

Review

Microbial bioinoculants and their role in plant growth and development

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The increase in the world's population, coupled with the limitations in the world's supply of natural resources and widespread degeneration of the environment presents a major challenge to agriculturalists. Chemical fertilizer is used to give the plant nutrient requirements within a short period to get faster results. Newly improved varieties of crops need high proportions of fertilizer. But chemical fertilizer has certain limitations and entails a lot of disadvantages. No doubt, the application of chemical fertilizer provides nutrition in high concentration in the soil and plants. When chemical fertilizer is applied, the entire contents would not be absorbed by the plants and the remaining parts would react in the soil. Part of it would be washed away and would contaminate water and some part of it would evaporate to the atmosphere; thereby the environment is polluted. Intense activity is involved in efforts to create plants that by themselves are able to fix nitrogen from the atmosphere – that is, convert nitrogen gas into nitrates that can be used by plant's metabolic machinery. At present, only certain plants called legumes are naturally able to do so and even legumes require the aid of symbiotic bacteria. Such a development would drastically curtail the amount of fertilizer required by agricultural crops (Tilak, 2001). The main factor is the price, biotechnology could provide an alternative to technologies that have harmful effects on the environment and it would have the potential of enhancing production on a sustainable basis (Al- Garni, 2006). Microorganisms are useful for biomineralization of bound soil and make nutrients available to their host and/or its surroundings. Nitrogen and phosphorus are major plant nutrients which occupy a key place in balanced use of fertilizer. Phosphorus is an important requirement of legumes for their nitrogen fixation process (Huda et al., 2007). All tropical legumes fix the atmospheric nitrogen by Rhizobium which requires optimum level of phosphorus in plant tissue. Their seedlings establish better in presence of mineral solubilizers because more of the tropical soils are phosphate fixing and make it unavailable to the plants (Dabas and Kaushik, 1998; Sahgal et al., 2004; Tilak et al., 2005; Hameeda et al., 2008; Gupta et al., 2007). It is due to the phosphate solubilizing organisms those solubilize the bound form of phosphorus and AM fungi acts as up-taker of phosphorus and make it available to the host plants. Microorganisms facilitate plant mineral nutrition by changing the amounts, concentrations and properties of minerals available to plants. These changes lead to change in growth, development and chemical composition of plant that are common and substantial enough to encourage the exploitation of plant microbe interaction for improvement of crop productivity. Possible approaches include both the introduction of foreign microorganisms and capitalization on the indigenous microflora. There are various groups of organisms that can be solubilize and/or leaching of phosphate, iron and other mineral metals. Since the chemical fertilizers are becoming important ingredient of the agricultural farming and production, need based technology should be given priority. As the production and manufacture cost of the chemical fertilizer are very high, its availability and uses are also becoming imperative. Biomineralizing phenomenon of the microbes is very important in this regard. Plant microbe interaction is an important phenomenon and also useful in the development of most suitable bioinoculant which may be able to improve the plant productivity under adverse condition. A large number of literatures are available regarding the microbial interaction and beneficial uses in plants of agriculture, horticulture and forestry. Keeping this in view, some important information regarding the biofertilizing potential of some important group of microbes and their application for the development of sustainable technology has been reviewed here.

Key words: Bioinoculants, phosphate solubiliser, nitrogen fixing bacteria, mycorrhiza, PGBR.

INTRODUCTION: TYPES OF BIOINOCULANTS AND THEIR ROLE

Phosphate solubilizing microorganisms

Phosphorus is added to soil in the term of phosphate fertilizer, part of which is utilized by the plant and the remainder converted into fixed and insoluble forms of phosphorus (Kumar et al., 2004; Afzal et al., 2005; Mehrvarz et al., 2008). There is nevertheless enough scope to use finely ground rock phosphate directly on the farm, especially in acidic soils. Since P availability from the phosphoric reserves; that is rock phosphate under neutral and alkaline conditions is scarce/negligible, the phosphate solubilizing microbes dissolving imprisoned/interlocked phosphates appear to have an important implication in Indian agriculture. A wide range of microorganisms from autotrophs to heterotrophs, diazotrophs to phototrophs, fungi and actinomycetes including mycorrhizas are known to have the ability to solubilize inorganic P from insoluble sources (Patil et al., 2002; Mehrvarz and Chaichi, 2008; Reis et al., 2008). From time to time the ability of different microorganisms to solubilize bound phosphates incorporated in agar soil or liquid media has been demonstrated. These reactions take place in rhizosphere and because solubilizing microorganisms dissolve more phosphates than they require for growth and metabolism, the surplus can be absorbed by plants. The mechanisms of conversion of the insoluble phosphorus by these organisms to available form include: altering the solubility of organic compounds to the ultimate soluble form through production of acids and H₂S, under aerobic and anaerobic conditions respectively, mineralizing organic compounds with release of inorganic phosphate (Gaiind and Gaur, 1991; Bijaya et al., 2003; Dubey, 2000). Phosphate solubilizing fungi improve the growth performance of the plants (Kehri et al., 2002; Manoharachary et al., 2008). A study showed among five fungi such as *Aspergillus niger* strain 1, *A. niger* strain 2, *A. niger* strain 3, *Aspergillus flavus* and *Aspergillus fumigates* when tested for their efficiency in solubilizing phosphate (that is tricalcium phosphate). The fungal isolate *A. niger* strain 1 found to be efficient in phosphate solubilization (Mahalingam and Thilagavathy, 2008). Vazquez et al. (2000) concluded that most of the bacterial species produced more than one organic acid whereas *A. niger* produced only succinic acid. The production of organic acids by rhizosphere microorganisms is possibly involved in the solubilization of insoluble calcium phosphate. Mechanical plant compost from Calcutta and an alluvial soil (Inceptisol) from West Bengal, India were used for isolation of P-solubilizers. Fourteen each of P-solubilizing bacteria and fungi were isolated from both sources. All the bacterial

isolates were spore-forming *Bacilli*. *Penicillium* and *Aspergillus* were the predominant fungal genera. P-solubilizers isolated from carbon rich compost exhibited higher solubilizing ability than soil isolates (Kole and Hajra, 1998).

Kumar et al. (2004) studied the influence of phosphate solubilizing microorganisms on Mussoorie rock phosphate (MRP) and 'aluminum phosphate' under two reactions (pH 5.1 and 6.2) categories of acid soils from upland and lowland areas soils were inoculated using suspension of fresh cultures of test isolates of bacteria and fungi, respectively. Fungi were superior to bacteria in releasing P from acidic soils. Ramesh et al. (1998) studied the influence of sources of phosphate, FYM and phosphate solubilizing microorganisms on yield and quality parameters of irrigated groundnuts was investigated at Hebbal, Bangalore. Pod, seed, oil and protein yields were highest when P was applied as 75% Mussoorie rock phosphate and 25% superphosphate, plus application of 9 tons farmyard manure/ha and seed inoculation with *Aspergillus awamori*. Narloch et al. (2002) studied the effect of phosphate-solubilizing fungi MSF-044, MSF-062 (*Penicillium* sp.) and MSF-087 (*Aspergillus* sp.) and soluble phosphate [Ca(H₂PO₄)₂·2H₂O] at 0.0, 4.5, 9.0, 17.5 35.0, 70.0 and 140.0 mg; kg⁻¹ P on dry matter production and P uptake of radish in non-sterilized soil under green house conditions. Isolates differed in their capacity to stimulate dry matter production of plants depending upon the P level. Isolates MSF-044 and MSF-062 were more efficient under low P (4.5 to 17.5 mg/kg⁻¹P), while the isolate MSF-087 only stimulated dry matter production in the highest P level (140.0 mg/kg⁻¹P). Plants inoculated with the isolate MSF-062 at 17.5 mg/kg⁻¹P presented no differences in dry matter compared to non-inoculated plants with 70.0 mg/kg⁻¹P. Phosphorus uptake by inoculated plants did not differ between treatments. Illmer and Schinner (1995) studied that two species *Penicillium aurantiogriseum* and *Pseudomonas* sp. (PI 18/89) with proven ability to solubilize inorganic phosphates were tested for their efficiency to dissolve calcium phosphates under non-sterile conditions. In laboratory experiments plant available phosphorus was only increased when phosphate solubilizing microorganisms disposed off sufficient nutrient supply. Field studies on forest soils in Australia indicated that phosphate-solubilizing microorganisms were too vulnerable and unreliable to be used in agriculture. Two solubilizing fungi identified were *Aspergillus terreus* and *A. niger* (Casanova et al., 2002). Reyes et al. (2006) studied the biodiversity of phosphate-solubilizing microorganisms (PSM) of rock phosphate mine in Tachira, Venezuela, a larger number of PSM were found in the rhizospheric than in the bulk soil.

