a large contribution to active soil organic C pools. It is quite recalcitrant and we know very little about its chemical structure.

Glomalin plays a vital part in sorption and sequestration of potentially toxic elements, reducing their bioavailability (Gonzalez-Chavez *et al.*, 2004). It has been suggested that this sequestration could be important for heavy metal biostabilization in heavy metal polluted soils (Khan, 2005). Glomalin attaches to soil and helps stabilize aggregates. Mycorrhizae may be of great significance for the survival of plants at HM polluted sites. Literature data on glomalin content in HM polluted soils are extremely scarce and there is a need to gather data on this aspect of mycorrhizoremediation, i.e. use of mycorrhizal plants in the phytoremediation of HM contaminated soils.

## AM AND PHYTOREMEDIATION

Despite the importance of the role that AM play in soil-microbe-plant interactions, relatively few studies have focused on their potential in phytoremediation efforts. This is first due to the fact that earlier phytoremediation studies focused on the predominantly non-mycorrhizal plant families such as *Brassicaceae* and *Caryophyllaceae*, and second AM have not been considered by earlier workers as important component of phytoremediation practices. It is possible to improve the phytoremediation capabilities of plants by inoculating them with appropriate AM fungi.

### Significance of arbuscular mycorrhizae

AM associations are important in natural and managed ecosystems due to their nutritional and non-nutritional benefits to their symbiotic partners. They can alter plant productivity, because AMF can act as biofertilizers, bioprotectants, or biodegraders (Xavier and Boyetchko, 2002). AMF are known to improve plant growth and health by improving mineral nutrition, or increasing resistance or tolerance to biotic and abiotic stresses (Clark and Zeto, 2000; Turnau and Haselwandter, 2002). Their potential role in phytoremediation of heavy metal contaminated

soils and water is also becoming evident (Chaudhry *et al.*, 1998; Khan *et al.*, 2000; Khan, 2001; Jamal *et al.*, 2002; Hayes *et al.*, 2003).

AMF modify the quality and abundance of rhizosphere microflora and alter overall rhizosphere microbial activity. Following host root colonization, the AMF induces changes in the host root exudation pattern, which alters the microbial equilibrium in the mycorrhizosphere (Pfleger and Linderman, 1994). These interactions can be beneficial or harmful to the partner microbes involved and to the plant, and sometimes may enhance plant growth, health, and productivity (Paulitz and Linderman, 1989; Lynch, 1990). Giovannetti and Avio (2002) reviewed and analysed important data on the main parameters affecting AM fungal infectivity, efficiency, and ability to survive, multiply and spread, which may help in utilizing obligate biotrophic AMF in biotechnological exploitation and sustainable agriculture. There is a need to understand and better exploit AM symbionts in the different world ecosystems.

Although AMF are ubiquitous, it is probable that natural AM associations are not efficient in increasing plant growth (Fitter, 1985). Cropping sequences as well as fertilization and plant pathogen management practices also dramatically affect the AMF propagules in the soil and their effects on plants (Bethlenfalvay and Linderman, 1992). The propagation system used for horticultural fruit and micro-propagated plants, can benefit most from AM biotechnology. Micropropagated plants can withstand transplant stress from *in vitro* to *in vivo* systems, if they are inoculated with appropriate AMF (Lovato *et al.*, 1996; Azcon-Aguilar *et al.*, 2002). In order to use AMF in sustainable agriculture, knowledge of the factors such as fertilizer inputs, pesticide use, soil management practices, etc. influencing AMF communities is essential (Bethlenfalvay and Linderman, 1992; Allen, 1991; 1992). This area deserves further research and efforts because sound scientific knowledge is necessary for the improvement of AM biotechnology aimed at selecting infective and efficient inoculants to be used as biofertilizers, bioprotectants, and biostimulants in sustainable agriculture, horticulture, and forestry.

