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Amazing Multiple Function Properties of Plant Growth Promoting Rhizobacteria in the Rhizosphere Soil

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ABSTRACT

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Plant–bacterial interactions in the rhizosphere are the determinants of plant health and soil fertility. These are associated with the rhizosphere, which is an important soil ecological environment for plant–microbe interactions. 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 exact mechanisms by which PGPR promote plant growth are not fully understood, but are thought to include (i) the ability to produce or change the concentration of plant growth regulators like indoleacetic acid, gibberellic acid, cytokinins and ethylene (Arshad and Frankenberger, 1993; Glick, 1995), (ii) asymbiotic N₂ fixation (Boddey and Dobereiner, 1995), (iii) antagonism against phytopathogenic microorganisms by production of siderophores (Scher and Baker, 1982), antibiotics (Shanahan *et al.*, 1992) and cyanide (Flaishman *et al.*, 1996), (iv) solubilization of mineral phosphates and other nutrients (De-Freitas *et al.*, 1997; Gaur, 1990). Another mechanism by which PGPR can inhibit fungal cell wall degrading enzymes, e.g., chitinase and β -1,3-glucanase. Biological control of soil-borne plant pathogens and the synthesis of antibiotics have also been reported in several bacterial species. This review begins with describing how the bacteria help plant directly and indirectly to producing hydrolytic enzyme, hormone, cyanide and siderophore.

Introduction

The concept of plant growth promoting rhizobacteria is now well established, both for growth promotion and biocontrol. Plant growth promoting rhizobacteria (PGPR) were first defined by Kloepper and Schroth (1978) to describe soil bacteria that colonize the roots of plants following inoculation

onto seed and they enhance plant growth. The ineffectiveness of PGPR in the field has often attributed to their inability to colonize plant roots (Lugtenberg, *et al.*, 2001). Plant growth-promoting rhizobacteria (PGPR) colonizing the surface or inner part of roots play an important positive role that directly

or indirectly influences plant growth and development (Gerhardt, K. E et al, 2009; Glick, B. R., 1999).

The mechanism by which PGPR increases crop performance is not well understood. There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism; suppression of plant disease (termed “Bioprotectants”), phytohormone production (termed “Biostimulants”), or improved nutrient acquisition (termed “Biofertilizers”).

Biofertilizer is a material containing microorganisms added to a soil to directly or indirectly make certain essential elements available to plants for their nutrition through synthesis of growth promoting substances or by enhancing the decomposition of plant residues. Various sources of biofertilizers include nitrogen fixers, phytostimulators, phosphate solubilizing bacteria, plant growth promoting rhizobacteria etc. Considerable progress has been made over the past two decades in evaluation of these technologies and development of application methods (Afzal and Asghari, 2008).

Microorganisms are important for agriculture in order to promote the circulation of plant nutrients and reduce the need of chemical fertilisers. The use of fertilizers, including chemical fertilizers and manures, to enhance soil fertility and crop productivity has often negatively affected the complex system of the biogeochemical cycles (Perrott *et al.*, 1992; Steinshamn *et al.*, 2004). Fertilizer use has caused leaching and run-off of nutrients, especially Nitrogen (N) and Phosphorus (P), leading to environmental degradation (Tilman, 1998; Gyaneshwar *et al.*, 2002). Plant growth promoting rhizobacteria (PGPR) accounts for about 2-5% of total the rhizobacteria

involved in plant growth promotion (Antoun and Kloepper, 2001).

Plant Growth-Promoting Rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria (PGPR) offer an environment-friendly means for increasing productivity and sustainability in agriculture. Many bacterial species, mostly associated with plant rhizosphere, have been tested and found to be beneficial for plant growth, yield, and crop quality. They have been called “plant growth promoting rhizobacteria (PGPR)”. PGPR are also termed as plant health promoting rhizobacteria (PHPR) or nodule promoting rhizobacteria (NPR) and are associated with the rhizosphere, which is an important soil ecological environment for plant–microbe interactions (Burr and Caesar, 1984). Generally, PGPR function in three different ways (Glick, 1995, 2001): synthesizing particular compounds for the plants (Dobbelaere *et al.*, 2003; Zahir *et al.*, 2004), facilitating the uptake of certain nutrients from the soil (Lucas *et al.*, 2004; Çakmakçi *et al.*, 2006), and lessening or preventing the plants from diseases (Jetiyanon and Kloepper, 2002; Raj *et al.*, 2003; Guo *et al.*, 2004; Saravanakumar *et al.*, 2008). These bacterial species are in the genera *Serratia*, *Pseudomonas*, *Burkholderia*, *Agrobacterium*, *Erwinia*, *Xanthomonas*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Alcanigenes*, *Arthrobacter*, *Acetobacter*, *Acinetobacter*, *Achromobacter*, *Aerobacter*, *Artrobacter*, *Azotobacter*, *Clostridium*, *Klebsiella*, *Micrococcus*, *Rhodobacter*, *Rhodospirillum*, *Flavobacterium* (Rodriguez and Fraga, 1999; Bloemberg and Lugtenberg, 2001; Esitken *et al.*, 2003).