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Six fungal strains belonging to the genus *Penicillium* and with high hydroxyapatite dissolution capacities were isolated from bulk soil of colonizer plants. Five of these strains had similar phenotypes to *Penicillium rugulosum* IR-94MF1 but they solubilized hydroxyapatite at different degrees with both nitrogen sources. From 15 strains of 'gram-negative bacteria' isolated from the rhizosphere of colonizer plants, 5 were identified as diazotrophic free-living encapsulated *Azotobacter* species able to use ammonium and/or nitrate to dissolve hydroxyapatite with glucose, sucrose and/or mannitol. Different nitrogen and carbohydrate sources are parameters to be considered to further characterize the diversity of PSM. Xiao et al. (2008) did the optimization for rock phosphate solubilization by phosphate-solubilizing fungi isolated from phosphate mines. The optimization for rock phosphate (RP) solubilization by phosphate-solubilizing fungi (*Candida krissii*, *Penicillium expansum* and *Mucor ramosissimus*) isolated from phosphate mines (Hubei, PR China) was investigated. The content of soluble phosphorus (P) released by these isolates was tested on the National Botanical Research Institute's phosphate growth medium (NBRIP) containing RP as sole P source. Results showed that the optimum conditions were at temperature of 32°C, shaking speed of 160 rpm, RP concentration of 2.5 g/l, EDTA concentration and 0.5 mg/ml. The content of soluble P gradually decreased with a larger particle size of RP with all the isolates. The content of soluble P was the highest when the initial pH for RP solubilization was 5.5 in the medium inoculated with *C. Krissii* which was different from that of 7.0 in the medium inoculated with P. The bacterial isolate *Micrococcus* spp. were found to be efficient in phosphate solubilization (Mahalingam and Thilagavathy, 2008). Phosphorus solubilization and immobilization data indicated that bacterial isolates solubilized more P than fungi, which in turn immobilized more phosphate (Alam et al., 2002). Wu et al. (2007) studied the biodegradability and mechanical properties of polycaprolactone composites encapsulating phosphate-solubilizing bacterium *Bacillus* sp PG01. The outcome of this work seems to suggest that by proper manipulation of composite compositions, the controlled release of the bacterial fertilizer (that is, *Bacillus* sp. PG01 cells) might be achievable. Mechanical plant compost from Calcutta and an alluvial soil (inceptisol) from West Bengal, India were used for isolation of P-solubilizers. Fourteen P-solubilizing bacteria were isolated from both sources. All the bacterial isolates were spore-forming *Bacilli* (Kole and Hajra, 1997). Line et al. (2006) used *Burkholderia cepacia* CC-A174 with a high ability for solubilizing tricalcium phosphate (TCP) to study the P-solubilization mechanism. They collected filtrates able to solubilize TCP from the cultures of strain CC-A174 and demonstrated that the P-solubilization increased 200 times during exponential growth when the pH decreased from 8 to 3. Wang et al. (2007) stated that 'inoculation'

with phosphate-solubilizing fungi diversifies the bacterial community in rhizospheres of maize and soybean. Application of phosphate-solubilizing microorganisms (PSMs) has been reported to increase P uptake and plant growth. However, no information is available regarding the ecological consequences of the inoculation with PSMs. Sharma and Prasad (2003) conducted a field experiment at Indian Agricultural Research Institute, New Delhi during 1996 to 1997 to 1998 to 1999 to study the effect of phosphate solubilizing bacteria (PSB) and incorporation of wheat and rice residue on the relative efficiency of diammonium phosphate (DAP) and Mussoorie rock phosphate (MRP) in three cycles of rice-wheat cropping system. The results of the present study; therefore indicate that low grade rock phosphate such as MRP can be advantageously utilized in rice-wheat cropping system when applied with PSB inoculation and incorporation of rice and wheat residues. Chen et al. (2006) studied the ability of a few soil microorganisms to convert insoluble forms of phosphorus to an accessible form is an important trait in plant growth-promoting bacteria for increasing plant yields. The use of phosphate solubilizing bacteria as inoculants increases the P uptake by plants. Mirik et al. (2008) used phosphate solubilizing bacteria three *Bacillus* species to control bacterial spot disease. Hameeda et al. (2008) studied the growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Five bacterial strains with phosphate-solubilizing ability and other plant growth promoting traits increased the plant biomass (20 to 40%) by paper towel method. Rivas et al. (2006) studied the biodiversity of populations of phosphate solubilizing *Rhizobia* that nodulates chickpea in different Spanish soils.

Within *Rhizobia*, two species nodulating chickpea, *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum* are known as good phosphate solubilizers.

Nitrogen fixing bacteria

Nitrogen is a necessary component which is used for the growth of the plant. Plants need a limited amount of nitrogen for their growth. The type of crops also determines the level of nitrogen. Some crops need more nitrogen for their growth while some crops need fewer amounts. The type of soil also determines which type of biofertilizers is needed for this crop. Nonsymbiotic nitrogen fixation is known to be of great agronomic significance. The main limitation to nonsymbiotic nitrogen fixation is the availability of carbon and energy source for the energy intensive nitrogen process. This limitation can be compensated by moving closer to or inside the plants, namely: in diazotrophs present in rhizosphere, rhizoplane or those growing endophytically. *Azotobacter* not only help in nitrogen fixation but also capable of producing antibacterial and antifungal compounds, hormones and

siderophores (Tilak, 1993). Biological nitrogen fixation is estimated to contribute 180×10^6 metric tons/year globally of which 80% comes from symbiotic associations and the rest from free-living or associative systems. The ability to reduce and siphon out such appreciable amounts of nitrogen from the atmospheric reservoir and enrich the soil is confined to bacteria and archaea. These include symbiotic nitrogen fixing forms namely: *Rhizobium*, the obligate symbionts in leguminous plants and *Frankia* in non-leguminous trees and non-symbiotic (free-living, associative or endophytic) N_2 -fixing forms such as *cyanobacteria*, *Azospirillum*, *Azotobacter*, *Acetobacter diazotrophicus*, *Azoarcus*, etc. (Tilak et al., 2005). The family Azotobacteriaceae comprises of two genera namely: *Azomonas* (non-cyst forming) with three species (*A. agilis*, *A. insignis* and *A. macrocytogenes*) and *Azotobacter* (cyst forming) comprising of 6 species, namely: *A. chroococcum*, *A. vinelandii*, *A. beijerinckii*, *A. nigricans*, *A. armeniacus* and *A. paspali*. *Azotobacter* is generally regarded as free-living aerobic nitrogen fixer. Yield improvement is attributed more to the ability of *Azotobacter* to produce plant growth promoting substances such as phytohormones IAA and siderophore azotoactin, rather than to diazotrophic activity (Tchan, 1984; Tchan and New, 1984). Members of the genus *Azospirillum* fix nitrogen under microaerophilic conditions, and are frequently associated with root and rhizosphere of large number of agriculturally important crop and cereals. Due to their frequent occurrence in the rhizosphere, these are known as associative diazotrophs (Okon, 1985; Tilak and Subba, 1987).

After establishing in the rhizosphere, *Azospirilla* usually, but not always, promote the growth of plants. Despite of their N_2 fixing capacity, the increase in yield is mainly attributed to improved root development due to the production of growth promoting substances and consequently increased rates of water and mineral uptake. *Azospirilla* can contribute towards long term goal of improving plant microbes interactions for salinity affected fields and crop productivity. *Azospirillum* plays a major role in osmoadaptation through increase in osmotic stress (Basan and Holguin, 1997). Two groups of nitrogen-fixing bacteria, that is *Rhizobia* and *Frankia* have been found. *Frankia* forms root nodules on more than 280 species of woody plants from 8 different families (Schwintzer and Tjepkema, 1990). However, its symbiotic relationship is not understood. Species of *Alnus* and *Casuarina* are globally known to form effective symbiosis with *Frankia* (Wheeler and Miller, 1991; Huss-Danell, 1990; Werner, 1992; Dommergues and Marco-Bosco, 1998). '*Rhizobia*' are defined as bacteria capable of forming root nodules on legumes, mediated by *nod* genes. The nitrogen-fixing symbiotic relationship has been exploited in agriculture to enhance crop and pasture growth without the addition of nitrogen fertilizers. For this reason, the majority of research in this field has focused on herbaceous crop and forage legumes of agricultural

significance (Jinturkar and kale, 2005). In contrast, few studies have been made of rhizobial associations among non-crop legumes, despite the fact that they may be ecologically important in the natural landscape (Boring et al., 1988). Worldwide, there are an estimated 17000 to 19000 legume species, although nodulating bacterial species have only been identified for a small proportion of these. *Rhizobia* are soil inhabiting bacteria with the potential for forming specific root structures called nodules. In effective nodules the bacteria fix nitrogen gas (N_2) from the atmosphere into ammonia (O'Gara and Shanmugam, 1976) which is assimilated by the plant and supports growth particularly on nutrient deficient soils. In nutrients [predominantly decarboxylic acids (Lodwig and Poole, 2003) and are protected inside the nodule structure (van Rhin and Vanderleyden, 1995)]. In infective nodules, no nitrogen is fixed, yet *Rhizobia* are still supplied with nutrient and in this situation the *Rhizobia* could be considered parasitic (Denison and Kiers, 2004).

The nitrogen fixing symbiotic relationship has been exploited in agriculture to enhance crop and pasture growth without the addition of nitrogen fertilizers. For this reason, the majority of research in this field and focused on herbaceous crop and forage legumes of agricultural significance. In contrast, few studies have been made of rhizobial associations among non-crop legumes, despite the fact that they may be ecologically important in the natural landscape (Boring et al., 1988). The first and foremost example of bioinoculant is the *Rhizobium* or legume inoculants which was first marketed in U.S.A during the early part of the century. In the present scenario legume – *Rhizobium* association has gained importance in the study of microbial inoculant. Biological nitrogen fixation (BNF) is an important attribute to symbiotic association of legume host with *Rhizobia*. To achieve maximum BNF out of any legume- *Rhizobium* association it is necessary to properly characterize and identify *Rhizobia* before they are made commercially available for field experiment (Sahgal and Johri, 2003). Symbiosis between leguminous plants and soil bacteria commonly referred to as *Rhizobia* is of considerable environmental and agricultural importance, since they are responsible for an estimated 180×10^6 tons per year of biological nitrogen fixation worldwide. Rapid industrialization is associated with land degradation. The available statistics reveals that the situation is alarming. Legume-*Rhizobium* associations have potential application in ecological restoration of such degraded land considering the potential if legume-*Rhizobium* symbiosis. Rhizobial inoculants have been used to improve plant and soil health for more than a century now. Inherent with the use of bioinoculants is the problem of variability in field performance and successful establishment of introduced strain on account of competition with the indigenous rhizobacterial population. Bioinoculant formulations are usually based on laboratory

screening followed by appropriate trials in the field (Sahgal and Johri, 2003). *Rhizobia* live in a mutualistic symbiotic relationship with legumes- a relationship that has existed and coevolved for 10 millions of years (Sprent, 1994). The nodulation process includes a complex array of signaling molecules, molecular recognition, and regulation, legumes secrete secondary metabolites known as flavonoid into the soil, *Rhizobia* which are motile are attached to these flavonoids and attached to the rhizoplane. The flavonoids also induce the bacteria to secrete specific signal molecules known as Nod factors (Werner, 2004). The nod factors bind to a receptor in the root -hair cell, and cause root hair curling and eventual penetration of the acterium into the root-hair cell. Nod factors are critical molecules for nodule formation. After entering the root hair, bacteria travel down an infection thread a plant structure made specifically for this purpose (Gage and Margolin, 2000). The growing infection thread branches as it reaches the developing nodule primodium formed by dividing cortical cells. This growth is also initiated by Nod factor which reactivate the cell cycle (Patriarea et al., 2004). In most cases *Rhizobia* then differentiate morphologically to form bacteroids which are usually larger than the free living bacteria and have altered cell walls, bacteroids are released from the infection thread and form symbiosomes in nodule cells (Oke and long, 1999).