The potential of arbuscular mycorrhizal fungi (AMF) to enhance plant growth is well recognized but not exploited to the fullest extent. They are rarely

found in nurseries due to the use of composted soil-less media, high levels of fertilizer and regular application of fungicide drenches. The potential advantages of the inoculation of plants with AM fungi in horticulture, agriculture, and forestry are not perceived by these industries as significant. This is partially due to inadequate methods for large-scale inoculum production. Monoxenic root-organ *in vitro* culture methods for AMF inocula production have also been attempted by various workers (Mohammad and Khan, 2002; Fortin *et al.*, 2002) but these techniques, although useful in studying various physiological, biochemical, and genetic relationships, have limitations in producing inocula of AM fungi for commercial purposes. Pot cultures in pasteurized soils, have been the most widely used method for producing AMF inocula but are time consuming, bulky, and often not pathogen free. To overcome these problems, soil-free methods such as soil-less growth media, aeroponics, hydroponics and axenic cultures of AM fungi have been used successfully to produce AMF-colonized root inocula (Sylvia and Jarstfer, 1994a; 1994b; Mohammad *et al.*, 2000; Mohammad and Khan, 2002). Substrate-free colonized roots produced by these methods can be sheared and used for large-scale inoculation purposes. Mohammad *et al.* (2004) compared the growth responses of wheat to sheared root and pot-culture inocula of AMF at different P levels under field conditions, and concluded that P fertilization can be substituted by AMF inoculum produced aeroponically to an extent of 5 kg/ha under field conditions.

#### **Arbuscular mycorrhiza-rhizobacteria interactions**

The increased microbial activity in the rhizosphere soil affects the plant. A range of stimulated rhizosphere microorganisms such as saprophytes, pathogens, parasites, symbionts, etc., carry out many activities which are important to plant health and growth. Some of these microbes affect plant root morphology and physiology by producing plant growth-regulating hormones and enzymes. Others alter plant nutrient availability and biochemical reactions undertaken by them.

AM fungi have differential effects on the bacterial community structure in the mycorrhizosphere (Paulitz and Linderman, 1989; Marschner and Baumann, 2003). AMF improve P-nutrition by

scavenging available P through the large surface area of their hyphae. PGPR (plant growth promoting rhizobacteria) may also improve plant P-acquisition by solubilizing organic and inorganic P sources through phosphatase synthesis or by lowering pH of the soil (Rodríguez and Fraga, 1999). Garbaye (1994) defined MHB (mycorrhization helper bacteria) as “bacteria associated with mycorrhizal roots and mycorrhizal fungi which collectively promote the establishment of mycorrhizal symbioses”.

There is growing evidence that diverse microbial populations in the rhizosphere play a significant role in sustainability issues (Barea, 2000; Barea *et al.*, 2002), and that the manipulation of AMF and certain rhizobacteria like PGPR and MHB is important. Vivas *et al.* (2003) used a dual AM fungus-bacterium inoculum to study the effect of drought stress induced in lettuce grown in controlled-environment chambers. Their results showed that there was a specific microbe-microbe interaction that modulates the effectiveness of AMF on plant physiology. The authors concluded that plants must be mycorrhizal in nutrient-poor soils and that mycorrhizal effects can be improved by co-inoculation with MHB such as *Bacillus* spp. Results of this study by Vivas and co-researchers show that co-inoculation of selected free-living bacteria isolated from adverse environments and AM fungi can improve the formation and function of AM symbiosis, particularly when the plant growth conditions are also adverse. Both AMF and PGPR complement each other in their role in N-fixation, phytohormone production, P-solubilization, and increasing surface absorption. Behl *et al.* (2003) studied the effects of wheat genotype and *Azobacter* survival on AMF and found that the genotype tolerant to abiotic stresses had higher AMF infection and a cumulative effect of plant-AMF-PGPR interaction was found. Similar observations were made by Chaudhry and Khan (2002; 2003), who studied the role of symbiotic AMF and PGPR N-fixing bacterial symbionts in sustainable plant growth on nutrient-poor heavy metal contaminated industrial sites and found that the plants surviving on such sites were associated with N-fixing rhizobacteria and had a higher arbuscular mycorrhizal infection, i.e. a cumulative and synergistic effect.

The MHB cannot be ignored when studying mycorrhizal symbioses in their natural ecosystems.