The common traits of growth promotion includes production or changes in the

concentration of plant hormones such as auxin, gibberellins, cytokinins and ethylene. Indole acetic acid (IAA) is one of the most physiologically active auxin. IAA released as secondary metabolite because of rich supplies of substrates exuded from the roots (Strzelczyk and Pokojska, 1984; Ahmad *et al.*, 2005) Microbial biosynthesis of IAA in soil is enhanced by tryptophan secreted from roots or decaying cells (Benezri *et al.*, 1998).

Phosphates and other nutrient are also solubilized by PGPR strains to increase the availability of 'P' for plants in soil with large amount of precipitated phosphates (Goldstein, 1986) and nitrogen fixation. These bacteria are also capable to suppress the growth of deleterious microorganisms by production of siderophores, β 1,3 glucanases, chitinases and antibiotics (Cattelan *et al.*, 1999). Siderophore producing bacteria promote plant growth indirectly by sequestering the limited iron in the rhizosphere and reduce the availability for growth of phytopathogens (Alexander and zeeberi, 1991). Plant growth benefits due to the addition of PGPR include increases in germination rate, root growth, yield, leaf area, chlorophyll content, nitrogen content, protein content, tolerance to drought, shoot and root weight, and delayed leaf senescence (Dobbelaere *et al.*, 2003; Cakmakci, 2005a, 2005b).

PGPR have been demonstrated to increase growth and productivity of many commercial crops including rice (Ashrafuzzaman *et al.*, 2009), wheat (Khalid *et al.*, 2004, Cakmakci *et al.*, 2007), cucumber (Maleki *et al.*, 2010), maize (Sandhya *et al.*, 2010), cotton (Anjum *et al.*, 2007), black pepper (Dastager *et al.*, 2010), and Bnana (Mia *et al.*, 2010) canola (de Freitas *et al.*, 1997), sugar beet (Cakmakci *et al.*, 1999), sugarcane (Sundara *et al.*, 2002),

conifer species (Bent *et al.*, 2002).

Significant increases in growth and yield of agronomically important crops in response to inoculation with PGPR have been reported many times (Kloepper *et al.*, 1980; Chen *et al.*, 1994; Zhang *et al.*, 1996; Amara and Dahdoh, 1997; Chanway, 1998; Pan *et al.*, 1999; Bin *et al.*, 2000; Biswas *et al.*, 2000; Asghar *et al.*, 2002; Vessey, 2003; Silva *et al.*, 2006). PGPR beneficial effects have been exploited in many areas including biofertilizers, microbial rhizoremediation and biopesticides (Adesemoye *et al.*, 2008)

Indirect Mechanisms

Indirect mechanisms used by PGPR include antibiotic protection against pathogenic bacteria, reduction of iron available to phytopathogens in the rhizosphere, synthesis of cell wall degrading enzymes, such as β -1, 3-glucanases, cellulases, proteases and chitinases are involved in the antagonistic activity of some biological control agents against phytopathogenic fungi (Chernin *et al.*, 1995; Dunn *et al.*, 1997; Ordentlich *et al.*, 1988; Harman *et al.*, 1993). Indirect growth promotion occurs through the elimination of pathogens by the production of cyanide (Owen and Zlor, 2001). PGPR have also been reported to be able to produce enzymes such as, lipase, by which they can lysis the cells of fungal pathogens (van Loon *et al.*, 2006). Dunne and collaborators (2000) showed that overproduction of extracellular protease in the mutant strains of *Stenotrophomonas maltophilia* W81 resulted in improved biocontrol of *Pythium ultimum*. PGPR also promote plant growth by suppressing growth of plant pathogens and deleterious rhizosphere microorganisms, thus freeing the plant from growth limitations that would have resulted because of the presence of these microorganisms

(Kloepper, 1992; Schippers *et al.*, 1987). These indirect mechanisms, such as suppression of harmful microorganisms and induced systemic resistance (ISR), are normally recognized as having a role in biological control (Kloepper, 1992; Dobbelaere *et al.*, 2003).

Direct Mechanisms

Direct promotion of growth by PGPR occurs when the rhizobacteria produce metabolites that promote plant growth such as auxins (Asghar *et al.*, 2002), cytokinins (Arkhipova *et al.*, 2005) and gibberellins (Gutierrez - Manero *et al.*, 2001; Joo *et al.*, 2004) as well as through the solubilization of phosphate minerals (Freitas *et al.*, 1997).

Plant growth promoting rhizobacteria and endophytes accelerates phytoremediation of metalliferous soils through modulation of (a) plant growth promoting parameters, (b) by providing plants with nutrients, and (c) controlling disease through the production of antifungal metabolites. Abbreviations: indole-3-acetic acid (IAA), indole-3-acetamide (IAM) pathway, indole-3-pyruvate (IPyA) pathway, methionine-S-adenosylmethionine (SAM), 1-aminocyclopropane-1-carboxylate (ACC), 1-aminocyclopropane-1-carboxylate synthase (ACS), phosphatase (Ptase), ammonia (NH₃), hydrogen cyanide (HCN). (Maa *et al.*, 2011)

Siderophore (Greek- literally means iron carrier)

Iron (Fe³⁺) is biologically important being a constituent of cytochrome and others heme or non-heme proteins and also a co-factor in various enzymes. When aerobic or facultative anaerobic microorganisms grow in an iron-deficient environment, they synthesize Fe³⁺ ion specific chelating

agents called siderophores (Goto, 1990). Siderophores are low molecular weight (500–1000 Da) compounds produced by fungi and bacteria, which bind with Fe³⁺ ions to be transported into the cell (Neilands, 1989). Based on their structure, the majority of the known siderophores have been grouped either as catecholates, produced only by bacteria, or as hydroxymates which are produced by fungi and bacteria.