Bacteroids are the nitrogen fixing cells and are incapable of cell division and further reproduction (Perret et al., 2000). A compactable Nod factor is not the only requirement for effective nodulation. Bacterial cell surface components such as lipopolysaccharides (LPS) cyclic β -glucans, exopolysaccharides (EPS), capsular proteins and K-antigens are also recognized by the plant and help determine host specificity (Spaink, 2002; Frayse et al., 2003; Mathis et al., 2005). If these components are not recognized by the host, then the process is disturbed to various degrees for example, of infection threads fail to form non fixing empty nodules (Nod⁺ Fix⁻) may result (Perret et al., 2000). The *Rhizobium* legume interactions have been reported to be very specific in nature. One of the major factors contributing for this specificity is the activity of plant root lectins. It has been speculated that the agrichemicals, accumulated due to extensive application to soil may protect the *Rhizobium* recognition sited on the root surface of legumes. As a result, the biological nitrogen fixation and consequently the yield of leguminous crops will be decreased due to reduced nodulation. Worldwide, there are an estimated 17000 to 19000 legume species (Martinez- Romero et al., 1991), although nodulating bacterial species have only been identified for a small production of these. Competition among the microorganisms determines the outcome of many biological events in nature and yet competitiveness is poorly understood. The *Rhizobium* legume symbiosis provides a good model system with which to study the molecular basis of bacterial competitiveness because

nodulation competitiveness is a ready quantifiable trait. About 170 million tons nitrogen is contributed annually through biological nitrogen fixation by many bacteria and some actinomycetes in association with some legumes and non-legumes. The latter techniques involves use of species *Rhizobia* that shows loose association with roots of non-legumes or enzymatic removal of cell wall of root cells that facilitates *Rhizobial* infection into the root cortex of treatment of seedling-root with phytohormones as 2, 4-D, NAA, IBA or cytokinins along with inoculation by *Azorhizobium cauinodans* or treatment with the signal compound (flavonoids) that are involved in nodule development and colonization by *Rhizobia*.

Rhizobia may invade the roots of non-legumes by specialized crack entry at the ruptured corners of the root that occur due to emerging lateral roots. The nitrogen fixation is attributed to presence of nod, nif and fixes genes that code for nitrogenase complex and other accessory proteins needed for proper functioning of the nitrogenase complex. A feeble nitrogen fixation was observed in paranodules of non-legumes since they lack the important O₂ scavenger and leghaemoglobin. Therefore, other possible sites of colonization as xylem vessels should be studied and possible techniques of transfer and expression of this gene in non- legumes should be perused. Though induced nodulation in non-legumes holds possibility of extending *Rhizobial* symbiosis and biological nitrogen fixation to cereals but this arena remains to be sheltered by numerous similar cascades of reactions present in legumes to be deciphered in non-legumes (Kalia and Gupta, 2002). Flavonoids released from plants play an important role as signal molecules in early stages of legume- *Rhizobium* symbiosis and in regulating the activities of soil microorganism. Flavonoids not only regulate transcription of *Rhizobial* nod genes, but also promote chemotaxis, growth, metabolism and symbiotic efficiency of *Rhizobia*; important effects on other soil microorganisms are evident from studies from how plants regulate *Rhizobia* and other soil microorganisms with natural plant products offer a basis of defining new concepts of rhizosphere ecology (Jain and Nainawatee, 2002). *Rhizobial* systematics has been determined from the beginning by association to the host plant. By 1980 the species names reflected those of their hosts (Skerman et al., 1980). Nevertheless, there were many strains that were unclassified, or indeed unclassifiable under this scheme most of these anomalies were included in the 'cowpea' *Rhizobia* group. This group eventually contained isolates from the majority of all nodulated legumes. This situation was widely considered unsatisfactory (Howieson and Brockwell, 2005).

The realization that transmissible genetic element-plasmid and symbiosis islands- could carry genes that conferred nodulation ability (Prakash et al., 1981; Fenton and Jarvis, 1994; Rao et al., 1994; Sullivar and Ronson, 1998) resolved one of the long standing problem of

Rhizobial systematics via strains with identical nodulation profiles could be different in biochemical and genetic tests and vice versa. *Rhizobium* classification is tentative and controversial since it is based on properties of isolates from less than 10% of known legumes. The genus is divided into 2 major groups (fast and slow growers) on morphological, physiological, symbiotic (cross-inoculation), and serological properties. All currently known *Rhizobia* are in the phylum proeobacteria, most in the class Alphaproteobacteria, which contains six Rhizobial families in a single order Rhizobiales. There are a number of species present in these rhizobial genera that have not been observed to form nodules, and therefore do not fit the functional definition of *Rhizobia*. These include many of the species that were formerly known as *Agrobacterium* for example *R. larrymoorei*, *R. rubi* and *R. vitis* (Young et al., 2001, 2004). However, there is recent evidence that other species formerly classified as *Agrobacterium* are capable of nodulation. For example *R. radiobacter* nodulates *Phaseolus vulgaris*, *Campylotropis* spp., *Cassia* spp. (Han et al., 2005) and *Wisteria sinensis* (Liu et al., 2005); both nodules and tumours were formed on *P. vulgaris* by *R. rhizogenes* strains containing a Sym plasmid (Velázquez et al., 2005). There are also other species, although classified within genera commonly considered to be represented entirely by nodulating strains, in fact include strains apparently devoid of nodulation ability. For example *Bradyrhizobium betae* forms tumours on *Beta vulgaris* (beetroot) but is not known to fix N₂ (Rivas et al., 2004). *Mesorhizobium thioanganeticum* is a sulfur-oxidising bacterium, and does not nodulate the tested legumes of *Clitoria ternatea*, *Pisum sativum* and *Cicer arietinum* (Ghosh and Roy, 2006). There are also non-symbiotic strains of *Mesorhizobium* (and other genera) that can become nodulating species by acquiring symbiosis genes.

The genus *Sinorhizobium* was recently reclassified to *Ensifer* on the basis of similarity of DNA sequences (Willems et al., 2003; Young et al., 2004). *Ensifer adhaerens* is a soil bacterium that attaches to other bacteria and may cause cell lysis (Casida, 1982). Although wild type *E. adhaerens* did not nodulate *P. vulgaris* or *Leucaena leucocephala*, it did so when transformed with a symbiotic plasmid from *Rhizobium tropici* (Rogel et al., 2001), demonstrating its capacity to become a rhizobial species. Other *E. adhaerens* strains were subsequently isolated those nodulated legumes naturally. These form a single clade with *Sinorhizobium* in 16S rRNA and *recA* phylogenies leading Willems et al. (2003) to suggest that these strains be reclassified as *Sinorhizobium adhaerens*. However, *Ensifer* (Casida, 1982) is the senior heterotypic synonym and thus takes priority (Young et al., 2001). This means that all *Sinorhizobium* spp. must be renamed as *Ensifer* spp. according to the Bacteriological code (Lapage et al., 1990). Symbiotic nitrogen fixing *Rhizobium* species are

able to specifically interact with leguminous plants. Both bacterial and plant genes are involved in the formation of root nodules on the host plants where atmospheric nitrogen is converted to ammonia (Dusha et al., 1987). In *Rhizobium melliloti*, genes coding for nitrogen fixation enzymes (*nif* genes) and for other functions of symbiotic nitrogen fixation (*fix* genes) are localized on a mega plasmid. Bacterial genes determining nodules has been found to be highly host specific for different *Rhizobium* species. Joshi et al. (2008) studied the effect of expression of *Bradyrhizobium japonicum* 61A152 *fegA* gene in *Mesorhizobium* sp., on its competitive survival and nodule occupancy on *Arachis hypogaea*. Nodulation is affected by temperature. It was adversely affected at both 28 and 37°C and the effect was more pronounced during the first third of the nodule formation (Dudeja and Khurana, 1989). Kinselection could be the main selective force maintaining nodulation and nitrogen fixation in *Rhizobium* bacteria (Olivieri and Frank, 1994). The effect of number of *Rhizobium* supplied on the subsequent numbers of nodules formed was tested. The final nodule numbers are related to the growth of the root system since the final number of nodules per gram of root is independent of the initial dose of inoculum, the effect of which was mainly to cause and increase in root growth (Bhaduri, 1951). The development of nodules involves a highly specific signal exchange between the two symbionts (Verma, 1992) cyclic adenosine 3'5'-monophosphate (cAMP) may have an important role in the sequence of biological events regulating nodule formation and functioning (Terakado et al., 1997).