They are quite common and, as Garbaye (1994) said, they are found every time they are looked for, and they seem to be closely associated with the mycorrhizal fungi in the symbiotic organs. They are adapted to live in the close vicinity of the AM fungi as high frequencies of MHB populations are isolated from the mycorrhizae. Some MHB isolates also promoted ectomycorrhizae formation in four conifers (Garbaye *et al.*, 1992), indicating that the MHB effect is not plant-specific. Various researchers showed that MHB's are fungus selective (Garbaye, 1994). Mosse (1962) showed that cell-wall degrading enzyme producing *Pseudomonas* sp. enhanced the germination of AM fungal spores of *Glomus mosseae* and promoted the establishment of AM on clover roots under aseptic conditions. These observations were later supported by other workers (Mayo *et al.*, 1986; Linderman and Paulitz, 1990).

Enriched soil microbial communities in the mycorrhizosphere are often organized in biofilms and probably horizontal gene transfer (HGT) between co-inhabiting microbial species and between plant-to-microbe occurs (van Elsas *et al.*, 2003). Many plant associated *Pseudomonas* rhizobacteria produce signal molecules for quorum sensing regulation, which were absent from soil-borne strains (Elasri *et al.*, 2001). This indicates that quorum sensing systems exist and are required in the mycorrhizosphere. Microbial colonies on the root surfaces consist of many populations or strains and positive and negative inter-population signaling on the plant root occur (Pierson *et al.*, 2002), which may play an important role in the efficiency of the use of biofertilizers.

In addition to the above described interactions between AM fungi and rhizobacteria, certain bacteria-like organisms (BLOs) reside in the AM fungal cytoplasm, first described by Mosse (1962). Khan (1971) illustrated AMF spores, collected from the semi-arid areas of Pakistan, containing one to ten small spherical ‘endospores’ without any subtending hyphae of their own. Ultra structural observations clearly revealed their presence in many field-collected AM fungal isolates. Because of their unculturable nature, further investigation of BLO's was hampered but current advanced electron and confocal microscopic and molecular analysis techniques have allowed us to know a bit more about their endosymbi-

otic nature. Minerdi *et al.* (2002) reported the presence of intracellular and endosymbiotic bacteria belonging to the genus *Burkholderia* (a genus known to fix N) in fungal hyphae of many species of *Gigasporaceae*. The authors used genetic approaches to investigate the presence of N-fixing genes and their expression in this endosymbiont and found *nifHDK* genes in the endosymbiont *Burkholderia* and their RNA messengers demonstrating that they possess a molecular basis for N-fixation. This discovery, as stated by Minerdi *et al.* (2002), indicates that a fungus which improves P-uptake, might also fix N through specialized endobacteria. Endosymbiont *Burkholderia* may have an impact on AMF-PGPR-MHB-Plant associations and metabolism. This finding suggests a new application scenario worth pursuing.

Recent methodological developments in molecular and microscopical techniques together with those in genomes, bioinformatics, remote-sensing, proteomics, etc. will be of help in understanding the complexity of interactions existing between diverse plants, microbes, climates, and soil.

#### Plant growth promoting rhizobacteria

Rhizobacteria include mycorrhization helper bacteria (MHB) and plant growth promoting rhizobacteria (PGPR), which assist AMF to colonize the plant roots (Klyuchnikov and Kozherin, 1990; Andrade *et al.*, 1997), P-solubilizers, free-living and symbiotic nitrogen fixers, antibiotic producing rhizobacteria, plant pathogens, predators and parasites (Sun *et al.*, 1999). The most common bacteria in the mycorrhizosphere are *Pseudomonas* (Vosátka and Gryndler, 1999), while different bacterial species exist in the hyphosphere.

Like AMF, rhizobacteria such as pseudomonads, are also ubiquitous members of the soil microbial community and have received special attention as they also exert beneficial effect on plant growth by suppressing soil-born pathogens, synthesizing phytohormones, and promoting plant growth (Weller, 1988; Glick, 1995; Kapulnik, 1996; Chin-A-Woeng *et al.*, 2003). Many fluorescent *Pseudomonas* strains have been reported as plant growth enhancing beneficial rhizobacteria. They are studied extensively in agriculture for their role in crop improvement as they stimulate plant growth either by producing plant growth promoting hormones, fixing atmospheric N<sub>2</sub>

or suppressing plant pathogens.