Siderophore as Biocontrol

Siderophores, low molecular weight compounds with high iron affinity, are produced by some microorganisms (also by most biocontrol agents) to solubilize and competitively acquire ferric ion under iron-limiting conditions, thereby making iron unavailable to other soil microorganisms which cannot grow for lack of it (Haas *et al.*, 2005; Loper *et al.*, 1997). The bacterium that originally synthesized the siderophores takes up the iron siderophore complex by using a receptor that is specific to the complex and is located in the outer cell membrane. Suppression of soil borne plant pathogens by siderophore producing *Pseudomonads* has been reported in some instances (Buysens *et al.*, 1996; Loper, 1988; Weger *et al.*, 1988).

Consequently, to survive in such environments, organisms secrete iron-binding ligands (siderophores) that can bind ferric iron and make it available to the host microorganisms. Although various bacterial siderophores differ in their abilities to sequester iron, in general, they deprive pathogenic fungi of this essential element since the fungal siderophores have lower affinity (Loper and Henkels, 1999; O'Sullivan and O'Gara, 1992).

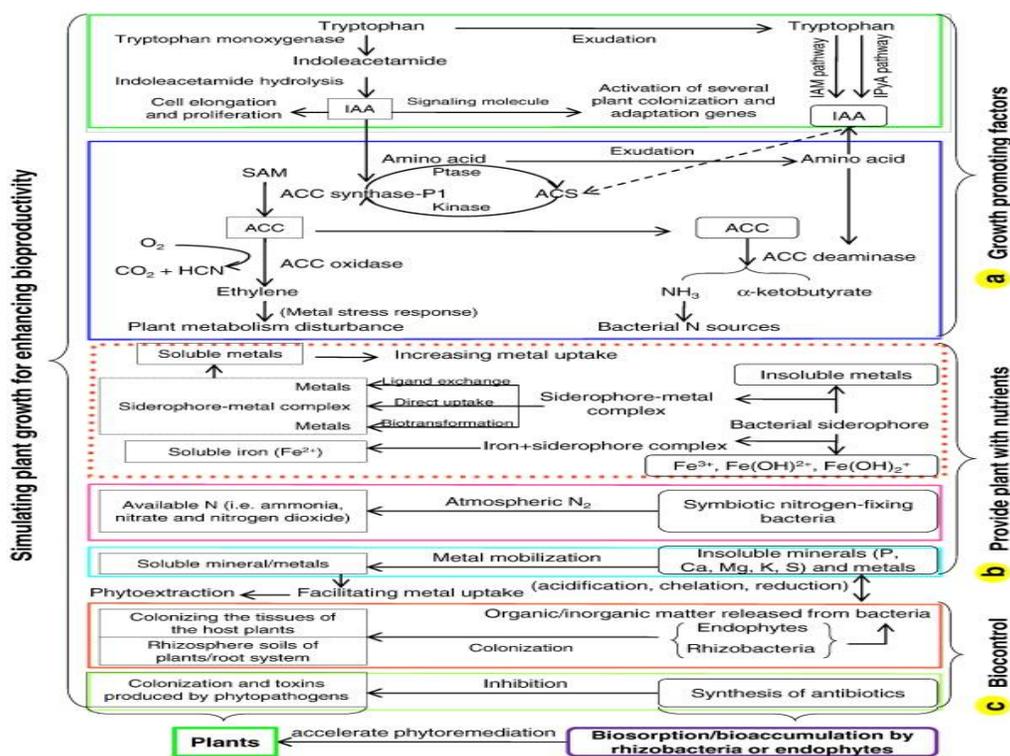
The production of siderophores by the biocontrol agents in quantities sufficient may to limited Fe³⁺ availability to the