Pauly et al. (2006) studied several reactive oxygen and nitrogen species are continuously produced in plants as by products of aerobic metabolism or in response to stresses. This compactable interaction initiated by a molecular dialogue between the plant and bacterial partners, leads to the formation of a novel root organ capable of fixing atmospheric nitrogen under nitrogen limiting conditions. Saha and Haque (2005) studied that the nitrogen fixing bacteria (*Rhizobium*) isolates were subjected to cultural, morphological, biochemical and nodulation test for characterization. Graham (1969) developed a selective medium for selective isolation and growth of *Rhizobium*. Qadri and Mahmood (2003) studied the characterization of *Rhizobia* isolated from some tree legumes growing at Karachi university campus. *Rhizobia* isolates from nodules of *Dalbergia sissoo* were acid – producing and fast growers, utilizes 11 carbon sources and resistant against the antibiotics Amoxicillin, Ampicillin, Cloxacillin, Erythromycin, Neomycin, Sulphamethoxazole, Trimethoprim and susceptible to Gentamycin and Tetracycline. The ability of a bacterial strain to form nodules on a particular species of host plant is thus not necessarily related to any particular set of serological characters amenable to the agglutination test (Kleczkowski and Thorton, 1944). Nehra et al. (2007) studied the characterization of heat resistant mutant

strains of *Rhizobium* sp (Cajanus) for growth, survival and symbiotic properties. Ahalwat and Dadarwal (1996) studied the Bacteriocin production by *Rhizobium* sp. and its role in nodule competence. *Rhizobium* associated with the tree legumes: Sahgal et al. (2004) studied the selection of growth promotory *Rhizobia* for *D. sissoo* from diverse soil ecosystem. 35 strains were isolated among them, 3 nodulated in *L. leucocephala*. Ultra structure study of root nodules was studied by Qadri et al. (2007). The internal structure of a mature nodule showed an epidermis, cortex, vascular region and a bacteroid region. Vascular bundles were amphicribal. A distinct periderm consisted of scleroid tissue could be observed in the cortex outside the vascular tissue. The bacteroid region contained infected and uninfected (interstitial) cells intermingled with each other. Infected cells of developing nodules as well as of mature nodules were vacuolated. Infection threads were also observed in the bacteroid zone.

Maheshwari and Kumar (1997) studied that although, *Rhizobia* are abundant in soil, all of them are not able to nodulate various types of legumes because of their specificity for each legumes, effective inoculant is required to develop individual crop. *Rhizobial* inoculants (as living fertilizer) composed of microbial inoculants or groups of microorganisms, which are able to fix atmospheric nitrogen required for the development of sustainable system (agriculture/forestry) for uncultivable land areas. The response of particular legume to stress is not a property of the host plant alone, it demands consideration of the inoculant *Rhizobia* and the process of symbiosis. Attempts are made to isolate, characterize, identify the suitable indigenous *Rhizobia* for their use as bioinoculant preparations for certain fast growing fuel wood tree species. Saha and Haque (2005) isolated *Rhizobium* from the healthy nodules. Pure culture of isolated bacteria was subjected to cultural, morphological, biochemical and nodulation tests for characterization. In all cases of inoculation nodule number was higher on plants cultivated on sterile soil mixed with *Rhizobium* than on natural soil. The present finding indicates that the plant growth was related to the number of nodules on roots of plant. Rathi et al. (2009) studied the influence of *Rhizobium leguminosarum* inoculation along with sulphur and micronutrients. Because many soil lack *Rhizobia* or the density of the *Rhizobia* is too low for profitable legume cultivation, legume seeds should be inoculated with large amounts of a host plant specific microsymbiont on their surface just before being sown to ensure efficient legume growth.

Diouf et al. (2003) have done the optimization of inoculation of *L. leucocephala* and *Acacia mangium* with *Rhizobium* under greenhouse conditions. The physiological stage of the bacterial culture had no effect on nodulation and growth of the seedlings of *A. mangium* inoculated and cultivated *in vitro* for four months. For *L. leucocephala*, the number of nodules was significantly

higher when the seedlings were inoculated with a bacterial culture in stationary phase. Grange and Hungria (2004) studied the genetic diversity of *Rhizobium* in indigenous common bean (*Phaseolus vulgaris*) in two Brazilian ecosystems and tested its nodulation in *Acacia leucocephala*. Twenty-five different profile combinations were obtained. *Rhizobium etli* was the predominant species, with 73 strains showing similar RFLP profiles; while 12 other strains differed only by the profile with one restriction enzyme. Fifty strains were submitted to sequencing of a 16S rDNA fragment and 34 clustered with *R. etli* including strains with RFLP-PCR profiles similar to those species or differing by one restriction enzyme. However, other strains differing by one or two enzymes were genetically distant from *R. etli* and two strains with identical profiles showed higher similarity to *Sinorhizobium fredii*. Other strains showed higher similarity of bases with *R. Tropici*, *R. Leguminosarum* and *Mesorhizobium plurifarum*, but some strains were quite dissimilar and may represent new species. Great variability was also verified among the sequenced strains in relation to the ability to grow in YMA at 40°C, in LB, to synthesize melanin *in vitro*, as well as in symbiotic performance including differences in relation to the described species, for example many *R. etli* strains were able to grow in LB and in YMA at 40°C, and not all *R. Tropici* were able to nodulate *Leucaena*. Rahangdale and Gupta (1997) studied the dual inoculation with nitrogen fixing *Rhizobium* and P- uptaker vesicular arbuscular mycorrhizal fungi association. They applied this inoculation to forest tree growing in Chhattisgarh was studied for their symbiotic association with forest trees at laboratory and nursery conditions. A very wide range of forest trees comprising leguminous and non leguminous were found to be associated with *Rhizobium* as well as VAM fungi.

The host specific strains of both organisms (12 strains of *Rhizobium* and 14 isolates of VAM) were isolated, purified and maintained in laboratory and glasshouse conditions. These inoculants were evaluated for their efficiency in increasing growth, nodulation, % colonization, phosphate-content, protein and sugar level of 4 different host plants (*Albizia lebbeck*, *D. sissoo*, *Albizia procera* and *Acacia nilotica*) at individual and dual inoculation level.

Arbuscular mycorrhizal fungi

The arbuscular mycorrhiza (AM) fungi are important rhizospheric microorganisms. They can increase the plant uptake of nutrients especially relatively immobile elements such as P, Zn and Cu (Ryan and Angus, 2003), and consequently, they increase root and shoot biomass and improve plant growth. It has been indicated that AM fungi can colonize plant roots in metal contaminated soil, while their effects on metal uptake by plants are

conflicting. They are ubiquitous in geographic distribution occurring with plant growing in all environmental conditions. VAM fungi occur over a broad ecological range from aquatic to desert environments. These fungi belongs to the genera *Glomus*, *Gigaspora*, *Acucospora*, *Scutellospora*, *entrophosphora*, *Modicella* and *Sclerocystis* are obligate symbionts and have not been cultured on nutrient media using standard microbiological techniques. They are multiplied in the roots of host plants and the inoculum is prepared using infected roots of host plants and the inoculum is prepared using infected roots and soil. Crop responses to VAM inoculation are governed by soil type, host variety, VAM strains, temperature, moisture, cropping practices and soil management practices. In general, field experiments with VAM have been the inability to produce clean pure inoculum on large scale. Field trails indicated that VAM inoculation increased yields at certain locations and the response varied with soil type, soil fertility particularly with available P status of soil and VAM culture (Tilak, 1993). In Fungal Biology in 21st century- 'roots' of nearly 90% plants form a symbiotic association with fungi called Mycorrhiza, (fungus root) contrary to popular belief, the luxuriance of rain forests is not because the rain forests soil is more fertile (as torrential rains over millennia leach out soluble minerals), but because the roots associate with fungi whose spreading hyphae increase the area of absorption of scarce nutrients and transport these to the plant in return for photosynthetically fixed carbon.

In the symbiotic interaction, the fungus enters the root cells to form specialized haustoria called arbuscules because they are highly branched tree like structure. Arbuscular mycorrhizal fungi also develop an extensive hyphal network external to the plant root which provides the physical link between the soil and root, drawing phosphorus and other minerals from the soil and translocating them to the root. The mechanisms that are responsible for the increased uptake from soil and transfer to host through the interface need to be identified, a proteome analysis based on separation of protein by two dimensional electrophoresis and their identification by mass spectrometry has been initiated to identify proteins involved in mycorrhizal development and functioning (Mamatha and Bagyaraj, 2002; Mamatha et al., 2002; Manoharachary, 2002). Mahmood et al. (2004) found some observations on mycorrhizae of *L. leucocephala* (Lam.) De Wit. In young roots of *L. leucocephala* infection hyphae formed appressorium, sending branches in different directions to colonize the host cell. The mycorrhizal fungus was non-septate and branched. Fungal cells (pelotons) were observed in the root cortex. Characteristic arbuscules commonly formed by VAM fungus were visible. The main hyphae branched and the branched hyphae terminated in an arbuscule. The cortical cells of the root showed large number of vesicles which were either globular or elliptical in shape. AM fungi also act as biocontrol agent for plant pathogen

(Raman et al., 2002). An investigation was undertaken to test the hypothesis that amending peat to increase its P buffer capacity and optimizing the P concentration of the amended medium for mycorrhizal activity will enhance its usefulness for raising mycorrhizal seedlings of tree species. The approaches entailed mixing a small quantity of soil of high P adsorbing capacity with peat and constructing a P sorption isotherm for the medium in order to establish solution P concentration near-optimal to optimal for mycorrhization of seedlings. A P sorption isotherm based on incubating the medium with graded amounts of P at 50% of available water-holding capacity was developed. Target solution P concentrations established using the approach enabled us to identify the optimal solution P concentration for mycorrhizal development on roots of our indicator plant *L. leucocephala* grown in the medium. Arbuscular mycorrhizal colonization, host growth, and P status of *L. leucocephala* pinnules measured at target solution P concentrations ranging from 0.12 to 1.0 mg/l revealed that AM fungal activity and symbiotic effectiveness was maximum at solution P concentration of 0.2 mg/l. Medium solution P concentrations in excess of 0.2 mg/l tended to depress AM fungal colonization, but colonization level did not decline below 43% (Peters and Habte, 2001).