Rhizospheric component of PGPR bacteria adhere to the root surfaces, use root exudates for growth, synthesize amino acids and vitamins, and establish an effective and enduring root colonization (Lugtenberg and Dekkers, 1999). However, quantitative and qualitative variations in root exudates during plant growth could affect the rhizospheric competency of introduced PGPRs. The development of AM symbiosis, also influence PGPR dynamics. *Pseudomonas fluorescens* 92rk increased mycorrhizal colonization of tomato roots by *Glomus mosseae* BEG12 suggesting that strain 92rk behaves as MHB (Toro *et al.*, 1997; Singh and Kapoor, 1998). Many researchers have reported additive effects on plant growth by AMF and PGPR (Edwards *et al.*, 1998; Galleguillos *et al.*, 2000; Gamalero *et al.*, 2003) but the mechanisms by which MHB and PGPR stimulate AM colonization are still poorly understood (Barea *et al.*, 1997). Various hypotheses have been suggested which include physical, chemical, physiological, and even direct stimulatory or antagonistic relations between AMF and other mycorrhizosphere microbes (Bansal *et al.*, 2002).

AM and PGPR symbioses not only induce physiological changes in the associated plant, but also modify the morphological architecture of the roots such as total root length and root tip numbers (Atkinson *et al.*, 1994). Gamalero *et al.* (2003) found the highest values of root architecture parameters in tomato roots inoculated with two strains of *P. fluorescens* (92rk and P190r) and AMF *Glomus mosseae* BEG12. The authors ascribed these findings both to modification of root architecture, due to PGPR and AMF, and to a greater absorption surface area due to extrametrical mycelium of AMF.

PGPR have also been shown to induce systemic resistance (ISR) to fungal, bacterial, and viral pathogens in various crops such as bean, tomato, radish, and tobacco (Zhang *et al.*, 2002).

#### Co-occurrence of AMF and PGPR/MHB

Co-existence of certain fungi and rhizobacteria has been known since 1896, when Janse (1896) reported bacteria and fungi in the same pieces of legumes roots. These fungi were also described as arbuscular mycorrhizal by Jones (1924), and it was Asai (1944) who first stated that root nodulation by the

rhizobacteria was dependent on the formation of mycorrhizae by the AM fungi. These earlier observations were confirmed by subsequent researchers (Filion *et al.*, 1999). Multifaceted interactions of AM fungi with various micro-organisms and micro-fauna in the mycorrhizosphere may be positive or negative (Facelli *et al.*, 1999). The positive/synergistic interactions between mycorrhizosphere AM fungi and various N-fixing and P-solubilizing bacteria is the basis of application of these microbes as biofertilizer and bio-protectant agents (Bansal *et al.*, 2002). These microbes are regulated by AMF for their own benefit, which in turn benefit the host plant. Meyer and Linderman (1986) reported enhanced mycorrhization of clover in the presence of PGPR rhizobacterium *Pseudomonas putida*. Similar observations were made later by several other researchers (Suresh and Bagyaraj, 2002). All these studies suggest that colonization of plant roots by AM fungi significantly influences the mycorrhizosphere microorganisms, including PGPR.

Duc *et al.* (1989) were the first to report that some pea mutants defective in nodulation also did not form AM symbiosis. Since the first report of legume nodulation mutants to be non-mycorrhizal, a range of nodulation-defective mutant legumes and non-legume mutated crops, have been tested (Barker *et al.*, 2002). Many recent reports have confirmed the similarities between nodulation and mycorrhiza-formation processes (Peterson and Guinel, 2000; Resendes *et al.*, 2001). There is enough evidence available now which indicates positive interaction between AM fungi and PGPR (Chanway *et al.*, 1991).