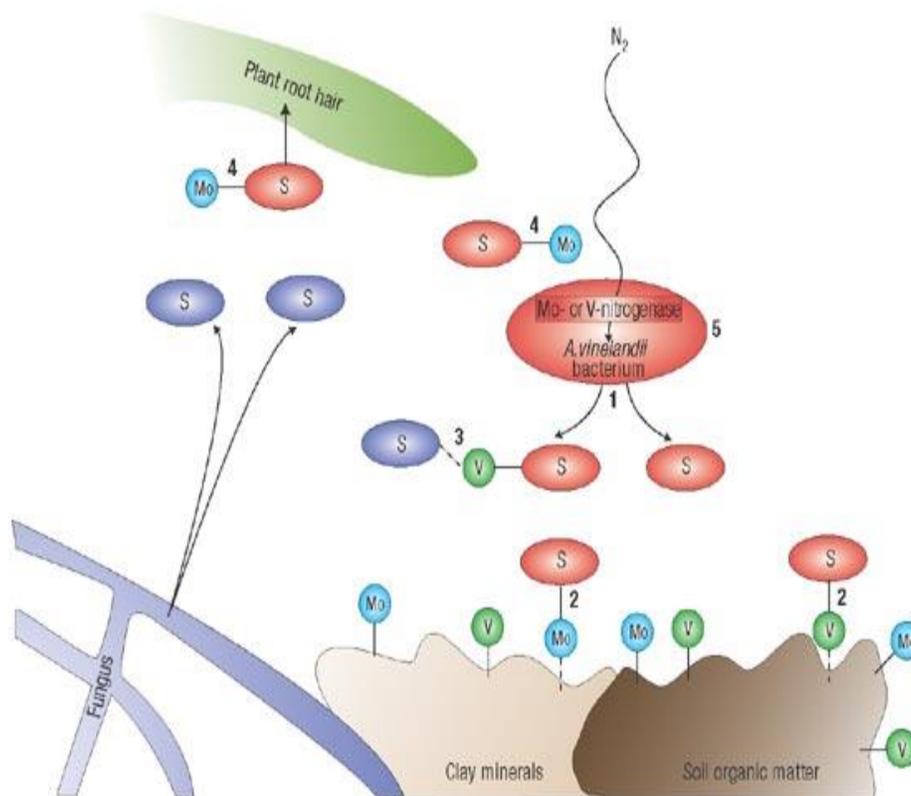
pathogen (Glick and Bashan, 1997) and is possible lead to induction of host resistance against the pathogen (Meziane *et al.*, 2005). Siderophore production is very common among *Pseudomonads* (O'Sullivan and O'Gara, 1992) *Frankia* (Boyer *et al.*, 1999) and *Streptomyces* sp. (Loper and Buyer, 1991). *Escherichia coli*, *Salmonella typhimurium* (Martinez *et al.*, 1990), *Actinobacillus pleuropneumoniae* (Diarra *et al.*, 1996), *Streptomyces* sp. (Imbert *et al.*, 1995), and *Arthrobacter flavescens* (Winkelmann, 1991) have also been shown to produce iron-chelating compounds. Siderophores are not only used in the process of plants acquiring iron and other metals, they also are found on bacteria in the body on cells and in some cases the siderophores rob our own blood cells of iron. *Escherichia coli* strains have been used in CAS assays to demonstrate the production of siderophores (Schwyn and Neilands, 1987). Production of siderophore and antifungal activity were simultaneously

exhibited by free-living rhizospheric isolates of *Azotobacter* (16.22%), fluorescent *Pseudomonas* (11.11%) and *Bacillus* (10%) (Ahmad *et al.*, 2008).

Under metal-limiting conditions, the bacterium *A. vinelandii* secretes metal-scavenging compounds (siderophores; S) (1). These siderophores scavenge the metals molybdenum and vanadium from unavailable complexes with clay, soil organic matter or other elements (2). The siderophores compete with siderophores produced by other organisms such as fungi for these metals (3).

The bacterium or plant roots readily take up the siderophore-metal complexes (4). Within the bacterium, the metal is incorporated into the enzyme nitrogenase (5), to allow the fixation of atmospheric nitrogen (N₂) that would otherwise be unusable to the bacterium. (Alexander and Zuberer, 1991).





Siderophore as Micro nutrient Provider

Plants are able to use bacterial iron siderophore complexes as a source of iron from the soil (Neilands, 1993; Wang *et al.*, 1993; Riquelme, 1996). The role of siderophores is to scavenge iron from the environment and make the mineral available to the cell (Neilands, 1995).

Poaceae (grasses) including agriculturally important species such as barley and wheat are able to efficiently sequester iron by releasing phytosiderophores via their root into the surrounding soil rhizosphere (Kraemer *et al.*, 2001). Thus, these plants are unable to uptake sufficient amounts of iron. Further, heavy metals that are accumulated in excess in plant tissues can cause changes in various vital growth processes and have negative effects on iron nutrition. Under such conditions, the siderophore producing rhizosphere bacteria might offer a biological rescue system that is

capable of chelating Fe³⁺ and making it available to plant roots (Fig. 2). The roots could then take up iron from siderophores–Fe complexes possibly via the mechanisms such as chelate degradation and release of iron, the direct uptake of siderophore–Fe complexes, and/or a ligand exchange reaction (Rajkumar *et al.*, 2010).

Several examples of increased Fe²⁺ uptake in plants with concurrent stimulation of plant growth as a result of PGPB inoculations have been reported (Burd *et al.*, 2000; Barzanti *et al.*, 2007; Carrillo-Castañeda *et al.*, 2003). Siderophores also promote bacterial IAA synthesis by reducing the detrimental effects of heavy metals through chelation reaction (Dimkpa *et al.*, 2008).