Osorio and Habte (2001) studied the synergistic influence of an arbuscular mycorrhizal fungus and a P solubilizing fungus on growth and P uptake of *L. leucocephala* in an oxisol. An investigation was carried out to assess the role that P solubilizing microorganisms play in the P nutrition of mycorrhizal and mycorrhiza-free *L. leucocephala* (Lam.). Soil microorganisms able to solubilize rock phosphate were isolated from the rhizosphere of *L. leucocephala* naturally, growing on three different soils of Hawaii. The isolates were screened for their ability to solubilize rock phosphate in culture medium. The highest activity was observed with one of the fungal isolates, which was identified as *Mortierella* sp. It was multiplied and further evaluated with or without the mycorrhizal fungus *Glomus aggregatum* in a highly, weathered soil for its effectiveness to enhance P uptake and growth of *L. leucocephala*. Phosphorus status of *L. leucocephala* pinnules monitored as a function of time revealed that plants colonized by both microorganisms had the highest P content followed by plants inoculated with the mycorrhizal fungus alone. Inoculation of soil with *Mortierella* sp. alone did not influence P content of plants measured at the time of harvest. However, *Mortierella* sp. increased the P content of mycorrhizal plants by 13% in the unfertilized soil and by 73% in the soil fertilized with rock phosphate. Shoot dry weight measurements showed that *Mortierella* sp. stimulated growth of nonmycorrhizal by 22%, while it stimulated the growth of mycorrhizal plants by 29% regardless of P fertilization.

The results suggest the existence of synergistic interaction between P solubilizing microorganisms and

mycorrhizal fungi, although the degree of synergism was more pronounced in terms of P uptake than in terms of growth.

Plant growth promoting microbes

Numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, stimulate plant growth by a plethora of mechanisms. These bacteria are collectively known as PGPR (plant growth promoting rhizobacteria). The search for PGPR and investigation of their modes of action are increasing at a rapid pace as efforts are made to exploit them commercially as biofertilizers. PGPR belongs to several genera for example *Actinoplanes*, *Agrobacterium*, *Alcaligenes*, *Amorphosporangium*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Cellulomonas*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium*, *Streptomyces* and *Xanthomonas*. PGPR are believed to improve plant growth by colonizing the root system and pre-empting the establishment of or suppressing 'deleterious rhizosphere micro organisms' (DRMO) on the root (Schroth and Hancock, 1981). Inoculating planting material with PGPR presumably prevents or reduces the establishment of pathogens (Suslow, 1982). Production of siderophores is yet another mechanism through which microbes influence plant growth. Siderophores are low molecular weight high affinity iron chelators that transport iron into the bacterial cells and are responsible increased plant growth by PGPR (Kloepper et al., 1988). Under iron deficient conditions, fluorescent siderophore iron complex (Hohnadel and Meyer, 1986) which creates an iron deficient environment deleterious to fungal growth. Plant growth promoting rhizobacteria (PGPR) are considered to promote plant growth directly or indirectly. PGPR can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of plant growth regulators (auxin, gibberellins and ethylene), siderophores, HCN and antibiotic production. Other microorganisms that are known to be beneficial to plants are the plant growth promoting rhizobacteria (PGPR). In addition to supplying combined nitrogen by biological nitrogen fixation, certain bacteria affect the development and function of roots by improving mineral and water uptake.

Considerable research is underway globally to exploit the potential of one such group of bacteria that belong to fluorescent *pseudomonad* (FLPs). This bacteria help in maintaining soil health, protect crop from pathogens and are metabolically and functionally (Lata et al., 2002; Lugtenberg and Dekkers, 1999). Most P corrugate, a form that grows at 4°C under laboratory conditions (Pandey and Palni, 1998), produces antifungals such as diacetylphloroglucinil and phenazine compounds that aid in phosphate solubilization. According to Gaur et al.

(2004), 50 to 60% of fluorescent *pseudomonads* recovered from the rhizosphere and endorhizosphere of wheat grown in Indo-Gangetic plains were antagonistic towards *Helminthosporium sativum*. Field trials of pseudomonad strain (GRP3) lead to yield increase from 5.6 to 18%. Rangarajan et al. (2001) analysed populations of *Pseudomonas* species have the potential to suppress the bacterial leaf and sheath blight causing pathogens. Recently, concern was showed on the use of FLPs in crop plants as the antifungal substances released by the bacterium, particularly 2, 4-diacetylphloroglucinol (DAPG) could affect the arbuscular mycorrhizal fungi (Andrade et al., 1995). Gaur et al. (2004) confirmed that DAPG producing *Pseudomonads* recovered from wheat rhizosphere did not adversely affect AM colonization. However, given the toxicity of DAPG, such an inhibition may probably be dependent on the amounts released by the bacterium. Filamentous fungi are widely used as producer of organic acids, particularly black *Aspergillus* and some species of *Penicillium*; these species were tested for solubilization of rock phosphate and have been reported for various properties of biotechnological importance such as biocontrol, biodegradation, phosphate solubilization and phosphate fertilization (Richa et al., 2007; Pandey et al., 2008; Manoharachary et al., 2005; Yadav et al., 2011). Environmental and health hazards associated with chemical fertilizer further make their possible use difficult. In the other hand success in agroforestry requires various inputs leading to proper establishment and growth of tree species. Selection of low demanding and fast growing tree species, fertilization, disease and pest management and proper protection of plants are some vital factors. Agroforestry ecosystem after a few years becomes self sustainable because it follows a 'feedback regulating system'.

The nutrient budget in such ecosystem is polycyclic in nature. Weathering of litter and biological cycle are some important processes of nutrient management. The contribution of nutrient from plant to soil varies from species to species and this is an important factor for selecting species for agroforestry. Opencast mining is generally practiced for commercial exploitation of coal. In this process, overburden materials that is, the overlying soil layer with existing vegetation are removed and deposited in another fresh area. Thus, the deposition of million tons of overburdens in the forms of rocks, shale, course tailing results in barren, biologically inert overburden dumps, called mine spoils. Revegetation of these mine spoils is essential for conservation of environment, biodiversity and to make the land productive. But, the revegetation of these mine spoils is difficult because, they are deficient in nutrients such as nitrogen (N), phosphorus (P) and devoid of organic matter with adverse physio-chemical properties. Root symbionts such as *Rhizobium* and arbuscular mycorrhizal fungi (AMF) are also reduced or absent in mine sites

(Noyd et al., 1995). High acidity in mine spoils due to oxidation of residual elemental or iron sulphur is also a constraint to revegetation which hampers root-growth of plant and reduces the population of beneficial microorganisms such as free-living N-fixers (Alexander, 1964; Armingier et al., 1996; Barnishel, 1977; Choudhury, 1996). In recent times, increased ecological awareness among researchers have resulted in search for innovative approaches for revegetation of mine area in India and abroad (Dugaya et al., 1996; Gupta et al., 1994; Kumar and Jena, 1996; Pandya et al., 1997; Prasad and Mahammad, 1990; Sonkar et al., 1998). Mining causes the destruction of natural ecosystems through removal of soil and vegetation and burial beneath waste disposal sites. The restoration of mined land in practice can largely be considered as ecosystem reconstruction- the re-establishment of the capability of the land to capture and retain fundamental resources (Jha et al., 2000). Mining is an extreme form of land degradation having devastating effects on flora and fauna and causing drastic changes in landform and hydrology. Because of large scale destruction of natural areas due to mining operations, a restoration strategy is needed as a part of the overall mining management plan. In restoration, emphasis is given first to build soil organic matter, nutrients and vegetation cover to accelerate natural recovery process. Tree plantations can be used as a tool for mine spoil restoration as they have ability to restore soil fertility and ameliorate microclimatic conditions.

Revegetation of mining overburden dump poses an ecological challenge. Overburden dumps are unstable and become polluted under unmanaged condition. It affects the original and habitat, increase heavy metal concentration and pollute the environment (Sengupta, 1993; Maiti, 2007). Phytostabilization is one way to restore the overburden dumps. As *Acacia auriculiformis* is able to adapt to stress environment, and presumed to be suitable for vegetating heavy metal contaminated soils, a large number open cast coal and iron mine operate in Orissa state, India and phytoremediation measure could be an ideal way to reforest and improve the soil condition of overburden dumps. This species can be used for the revegetation of overburden dumps of open cast mines of Orissa. Soil microorganisms play an important role in ameliorating soil condition that facilitates plant productivity. The effect of soil AM fungi on establishment and growth of *A. auriculiformis* in waste land soil is also reported (Giri et al., 2004). Legume is a natural mini-nitrogen manufacturing factory in the field and can play a vital role in increasing indigenous nitrogen production. Legume help in solubilizing insoluble P in soil, improving the soil physical environment, increasing soil microbial activity, and restoring organic matter and also has smothering effect on weed. The carryover of N derived from legume grown, either in crop sequence or in intercropping system for succeeding crops is also important. In a country like India, where the average

consumption of plant nutrients from chemical fertilizers on national basis is very low, the scope for exploiting direct and residual fertility due to legumes has obviously a great potential (Ghosh et al., 2007). It is known that a large number of seedlings of forest species useful for afforestation in dry deciduous conditions are required for raising plantations.