There are several reports on the interactions between AMF and *Rhizobium* species (Albrecht *et al.*, 1999) which suggest that the interaction is synergistic, i.e. AM fungi improve nodulation due to enhanced P-uptake by the plant. In addition to this principal effect of AMF on P-mediated nodulation, other secondary effects include supplying trace elements and plant hormones, which play an important role in nodulation and N<sub>2</sub>-fixation. Current researches are directed to gaining understanding on the role AMF play in the expression of *Nod* genes in *Rhizobia* (Harrison, 1997). Barea and his associates (Azcon-Aguilar and Barea, 1994; Azcon-Aguilar *et al.*, 2002; Barea, 2000; Barea *et al.*, 2002) achieved many significant findings in this area. Synergistic interac-

tions between AMF and asymbiotic N<sub>2</sub> fixing bacteria such as *Azobacter chroococum*, *Azospirillum* spp. and *Acetobacter diazotrophicus* have also been reported by many researchers (Suresh and Bagyaraj, 2002). Synergistic interactions between P-solubilizing bacteria and AMF and their effect on plant growth were studied by many workers during the last three decades. Duponnois and Plenchette (2003) studied the effects of MHB *Pseudomonas montellii* strain HR13 on the frequency of AM colonization of Australian *Acacia* species and reported a stimulatory effect. The authors recommend dual inoculation to facilitate controlled mycorrhization in nurseries where *Acacia* species are grown for forestation purposes. Modern researches have provided evidence that the genetic pathway of AM symbiosis is shared in part by other root-microbe symbioses such as N-fixing rhizobia (Peterson and Guinel, 2000).

Many researchers reported unsuccessful attempts to select an appropriate *Rhizobium* strain for inoculating legumes owing to the failure of the selected strain(s) to survive and compete for nodule occupancy with indigenous native strains under low P and moisture contents (Bottomley, 1992). In this context, the role of AM fungi as P suppliers to legume root nodules appears to be of great relevance. Requena *et al.* (1997) found a specific AM fungus, *Rhizobacterium* sp. combination for effective nodulation and N<sub>2</sub> fixation in a mycotrophic legume *Anthyllis cytisoides* in Mediterranean semi-arid ecosystems in Spain. They reported *Glomus intraradices* to be more effective with *Rhizobium* sp. NR4, whereas *G. coronatum* was more effective when co-inoculated with strain NR9. Such specificity in interactions between AM fungi, *Rhizobium* and PGPR have been described by various researchers, indicating that it is important to consider the specific functional compatibility relationships between AMF, PGPR and MHB and their management when used as biofertilizers.

New techniques applied in molecular ecology have resulted in the identification of members of unculturable Archea in the mycorrhizospheres but their role in the hyphosphere is not known (Bomberg *et al.*, 2003). This means, as pointed out by Sen (2003), that analyses of PGPR distribution and activities must now be extended to accommodate *Crearchaeotal* microbes as well. Areas like

host-microbe-specificity and microbial-linked control of plant diversity and productivity still needs to be elucidated at gene, organismal, and ecosystem scales (Sen, 2003).

## CONCLUSION

AM are ubiquitous and most plants are colonized by AMF in nature, i.e., mycorrhizosphere is the rule, not the exception. Thus if we are to understand the rhizosphere reactions and interactions, we must understand the mycorrhizosphere. Mycorrhization helper bacteria (MHB) might be exploited to improve mycorrhization, and AMF to improve nodulation and stimulate PGPR. It is anticipated that future commercial biofertilizers would contain PGPR, MHB, and AMF. Requena *et al.* (1997) found that AM fungus *Glomus coronatum*, native in the desertified semi-arid ecosystems in the southeast of Spain, was more effective than the exotic *G. intraradices* in AM/PGPR co-inoculum treatments. The indigenous isolates must be involved. This area merits greater attention. More extensive field investigations on this multi-agent biofertilizer will make this a popular technology among field workers in agriculture, forestry and horticulture. Manipulation of microorganisms in the mycorrhizosphere for the benefit of plant growth requires research at the field level (Khan, 1975; 2002b). In order to exploit microbes as biofertilizers, biostimulants and bioprotectants against pathogens and heavy metals, ecological complexity of microbes in the mycorrhizosphere needs to be taken into consideration and optimization of rhizosphere/mycorrhizosphere systems need to be tailored. Smith (2002) stressed the need to better integrate information on root and soil microbe distribution dynamics and activities with known spatial and physiochemical properties of soil. This, as pointed out by Smith (2002), should be achieved through greater collaborative efforts between biologists, soil chemists and physicists.

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