Chemical compounds produced by microorganisms in the rhizosphere can also increase the availability and uptake of iron. Plants such as oats are able to assimilate iron

via these microbial siderophores. It has been demonstrated that plants are able to use the hydroxamate-type siderophores ferrichrome, rodotorulic acid and ferrioxamine B; the catechol-type siderophores, agrobactin; and the mixed ligand catechol-hydroxamate-hydroxy acid siderophores biosynthesized by saprophytic root-colonizing bacteria. All of these compounds are produced by rhizospheric bacterial strains, which have simple nutritional requirements, and are found in nature in soils, foliage, fresh water, sediments, and seawater (Carrillo-Castañeda *et al.*, 2002). Several studies have demonstrated that production of siderophore by PGPR was most effective in controlling the plant root pathogens (Mullen, 1998; Diaz *et al.*, 2002; and Dey *et al.*, 2004). *B. subtilis*, *B. amyloliquefaciens*, and *B. pumilus* have a background of being biological control agents against diverse soil pathogens (El-Hassan and Gowen, 2006; Liu *et al.*, 2008; Szczech and Shoda, 2006; Yu *et al.*, 2002;).

Siderophore production enables bacteria to compete with pathogens by removing iron from the environment (O' Sullivan and O'Gara 1992; Persello-Cartieaux *et al.* 2003). Although various bacterial siderophores differ in their abilities to sequester iron, in general, they deprive pathogenic fungi of this essential element since the fungal siderophores have lower affinity (Loper, and Henkels. 1999; O'Sullivan, and Gara. 1992). *E. coli* isolated and characterized from endorhizosphere of sugarcane (*Saccharum* sp.) and rye grass (*Lolium perenne*) is found to produce maximum siderophores and thus is found to help in the growth of the plants (Gangwar, et al 2009).

Phosphate Solubilizing Bacteria (PSB)

Phosphorus is second only to nitrogen in

mineral nutrients most commonly limiting the growth of terrestrial plants. Phosphorus (P) is one of the major essential macronutrients for plants and is applied to soil in the form of phosphatic fertilizers.

However, a large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly and becomes unavailable to plants (Goldstein, 1986) Phosphate solubilizing bacteria (PSB) are the group of common PGPR in rhizosphere. Secretion of organic acids and phosphatases to solubilize insoluble phosphate to soluble forms are common in this group (Kim *et al.*, 1998).

Although several phosphate solubilizing bacteria occur in soil, their numbers are not adequate to compete with other bacteria commonly established in the rhizosphere (Glick *et al.*, 1995) Moreover, the population of inorganic P-solubilizing microorganism is very low, less than 10²cfu g⁻¹of soil. Therefore the number of PSM is more important in the rhizosphere than in non -rhizosphere soil (Kucey *et al.*, 1989). Microorganisms are involved in a range of processes that affect the transformation of soil P and are thus an integral part of the soil P cycle..

In particular, soil microorganisms are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization (Hilda and Fraga, 1999). Currently, the main purpose in managing soil phosphorus is to optimize crop production and minimize P loss from soils. Recently, phosphate solubilizing microorganisms have attracted the attention of agriculturists as soil inoculums to improve the plant growth and yield (Young, 1994; Young *et al.*, 1998; Goldstein *et al.*, 1999; Fasim *et al.*, 2002).

Phosphate-solubilizing bacteria are common in rhizospheres (Nautiyal *et al.*, 2000; Vazquez *et al.*, 2000b). Bacterial strains belonging to genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aerobacter*, *Flavobacterium* and *Erwinia* have the ability to solubilize insoluble inorganic phosphate (mineral phosphate) compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyl apatite and rock phosphate (Goldstein, 1986; Rodríguez and Fraga, 1999; Rodríguez *et al.*, 2006). Strains from genera *Pseudomonas*, *Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers, while tricalcium phosphate and hydroxyl apatite seem to be more degradable substrates than rock phosphate (Arora and Gaur, 1979; Banerjee *et al.*, 2006; Halder and Chakrabarty, 1993; Illmer and Schinner, 1992; Rodríguez and Fraga, 1999;).

Four strains, namely *Arthrobacter ureafaciens*, *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis* and *Delftia sp.* have been reported for the first time by (Chen *et al.*, 2006) as phosphate-solubilizing bacteria (PSB) after confirming their capacity to solubilize considerable amounts of tricalcium phosphate in the medium by secreting organic acids. Bacterial strains *Azotobacter vinelandii* and *Bacillus cereus* when tested *in vitro* are found to solubilise Phosphate and thus help in the growth of plant (Husen, 2003). *Bacillus megaterium* from tea rhizosphere is able to solubilize phosphate and thus it helps in the plant growth promotion (Chakraborty, 2006). Solubilisation of insoluble phosphorous compounds in the rhizosphere by microorganisms is another important means of achieving plant growth promotion (Gull *et al.*, 2004). The most efficient Phosphate Solubilising Microorganisms belong to genera *Bacillus*, *Rhizobium* and

Pseudomonas amongst bacteria, and *Aspergillus* and *Penicillium* amongst fungi. Within rhizobia, two species nodulating chickpea, *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum*, are known as good phosphate solubilizers (Rivas, 2006).

Hydrolytic Enzymes

A variety of mechanisms have been reported to contribute to the biocontrol activity of microbes and it is, for example, known that cell wall degrading enzymes, such as β -1,3-glucanases, cellulases, proteases and chitinases are involved in the antagonistic activity of some biological control agents against phytopathogenic fungi (Chernin *et al.*, 1995; Dunn *et al.*, 1997; Harman *et al.*, 1993; Ordentlich *et al.*, 1988).