Microbial application at the nursery stage was also found to be useful in enhancing productivity in some forest trees like *Albizia*, *Acacia* and *Dalbergia* etc. (Rahangdale and Gupta, 1998; Sahgal et al., 2004; Thatoi et al., 1993; Verma, 1995). *A. auriculiformis*, *A. nilotica* and *A. leucocephala* are three legumes suitable for agroforestry because of their litter contains more than 2% nitrogen (Puri, 1960).

These trees can easily be grown at an elevation of 0 to 500 m receiving rainfall 200 to 1400 MM annually (Mishra et al., 2004). These three species can easily withstand 4 to 6 months of dry season. It is however equally important to screen some suitable bioinoculants for these species.

An assessment of the current state of bacterial inoculants for contemporary agriculture in developed and developing countries is critically evaluated from the point of view of their actual status and future use. A biotechnological goal is to use a combined inoculation of selected rhizosphere microorganisms to minimize toxic effects of pollutants and maximize plant growth and nutrition. Selected combinations of microbial inocula enhanced the positive effect achieved by each microbial group, improving plant development and tolerance in polluted soils.

The application of bioinoculants like AM fungi and one or two of the plant growth promoting rhizobacteria such as *Azospirillum*, *Agrobacteria*, *Rhizobium*, *Pseudomonas*, several gram positive *Bacillus* species is an environment-friendly, energy efficient and economically viable approach for reclaiming wastelands and increasing biomass production. *Rhizobium* and phosphate solubilizing microbes may affect AM fungi and their plant host by various mechanisms such as effects on the receptivity of the root, effect on the root fungus recognition, effects on the fungal growth, modification of the chemistry of the rhizospheric soil and effects on the germination of the fungal propagules (Al-Garni, 2006). On the other hand AM fungi are known to enhance nodulation and N fixation by legumes (Amora-Lazecario et al., 1998; Johansson et al., 2004). Moreover AM fungi, *Rhizobium* and phosphate solubilisers often act synergistically on infection rate, mineral nutrition and plant growth.

Development of model systems and sensors for effective N and P utilization and newer probes to assess microbial diversity and microbial enzyme in model systems would generate information on the role of organics and biofertilizers in integrated nutrient management in sustainable agriculture (Tilak, 2001).

MICROBIAL CONSORTIUM AND APPLICATION

Raja et al. (2006) concluded the combination of bioinoculants is a major cause for success of both the plant establishment and the sustainability of bioinoculants and confirms the beneficial effects of microbial consortium over conventional single inoculant application method. Fertilizer application enhanced the efficiencies of N, P and K uptake, whereas reduced their usage efficiencies. Though soil type did not affect microbial inoculation response, fertilizer application significantly affected plant response to microbial inoculation (Muthukumar and Udaiyan, 2006). The microbial inoculants were used in single form or in combinations (Prabakaran and Ravi, 1996; Gupta et al., 1999; Amutha and Kannaiyan, 2004; Aseri and Rao, 2005; Zaidi and Khan, 2006; Anil et al., 2007; Gaikwad et al., 2008). The effects of the inoculation of *Rhizobium* and phosphate solubilizing bacterium (PSB; *Bacillus megaterium* var. *phosphaticum*), singly or in combination gave better result than the uninoculated control (Sengupta et al., 2002; Marimuthu et al., 2002; Jat et al., 2003; Mathew and Hameed, 2003; Kashyap et al., 2004; Purbey and Sen, 2005). Singh and Tilak (2001) said that the synergistic effect of combined phosphorus fertilizers and inert sources of natural P (varisite, strengite, fluorapatite, hydroxyapatite, and tricalcium phosphate) along with bioinoculants (namely: phosphate solubilizing rhizobacteria and arbuscular mycorrhizas) is discussed. Sumana and Bagyaraj (2002) studied the interaction between VAM fungus and nitrogen fixing bacteria and their influence on growth and nutrition on different tree species. Effect of three *Penicillium* spp namely: *P. islandicum*, *P. olivicolor*, and *P. restrictum*, one AM *Glomus* sp. and rock phosphate on the growth and yield of wheat was evaluated on pot culture containing sterilised soil (Gupta and Baig 2001). Soil compaction induced a limitation in root and shoot growth that was reflected by a decrease in the microbial population and activity. Our results show that bacterial population was stimulated by the decrease in soil bulk density (Canbolat et al., 2006). Enhancement of growth, nodulation and N₂-fixation in *D. sissoo* roxb. by *Rhizobium* and *Glomus fasciculatum* in the form of dual inoculation was studied by Rao et al. (2003). The structure of the plant root system contributes to the establishment of the rhizosphere microbial population.

The interactions of plant roots and rhizosphere microorganisms are based largely on interactive modification of the soil environment by processes such as water uptake by the plant, release of organic chemicals to the soil by the roots, microbial production of plant growth factors and microbial mediated availability of mineral nutrients. Within the rhizosphere, plant roots have a direct influence on the composition and density of the soil microbial community known as rhizosphere effect. Microorganisms in the rhizosphere may undergo

successional changes as the plant grows from seed germination to maturity. During plant development, a distinct rhizosphere succession results in rapidly growing, growth factor requiring and opportunistic microbial populations. These successional changes correspond to changes in the materials released by the plant roots, the rhizosphere during plant maturation. Initially, carbohydrate exudates and mucilaginous materials support the growth of large populations of microorganisms within the grooves of the epidermal plant cells, on the root surface and within mucilaginous layers of the roots. Microorganisms need more time to fix and establishing themselves in soil. The present finding showed that phosphate-solubilizing microorganisms can interact positively in promoting plant growth as well as P uptake of maize plants, leading to plant tolerance improving under water deficit stress conditions (Ehteshami et al., 2007). Highest N and P uptake by *V. radiata* was recorded after treatment with a combination of *B. circulans*, *C. herbarum* and *G. fasciculatum* in the presence of MRP. Generally, the PSM population increased after AM fungus inoculation (Singh and Kapoor, 1999). Varma and Mathur (1989) studied the effects of interactions between *Azotobacter chroococcum* and *Pseudomonas striata* on their growth pattern were studied. Plant roots have a direct effect on the surrounding microbial populations; microorganisms in the rhizosphere have marked an influence on the growth of plants. In the absence of appropriate microbial populations in the rhizosphere, plant growth may be impaired.

Microbial populations in the rhizosphere may benefit the plant in a variety of ways, mineral nutrients; synthesis of vitamins, amino acids, auxins, cytokinins, and gibberellins, which simulate plant growth; and antagonism with potential plant pathogens through competition and development of amnesal relationships based on production of antibiotics. Organisms in the rhizosphere produce organic compounds that affect the proliferation of the plant root system. Microorganisms synthesize auxins and gibberlin-like compounds, and these compounds increase the rate of seed germination and the development of root hairs that aid plant growth. Rhizosphere microorganisms increase the availability of phosphate through solubilization of materials that would be otherwise be unavailable to plants. Plants have been shown to exhibit higher rates of phosphate uptake when associated with rhizosphere microorganisms than in sterile soils. The principal mechanism of increasing phosphate availability is the microbial production of acids that dissolve apatite (a common mineral group including calcium fluophosphate), releasing soluble forms of phosphorous. The immobilization of nitrogen by microorganisms in the rhizosphere accounts for an appreciable loss added nitrogen fertilizer intended for plant use. Although diverse and complex, the majority of interactions on the rhizosphere are mutually beneficial to

both plants and microorganisms and are synergistic in character. Response of *L. leucocephala* to inoculation with *Glomus fasciculatum* and/or *Rhizobium* was studied in a phosphorus deficient unsterile soil. *G. fasciculatum* only inoculation improved nodulation by native *Rhizobia* and *Rhizobium* only treatment improved colonization of roots by native mycorrhizal fungi.

Dual inoculation with both the organisms improved nodulation, mycorrhizal colonization, dry weight, nitrogen and phosphorus content of the plants compared to single inoculation with either organism.

ROLE OF MICROBIAL APPLICATION ON GROWTH AND DEVELOPMENT OF TREE LEGUMES

Duponnois et al. (2000) studied ectomycorrhization of six *A. auriculoformis* provenances from Australia, Papua, New Guinea and Senegal in glass house conditions and its effect on plant growth and on the multiplication of plant parasitic nematode. The results suggest the provenances of *A. auriculoformis* that are well adapted to the indigenous environmental conditions must be selected for their mycorrhizal dependency and for their resistance to plant parasitic nematodes. More over the ectomycorrhizal symbiosis has to be considered as an important component of the cultural system. Roux et al. (2009) studied the *Bradyrhizobia* nodulating the *A. mangium* × *A. auriculoformis* interspecific hybrid are specific and differ from these associated with both parental species. Inoculation experiments performed under *in vitro* or green house conditions showed that all strains were infective with their original hosts but exhibited very variable degrees of effectively according to the host plant tested. They concluded that there is still a high potential for isolating and testing new strains from hybrid to be used as inoculants in the context of large scale afforestation. Giri et al. (2009) studied the effect of inoculation of two arbuscular mycorrhizal fungi, *Glomus fasciculatum* and *Glomus macrocarpum*, alone and in combination, on establishment and growth of *A. auriculoformis* in a wasteland soil was studied under nursery and field conditions. Arbuscular mycorrhiza colonized plants showed significant increment in height, biomass production and girth as compared to non mycorrhizal plants. All growth parameters were higher on dual inoculation of *G. fasciculatum* and *G. macrocarpum* as compared to uninoculated plants under both nursery and field condition. Sharma et al. (2001) studied the mycorrhizal dependency and growth responses of *A. nilotica* and *A. lebbeck* to inoculation by indigenous AM fungi as influenced by available soil P levels in a semi-arid Alfisol wasteland. A series of available phosphorus (Olsen) levels ranging from 10 to 40 ppm were achieved in a semi-arid soil.

The influence of the levels of phosphorus on the symbiotic interaction between two subtropical tree \

species, *A. nilotica* and *A. lebbeck* and a mixed inoculum of indigenous arbuscular mycorrhizal (AM) fungi was evaluated in a greenhouse study. The extent to which the plant species depended on AM fungi for dry matter production decreased as the levels of soil P increased, but the degree of this decrease differed in the two species tested. *A. nilotica* colonized by AM fungi showed a significant increase in shoot P and dry matter at a soil P level. Vijaya and Srivasuki (1999) studied the effect of inoculum type and inoculation dose on ectomycorrhizal development and growth of *A. nilotica* seedlings inoculated with *P. tinctorius* in a nursery. Fumigated nursery bed on a sandy loam was inoculated with the ectomycorrhizal fungus *P. tinctorius* and seeded with *A. nilotica*. Two types of inoculum were compared: mycelium grown in a vermiculite/peat mixture and mycelium grown in liquid medium and entrapped in a calcium alginate gel with different quantities of mycelium. At the end of the first growing season the alginate inoculum at the dose of 4 g mycelium (dry weight) per m² proved to be the most efficient. The top dry weight of the seedlings in this treatment was 2.0 fold that of the non-inoculated fumigated controls. This inoculation treatment also ensured 90% mycorrhizal infection by *P. tinctorius*. Line et al. (2006) studied the growth of mycorrhized seedlings of *L. leucocephala* (Lam.) de Wit. in a copper contaminated soil. Due to the low infectivity potential of arbuscular mycorrhizal fungi (AMF) in a mining area located at the State of Bahia, Northeastern Brazil, the effect of mycorrhization on the seedlings of *L. leucocephala* was investigated, in order to use this species for revegetation of the area. Proportions of copper contaminated soil higher than 50% inhibited plant growth, development of the AMF in the roots and consequently, the benefit of mycorrhization.

The pattern of Cu and P absorption in *Leucaena*, associated or not with *G. etunicatum* is maintained when the soil is up to 50% contaminated. Barreto and Fernandes (2001) used *Leucaena leucocephala* in alley cropping systems to improve Brazilian coastal tableland soils. Decreases of the soil density and increases of the porosity were also observed in response to legume incorporation. These effects were most noticeable in the superficial layers. Forestier et al. (2001) studied the effect of *Rhizobium* inoculation methodologies on nodulation and growth of *L. leucocephala*. Their aim of the study was to evaluate the effect of five methods of *Rhizobium* inoculum application on nodulation and nitrogen fixation in *L. leucocephala* seedlings cultivated for 6 months in the greenhouse. Plants inoculated with alginate beads were significantly more developed and more nodulated than plants inoculated with the other methodologies used. Bala and Giller (2001) suggested that the host range and specificity is reported of a genetically diverse group of *Rhizobia* isolated from nodules of *L. leucocephala* and other tree species. Nodule number and nitrogen content was measured in seedlings of herbaceous and woody

legume species after inoculation with rhizobial strains isolated from tropical soils, to establish symbiotic effectiveness groups for Rhizobial strains and their hosts. The complex nature of cross-nodulation relationships between diverse rhizobial strains and legume hosts is highlighted. Host plants inoculated with effective rhizobial strains showed better nitrogen use efficiency than plants supplied solely with mineral nitrogen. Wang et al. (1999) studied the genetic diversity of *Rhizobia* from *Leucaena leucocephala* nodules in Mexican soils. *Leucaena* species are leguminous plants native to Mexico. Using two *L. leucocephala* cultivars grown in different soils, we obtained 150 isolates from the nodules.

Twelve rDNA types were identified which clustered into groups corresponding to *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* by restriction fragment length polymorphism (RFLP) of amplified 16S rRNA genes. Types 2, 4, 5, 6, 10, 11 and 12 were distinct from all the defined species. Others had patterns indistinguishable from some recognized species. Most of the isolates corresponded to *Sinorhizobium* 12. The affinities of host cultivars for different Rhizobial groups and the impact of soil cultivation and the soil populations of *Rhizobia* were analysed from the estimation of isolation frequencies and diversity. The results showed differences in Rhizobial populations in cultivated and uncultivated soils and also differences in *Rhizobia* trapped by *L. leucocephala* cv. *cunningham* or *peruvian*. *A. nilotica* nodulate with fast-growing *Rhizobium* (Dreyfus and Dommergues, 1981). The nitrogen-fixing potential of the species is still unknown. *A. auriculiformis* produces profuse bundles of nodules, which suggests a good nitrogen-fixing potential. Sharma and Ramamurthy (2000) conducted studies under controlled pathogenic conditions to assess the compatibility and nitrogen fixing potential of *Rhizobium* and *Bradyrhizobium* strains for *A. auriculiformis*. Of the 17 strains from different sourced tested only 10 were able to produce visible nodules and their nodulation frequency was also different. Strains isolated from other species of *Acacia* were not particularly more compatible with *A. auriculiformis* than strains isolated from other plant species. There was a significant increase in growth and associated parameter in the nodulated seedlings over that in seedlings without root nodules. However, nodules number appeared to be a poor indicator of the nitrogen fixing potential of strains, as it poorly correlated with nitrogenase activity, seedling dry weight and other growth related biometric parameters. *A. leucocephala* (*L. leucocephala*) has been the focus of a great deal of research in the past few decades for its nitrogen fixing potential. The acetylene reduction method (Hogberg and Kvarnstrom, 1982) and the difference method (Sanginga et al., 1985), which has been used to evaluate nitrogen fixation by *L. leucocephala*, give figures in the range of 100 to 500 kg N₂ ha⁻¹ yr⁻¹. The high nitrogen-fixing potential of this tree is related to its abundant nodulation under specific soil conditions, in which the nodule dry

weight was reported to reach approximately 51 kg ha⁻¹ in a stand of 830 trees ha⁻¹ (Hogberg and Kvarnstrom, 1982), and approximately 63 kg ha⁻¹ in a stand of 2,500 trees ha⁻¹ (Lulandala and Hall, 1986). *L. leucocephala* generally nodulates with *Rhizobium* (Halliday and Somasegaran, 1982) and occasionally nodulates with *Bradyrhizobium* (Dreyfus and Dommergues, 1981).

The *Rhizobium* strain specific to *L. leucocephala* is not generally found in soils. This explains the positive response to inoculation obtained in most soils where the level of nutrients (other than nitrogen) is high enough to satisfy the tree's requirements. *Leucaena* has often been regarded as a kind of "miracle tree", an appellation that makes sincere scientists wince. However, such names as subabul in India, its sensitivity to soil acidity and its high nutrient demand are reflected in its poor performance in infertile soils. Purohit et al. (2007) studied the ecology of soil fungi in *A. nilotica* based agroforestry systems of Rajasthan, India. The present study is aimed to quantify the spatial and temporal variations in the biomass and population of soil fungi in the *A. nilotica* sub. *indica* based traditional agro forestry systems. Soil in the vicinity of the tree possessed higher fungal biomass and population. Increase in electrical conductivity and pH towards alkalinity reduced fungal biomass. Thicker hyphae (12 to 16 μ) were predominant. In the areas under canopy and thinner (8 to 12 μ) in open area. Soil moisture controls fungi hyphal growth and density. Soil organic carbon reduces fungal biomass significantly in the vicinity of the tree than in the open area. Soil nitrogen is high in the under canopy area of the tree. Fungal population and biomass were linearly related. Gupta et al. (2011) studied the influence of mineral solubilizing microbes on growth and biomass of *A. auriculiformis* and developed a possible consortium for the species. A good example of the spread and role of *leucaena* in rural development is given by the work of Manibhai Desai, Director of the non-profit Bharatiya Agro Industries Foundation (BAIF) of Pune, India. He has inspired a generation of Indian scientists to dedicate their efforts to improving the livelihood of the rural poor. *Leucaena* is a major tool in this programme of revegetation, water management and animal improvement, a programme that earned Desai the prestigious Magsaysay Award in 1985. Often featured in their excellent publication, The BAIF Journal, *leucaena* is the subject of extensive development and research activities at BAIF. The giant *leucaenas* were carefully appraised in many types of management systems prior to large-scale seed increase (to 40 tons by 1986, equivalent to 800 million seeds) and distribution among India's rural community. Desai and his scientific staff have unquestionably inspired tree planting in the past decade on a scale previously believed impossible for the small farmer (Brewbaker, 1987).

Huda et al. (2007) studied effects of phosphorus and potassium addition on growth and nodulation of *D. sissoo* in the nursery. A study was conducted to test the

influence of different inorganic fertilizers (phosphorous and potassium) on the nodulation and growth of *Dalbergia sissoo* grown in the nursery. Before seeds sowing, different combinations of P, K fertilizers were incorporated with the nutrient-deficient natural forest soils, and then amended with cow dung (soil: cowdung = 3:1). Nodulation status (nodule number, shape, fresh weight, dry weight and color) in the roots and the plant growth parameters (length of shoot and root, collar diameter, fresh and dry weight of shoot and root) were recorded 60 days after seeds sowing. Nodulation status and growth of the plants varied significantly in the soils amended with fertilizers in comparison to the control. From the study, it is revealed that PK at the rate of 160 kg/hm² fertilizer with soil and cowdung mixture is recommended for optimum growth and nodule formation of *D. sissoo* in degraded soils at a nursery level. Bouillet et al. (2008) did the mixed-species plantations of *Acacia mangium* and *Eucalyptus grandis* in Brazil. Nitrogen accumulation in the stands and biological N₂ fixation. Dash and Gupta, (2011) analysed the roots of tree legumes grown in different pots inoculated with mineral solubilizing bacteria and fungi to evaluate AM infection and colonization. As compared to fungal inoculation, bacterial inoculation encouraged increase in percent AM colonization in the roots.