Bacterial strains that produce different hydrolytic enzymes such protease, lipase, pectinase, amylase also inhibited the growth of pathogenic fungi *F. culmorum* and *F. oxysporum*. Nielson and Sorensen (1999) demonstrated that *P. fluorescens* antagonistic to *R. solani* and *Pythium ultimum*, and produced lytic enzymes. Understanding the mode of action of biocontrol agents is a prerequisite for: (i) developing rational procedures in order to select more effective antagonistic microbial strains, (ii) developing appropriate production and formulation methods that enhance biocontrol activity, and (iii) fulfilling some requirements of the toxicological and registration packages needed for commercial development (Jijakli and Lepoivre, 1998).

Biological control of plant diseases can be attained through reduction of inoculum quantity or disease causing capacity of a pathogen with use of one or more organisms except man (Cook and Baker, 1983). A microorganism can exert antagonism

towards a plant pathogen directly by producing substances acting directly on one or more stages of the life cycle of the pathogen (Cook and Baker, 1983) or indirectly by activating mechanisms of host resistance towards the pathogen (Van Loon *et al.*, 1998). The growth inhibition noticed in the volatile compound assay may be attributed to cyanogenesis from glycine, resulting in the production of HCN, which is volatile in nature and plays a key role in the inhibition of phytopathogenic fungi namely *Sclerotium rolfsii*, *Rhizoctonia solani* and *Pythium* sp. under *in vitro* conditions (Bakker and Schipper, 1987).

Indole Acetic Acid

Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, and *Rhizobium* as well as *Alcaligenes faecalis*, *Enterobacter cloacae*, *Acetobacter diazotrophicus* and *radyrhizobium japonicum* have been shown to produce auxins which help in stimulating plant growth (Patten and Glick, 1996). IAA is the most common and best characterized phytohormone. It has been estimated that 80% of bacteria isolated from the rhizosphere can produce plant growth regulator IAA (Patten and Glick, 1996). Indole acetic acid (IAA) is one of the most physiologically active auxins. Auxins are produced by plants (Arshad and Frankenberger, 1991) and several microorganisms including bacteria (Barea *et al.*, 1976) and fungi (Dvornikova *et al.*, 1970).

Indole-3-acetic acid (IAA) is a common product of L-tryptophan metabolism by several microorganisms including PGPR (Frankenberger and Brunner, 1983; Lynch, 1985). Microorganisms inhabiting rhizospheres of various plants are likely to synthesize and release auxin as secondary metabolites because of the rich supplies of

substrates exuded from the roots compared with non rhizospheric soils (Kampert *et al.*, 1975; Strzelczyk and Pokojaska-Burdziej, 1984). Plant morphogenic effects may also be a result of different ratios of plant hormones produced by roots as well as by rhizosphere bacteria (Muller *et al.*, 1989).

IAA is the main auxin in plants, controlling many important physiological processes including cell enlargement and division, tissue differentiation, and responses to light and gravity. Bacterial IAA producers (BIPs) have the potential to interfere with any of these processes by input of IAA into the plant's auxin pool. The consequence for the plant is usually a function of the amount of IAA that is produced. A root, for instance, is one of the plant's organs that is, most sensitive to fluctuations in IAA and its response to increasing amounts of exogenous IAA extends from elongation of the primary root, formation of lateral and adventitious roots, (Finnie and Van Staden, 1985). It is now generally agreed that indole-3-acetic acid (IAA) is the major and most abundant auxin in plants.

IAA plays a key role in the regulation of plant growth and development (Moore, 1989; Luthen *et al.*, 1999; Davies, 1995). Over the last few years significant progress has been made in understanding the IAA-induced signal transduction pathway (Shahab and Ahmed, 2008; Venis and Napier, 1991). Although other auxins, such as indole-3-acetic acid) indole 3 butyric acid (IBA) and phenyl acetic acid (PAA) have also been identified in plants (Normanly, 1997) little is known about their physiological function. It is presumed that PGPR producing plant growth regulators play a critical role in plant growth promotion. Several studies have demonstrated the potential of rhizobacteria to synthesize auxins *in vitro* (Arshad and

Frankenberger, 1993; and Benizri *et al.*, 1998). Asghar *et al.*, (2002) and Khalid *et al.*, (2004) reported that addition of L-tryptophan (L-TRP) as an auxin precursor substantially increased auxin production.

The tryptophan increases the production of IAA in *Bacillus amyloliquefaciens* FZB42 (Idris *et al.*, 2007). Tien *et al.* (1979) showed that *Azospirillum* is able to produce auxins when exposed to tryptophan. Karnwal (2009) tested *Fluorescent Pseudomonas* isolates for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan and found that for both strains, indole production increased with increases in tryptophan concentration. Yasari and Patwardhan (2007) reported that application of *Azotobacter* and *Azospirillum* strains increased canola yield (21.17%), pod per plant (16.05%), number of branches (11.78%) and weight of 1000grain (2.92%).