ROLE OF MICROBIAL APPLICATION OF ESTABLISHMENT OF TREE LEGUMES IN WASTELANDS AND UNDER STRESS CONDITION

Mining causes the destruction of natural ecosystems through removal of soil and vegetation and burial beneath waste disposal sites. The restoration of mined land in practice can largely be considered as ecosystem reconstruction - the reestablishment of the capability of the land to capture and retain fundamental resources. In restoration planning, It is imperative that goals, objectives, and success criteria are clearly established to allow the restoration to be undertaken in a systematic way, while realizing that these may require some modification later in light of the direction of the restoration succession (Jha et al., 2000; Tilak, 2001). A restoration planning model is presented where the presence or absence of topsoil conserved on the site has been given the status of the primary practical issue for consideration in ecological restoration in mining. Examples and case studies are used to explore the important problems and solutions in the practice of restoration in the mining of metals and minerals. Even though ecological theory lacks general laws with universal applicability at the ecosystem level of organization, ecological knowledge does have high heuristic power and applicability to site-specific ecological restoration goals. The concept of adaptive management and the notion that a restored site be regarded as a long-term experiment is a sensible

perspective. Unfortunately, in practice, the lack of post-restoration monitoring and research has meant few opportunities to improve the theory and practice of ecological restoration in mining. Vegetative material used in reclamation shall consist of grasses, legumes, herbaceous or woody plants, shrubs, trees or a mixture thereof which is consistent with site capabilities such as drainage, pH, soil depth, available nutrients, soil composition and climate. Such vegetation should be designed to provide a cover consistent with the stated land-use objective and which does not constitute a health hazard (Aseri and Rao, 2005). Mining is an extreme form of land degradation having devastating effects on flora and fauna and causing drastic changes in landform and hydrology. Because of large-scale destruction of natural areas due to mining operations, a restoration strategy is needed as a part of the overall mining management plan. In restoration, emphasis is given first to build soil organic matter, nutrients and vegetation cover to accelerate natural recovery process.

Tree plantations can be used as a tool for mine spoil restoration as they have ability to restore soil fertility and ameliorate microclimatic conditions. We discuss here various approaches of ecosystem restoration on mine spoil, criteria for the selection of plantation species and future research needs in this regard (Singh and Bhati, 2005). While *Rhizobia* and mycorrhizal fungi were a ubiquitous component of the soil biota in all undisturbed woodland soils they were absent or poorly represented in the stockpiled topsoils and some of the rudimentary soils formed in waste rock at the mine site. Kahlon et al. (2006) studied the effect of phosphorus, zinc, sulphur and bioinoculants on yield and economics of cowpea. An incubation study was conducted to test the suitability of Bokaro fly ash, alone or in combination with lignite, charcoal and farmyard manure, to act as a carrier for the *Rhizobium* inoculant. *R. leguminosarum* broth containing 108 cells/ml was mixed with the prospective carriers, packed in polythene and maintained at room temperature. Charcoal and lignite produced the highest populations of *Rhizobium*, possibly due to its ability to absorb toxic compounds and aerate the medium. Fly ash in combination with charcoal (1:1 or 1:3) had the highest population. The *Rhizobium* increased up to 45 days and then remained constant. It is concluded that 25% fly ash in combination with lignite or charcoal can be used as a carrier for bioinoculants (Lal and Mishra, 1998). Revegetation research at Ranger mine concludes that *Rhizobium* treatment is of little benefit because acacias are the easiest species to establish on waste rock dumps (Batterham, 1998). Many processes other than initial establishment, however, will govern long-term success of rehabilitated areas (Reddell and Milnes, 1992). Nitrogen deficiency in mine soils can be amended via the establishment of nitrogen fixing legumes and root nodule bacteria that is *Rhizobium*. Legumes are only effective if an association is formed with the appropriate strain of

Rhizobia (Bell, 2002). If local nitrogen fixing species are to be reintroduced to the mined area, their effective inoculation will be catalysed through the replacement of fresh surface soil or through the careful selection, collection and inoculation of the plants with the appropriate strains. The contribution of plant available nitrogen and of organic matter by Acacias to a re-establishing ecosystem through mechanisms such as litter cycling may rely on symbiosis with *Rhizobia* (Coleman et al., 1983).

Revegetation of the mine spoils is essential for conservation of environment, biodiversity and to make the land productive. But, the revegetation of these mine spoils is difficult because, they are deficient in nutrients such as nitrogen (N), phosphorus (P) and devoid of organic matter with adverse physico-chemical properties. Root symbionts such as *Rhizobium* and arbuscular mycorrhizal fungi (AMF) are also reduced or absent in mine sites (Noyd et al., 1995). High acidity in mine spoils due to oxidation of residual elemental or iron sulphur is also a constraint to revegetation, which hampers root-growth of plant and reduces the population of beneficial microorganisms such as free-living N-fixers (Alexander, 1964; Arminger et al., 1996; Barnishel, 1977; Choudhury, 1996). In the recent times, increased ecological awareness among researchers, have resulted in search for innovative approaches for revegetation of coal mine area in India and abroad (Dugaya et al., 1996; Gupta et al., 1994; Kumar and Jena, 1996; Pandya et al., 1997; Prasad and Mahammad, 1990; Sonkar et al., 1998). The use of native and indigenous plant species have been emphasized in revegetation programs with a view to maintain essential processes and life support system, preservation of genetic diversity and to ensure sustainable utilization of species and ecosystem (Banerjee et al., 1996). Plant species also emerge naturally on the barren mined land after certain intervals of time from the initiation of dump, but succession of plant species under such situation proceeds at a much slower rate (Bradshaw and Chadwick, 1980; Roberts et al., 1981). Therefore, it is essential to understand the structure and function of an ecosystem with its primary and secondary succession patterns for a successful revegetation programs. A study was conducted to determine the effect of unamended and variously amended tailings on the survival, growth and metal uptake of different plant species for the phytostabilization of Rajpura-Dariba mine tailings in Rajasthan, India. The species studied were: *A. auriculiformis*, *A. nilotica*, *Aegle marmelos*, *A. lebbeck*, *Bauhinia purpurea*, *Boswellia serrata*, *Caesalpinia bonduc*, *Cassia fistula*, *Casuarina equisetifolia*, *Dendrocalamus strictus*, *Eucalyptus citriodora*, *Lawsonia inermis*, *Parkinsonia aculeata*, *Peltophorum ferrugineum*, *Stylosanthes hamata*, *Withania somnifera*, *Ziziphus mauritiana*, *Vigna unguiculata*, *Triticum aestivum* and *Brassica campestris*.

The conditioning was carried out with inorganic fertilizer

(NPK) and farmyard manure and capping with 15% soil. It was found that *A. auriculiformis*, *L. inermis*, *E. citriodora*, *P. aculeata* and *W. somnifera* were the species that survived and grew in the inhospitable tailing surface though after suitable amelioration.

The results indicate that for successful phytoremediation of metal polluted sites, a strategy should be considered that combines rapid screening of plant species possessing the ability to tolerate and accumulate heavy metals with agronomic practices that enhance shoot biomass production and/or increase/decrease in metal bioavailability in the rhizosphere (Archana and Aery, 2005). Archer and Cladwell (2004) studied the response of six Australian plant species to heavy metal contamination at an abandoned mine site. This investigation was carried out to assess the potential suitability of certain Australian plants for use in the phytoremediation of derelict mine sites. A revegetation trial was conducted to evaluate the feasibility of growing a legume species, *Prosopis juliflora* L., on fly ash ameliorated with combination of various organic amendments, blue-green algal biofertilizer and *Rhizobium* inoculation. Plants accumulated higher amounts of Fe, Mn, Cu, Zn and Cr in various fly ash amendments than in garden soil. Further, inoculation of the plant with a fly ash tolerant *Rhizobium* strain conferred tolerance for the plant to grow under fly ash stress conditions with more translocation of metals to the above ground parts. The results showed the potential of *P. juliflora* to grow in plantations on fly ash landfills and to reduce the metal contents of fly ash by bioaccumulation in its tissues (Rai et al., 2004).

COMMERCIALLY AVAILABLE BIOFERTILIZER AND APPLICATION

Russian microbiologists who developed a product called phosphobakterin and tested it on various crops between 1940 to 1960 observed noticeable increases in yields and better phosphorus uptake in inoculated plants. Subsequently, several investigators in other countries have repeatedly attempted to test the value of seed inoculation with phosphate dissolving microorganisms on growth and yield of plants. Recently at I.A.R.I, a carrier based 'microphos' bio fertilizer containing *Pseudomonas striata* has been prepared and tested on crops (Poi, 2008; Mehrvarz and Chaichi, 2008). Application of liquid inoculants to seedlings was better than seed inoculation. Odee et al. (2002) recommend that seedlings raised in the nursery should be inoculated with a liquid inoculant immediately or soon after germination. Turan et al. (2006) evaluated the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. In this study, the capacity of phosphate solubilizing bacterial strain, *Bacillus* (FS3) and fungal isolates, *Aspergillus* FS9 and FS11 have been tested in

National Botanical Research Institutes. The result suggested that phosphate solubilizing bacteria FS3 and fungal strain FS9 have great potential for use bio-fertilizer development in agriculture. Several phosphate solubilizers, including bacteria, actinomycetes and fungi have been isolated. For the convenience of culturing, maintenance, preservation and manipulation bacteria are selected for preparation and use as biofertilizers (Fallah et al., 2007; Xiao et al., 2008). The preparation of the biofertiliser containing *Bacillus megaterium* var. *phosphatum* is known as phosphobacterin cultures obtained from USSR, FOSFO 24 a Czechoslovakian culture and an indigenous culture isolated from *Cassia accidentals*.

Laboratory experiments, trials and tests had to be carried out to get suitable end product that would ideally suit the different conditions of the soil.

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