Hydrogen Cyanide

The HCN production is found to be a common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) in the rhizospheric soil and plant root nodules (Ahmad *et al.*, 2008; Charest *et al.*, 2005). Cyanide is a secondary metabolite produced by gram negative bacteria (Askeland and Morrison, 1983). Hydrogen cyanide (HCN) and CO₂ are formed from glycine and catalyzed by HCN synthase (Castric, 1994).

HCN productions by strains of *Pseudomonas* suppress diseases while mutant strain defective in synthesis of HCN lost the ability to protect plants from diseases (Voisard *et al.*, 1989; Sacherer *et al.*, 1994). Hydrogen cyanide (HCN) has been demonstrated in a small number of bacterial species, such as *Pseudomonas aeruginosa*, *Pseudomonas asuorescens* and *Chromobacterium violaceum* (Askeland and

Morrison, 1983; Castric, 1975; Knowles and Bunch, 1986).

Ecological role for bacterial cyanogenesis has been discovered in the case of the root-colonizing and plant-beneficial *P. fluorescens* strain CHA0 (Voisard *et al.*, 1989), which protects several plants from fungal root diseases (Voisard *et al.*, 1994; Schnider *et al.*, 1995). HCN production by strain CHA0 accounts for part of the strain's biocontrol capacity, for example the suppression of tobacco black root rot caused by *Thielaviopsis basicola* (Laville *et al.*, 1998; Voisard *et al.*, 1989). Iron sufficiency is important for both HCN production and disease suppression (Keel *et al.*, 1989; Voisard *et al.*, 1989).

PGPR as Biocontrol

Fungicides were the essential trial as seed treatment for controlling damping-off and root rot diseases for a long time. However, fungicidal treatments cause hazards to human health and increase environmental pollution. Therefore there are needed to alternative fungicidal. Pesticides and fertilizers cause damage to the environment and effect human health (Perkins and Patterson, 1997). As a consequence, there is a trend toward finding ways to minimize the use of fungicides (Maas and Galletta, 1997) seed treatments.

Biological control of crops diseases and pests using microbial inoculants is being increasingly recognized as a viable, eco-friendly alternative that limits the massive use of synthetic chemical pesticides (Charan *et al.*, 2011). Plant-associated bacteria can be used directly for biological control of soil borne plant pathogens or indirectly for the productions of active substances (e.g., antibiotics, hydrolytic enzymes, osmoprotective substances (Berg, 2002).

The antagonistic mechanisms towards fungal pathogens in vitro include their glucanolytic; chitinolytic, cellulolytic, proteolytic and pectinolytic activity (Berg, 2003).

The use of biocontrol agents (Elad and Shtienberg, 1996) and alternative treatments (e.g., cultural practices, cover crops, and organic amendments) are perceived to be less harmful than conventional fungicides and may be an alternative in controlling plant diseases (Cutler and Hill, 1994). Numerous researches have been focused on searching and selecting antagonist microorganisms on diverse soil pathogens. Among the most used are bacteria's like *Bacillus*, *Pseudomonas*, and *Streptomyces*, fungi of the *Trichoderma*, *Penicillium*, *Gliocladium*, *Aspergillus*, *Rhizopus* genera. These microorganisms, natural inhabitants of diverse substrates, in laboratory tests (in vitro) as well as in the greenhouse and field, have demonstrated antagonistic activity on a wide ranging group of pathogens such as *Sclerotium rolfsii*, *S. cepivorum*, *Rhizoctonia solani*, *Pythium ultimum*, *Phytophthora parasitica*, and *M. phaseolina* (Adekunle *et al.*, 2001; Bell *et al.*, 1982; Balasundaram and Sarbhoy, 1988; Harrison and Stewart, 1988; Hussain *et al.*, 1990; Singh *et al.*, 2008).

Application of biological control using antagonistic microorganisms against seed and root rot pathogens proved to be successfully and its efficiency in controlling many diseases and improving vegetative growth and yield quality of many crops was recorded (Adams, 1990; Farzana and Ghaffar, 1991; Callan *et al.*, 1997). Coating seeds of many crops with bio control agents such *Trichoderma* spp., *Bacillus subtilis*, *Pseudomonas florocense* were the most effective treatments for controlling many seed and soil borne pathogens (Adams, 1990; Abd -Kareem, 2002; Harman *et al.*,

1989; Lacicowa and Pieta, 1996; Ragab *et al.*, 1999).

However, biological seed treatments may not provide adequate seed protection under all condition as bio-protection may be fail to establish on seed or in the rhizosphere at sufficient level for disease control (Baird *et al.*, 1994; Osburn and Scharoth, 1989;). *Bacillus* spp. is one of the biological control agents that has shown inhibitory effects against a considerable number of plant pathogens, and the antibiotics that it produces are generally assumed to be responsible for the control activity (Helbig *et al.*, 1998; Krebs *et al.*, 1998). Some authors have suggested that the use of antimicrobially active species and strains of the genus *Bacillus*, or the use of their metabolites, may be an alternative or supplementary method to chemical plant protection (Berger *et al.*, 1996; Handelsman *et al.*, 1990; Klich *et al.*, 1994; Sharga and Lyon, 1998). Many of these bacilli are generally soil-inhabiting bacteria or exist as epiphytes and endophytes in the spermosphere (Walker *et al.*, 1998) and rhizosphere (Handelsman *et al.*, 1990; Kajimura *et al.*, 1995; McKeen *et al.*, 1986). For this reason, *Bacillus* species are ideal candidates for use as biocontrol agents in seed treatment programs against soil-borne pathogens (Walker *et al.*, 1998).

B. subtilis, *B. amyloliquefaciens*, and *B. pumilus* have a background of being biological control agents against diverse soil pathogens (El-Hassan and Gowen, 2006; Liu *et al.*, 2008; Szczech and Shoda, 2006; Yu *et al.*, 2002;).

The effects of *Pseudomonas* spp. in plant growth promotion have been observed (Lemanceau, 1992). The beneficial effects of these bacteria have been attributed to their ability to promote plant growth and to protect the plant against pathogenic micro

organisms. The fluorescent *Pseudomonads* have been applied successfully to suppress *Fusarium* wilts of various plant species (Lemanceau and Alabouvette, 1993). Moreover, various secondary metabolites secreted by *Pseudomonas* spp., including HCN and siderophores, have been found to be inhibitory against different phytopathogens (Bagnasco *et al.*, 1998; Siddiqui, 2006).

Enhancement of plant-pathogen biological control agents may improve alternative measures instead of chemical measures. For example, several attempts have been made to use the biocontrol approach against severe diseases of crop plants. Several studies have reported the antagonistic effects of *Streptomyces* spp. on plant pathogens, for example: *S. rochei* on Phytophthora root rot of pepper (Ezziyyani *et al.*, 2007); *S. griseoviridis* on *Fusarium* root rot and wilt of tomato (Minuto *et al.*, 2006); *S. platensis* on *R. solani* leaf blight/seedling blight of rice (Wan *et al.*, 2008); *S. hygroscopicus* on *Colletotrichum gloeosporioides* anthracnose of several crops (Prapagdee *et al.*, 2008).

fluorescent Pseudomonas has been suggested as potential biological control agent due to its ability to colonize rhizosphere and protect plants against a wide range of important agronomic fungal diseases such as black root-rot of tobacco (Voisard *et al.*, 1989), root-rot of pea (Papavizas and Ayers, 1974), root-rot of wheat (Garagulia, 1974), damping-off of sugar beet (Fenton *et al.*, 1992; Shanahan *et al.*, 1992; Kumar *et al.*, 2002) and as the prospects of genetically manipulating the producer organisms to improve the efficacy of these biocontrol agents (Dowling and Gara, 1994).

Several reports have shown the potential of *Pseudomonas* species as BCAs for

controlling plant and fruit diseases (Botelho and Hagler, 2006; Jayaraj *et al.*, 2007; Okubara *et al.*, 2004; Trivedi *et al.*, 2008; Walsh *et al.*, 2001;).

In conclusion, chemical fertilization is a very common method of providing plants with their necessary nutrients because of its rapid effects on plant growth and yield production. However, there are important issues regarding the use of chemical fertilizers, as their improper and excess use can adversely affect the environment. Accordingly, it is important to indicate the contribution of chemical and biological fertilization to the plant growth. PGPR are likely to be of great interest in sustainable crop protection and growth promotion. However, the effective use of these rhizobacteria sometimes fails because PGPR are unable to recolonize the rhizosphere of the inoculated plants. Moreover, the soil persistence of bacteria may be influenced by a number of biotic and abiotic factors. It has also been reported that PGPR isolated from native rhizosphere are more effective in growth enhancement and crop protection than other strains because of better adaptability of bacterial strains. A promising strategy for the replacement and/or supplement of chemicals is the implementation of Biocontrol technology, used individually or as an integrated control component (Akrami *et al.*, 2009).

Application of PGP Bacteria in organic farming can be effectively used to increase the efficiency of fertilizer similar to mineral fertilizer. In view of environmental pollution in the case of excessive use of mineral fertilizers and high production costs of N and P fertilizers, bacteria tested in our study may suited alone or in combination to achieve sustainable and ecologically safe agricultural production in the Andaman region.

Plant pathogens include fungi are the most visible threats to sustainable food production. The decreasing efficacy of the fungicides as well as risks associated with fungicide residues on the leaves and fruit, have highlighted the need for a more effective and safer alternative control measures. In recent years, biological control of plant pathogens has received increasing attention as a promising supplement or alternative to chemical control.

Biological control of plant disease is defined as "The involvement of the use of beneficial microorganisms, such as specialized fungi or yeast or bacteria to attack and control the plant pathogens (i.e., fungi, bacteria, nematodes or weeds) and the diseases they are causing (Cook, 1994 and Fravel, 2005). Biological control is a potent means of reducing the damage caused by plant pathogens (Jeyarajan and Nakkeeran, 2000 and Haggag; Wafaa, 2002). A promising strategy for the replacement and/or supplement of chemicals is the implementation of Biocontrol technology, used individually or as an integrated control component (Akrami *et al.*, 2009).

The appropriate use of fertilization, which is a combination of chemical and biological fertilization, can very much contribute to the enhanced food production in the world, while economically and environmentally recommendable. These strains could be useful in the formulation of new inoculants, improving the cropping systems into which it can be most profitably applied.

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