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Review

Biocontrol potentiality of plant growth promoting bacteria (PGPR) - *Pseudomonas fluorescens* and *Bacillus subtilis*: A review

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Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere. The rhizosphere concept was first introduced by Hiltner to describe the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities. A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates creating a very selective environment where diversity is low. Since, bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization. The present review deals with the following topics: Plant growth promoting rhizobacteria (PGPR), occurrence of PGPR, nitrogen fixation by PGPR, *Bacillus* species, *Pseudomonas* species, Production of plant growth promoting substances by PGPR isolates, PGPR as biocontrol agent and antagonistic activity of PGPR isolates against phytopathogens.

Key words: Biocontrol, plant growth promoting rhizobacteria (PGPR), *Pseudomonas fluorescens*, *Bacillus subtilis*.

INTRODUCTION

Agriculture is heavily dependent on the use of chemical fertilizers and pesticides to achieve higher yields. This dependence is associated with problems such as environmental pollution, health hazards, interruption of natural ecological nutrient cycling and destruction of biological communities that otherwise support crop production. Hence, crop production and pest and disease management have to be achieved in shorter intervals of time with fewer detrimental inputs. The use of bioresource to replace chemical fertilizers and pesticides is growing. In this context, plant growth promoting microorganisms are often novel and potential tools to provide substantial benefits to agriculture (Sivasakthi et al., 2013).

Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism

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which is known as rhizosphere. The rhizosphere concept was first introduced by Hiltner to describe the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities. The original concept has now been extended to include the soil surrounding a root in which physical, chemical and biological properties have been changed by root growth and activity (McCully, 2005; Sivasakthivelan and Saranraj, 2013).

A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates creating a very selective environment where diversity is low (Gracia et al., 2011). Since, bacteria are the most abundant microorganisms in the rhizosphere; it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization (Barriuso et al., 2008).

Plant growth promoting rhizobacteria (PGPR) are free - living, soil - borne bacteria, which enhance the growth of the plant either directly or indirectly (Kloepper et al., 1980; Glick and Ibid, 1995). The direct mechanisms involve nitrogen fixation, phosphorus solubilization, HCN production, production of phytohormones such as auxins, cytokinins and gibberellins and lowering of ethylene concentration (Glick and Ibid, 1995; Glick et al., 1999). Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas* and *Rhizobium* as well as *Alcaligenes faecalis*, *Enterobactercloacae*, *Acetobacter diazotrophicus* and *Bradyrhizobium japonicum* have been shown to produce auxins which help in stimulating plant growth (Patten and Glick, 2002).

There are many reports on plant growth promotion and yield enhancement by plant growth promoting rhizobacteria (PGPR) (Lugtenberg et al., 2001). The mechanisms of plant growth promotion by PGPR include: the ability to produce phytohormones, N_2 fixation, antagonism against phytopathogens and solubilization of insoluble phosphates (Lugtenberg and Kamilova, 2009). It was also suggested that PGPR can also prevent the deleterious effects of stresses from the environment (Paul and Nair, 2008).

Bacteria associated with plants can be either harmful or beneficial plant growth promoting rhizobacteria (PGPR) may promote growth directly, by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron or production of plant growth regulators, phytohormones (Kloepper, 1997). Some bacteria support plant growth indirectly by improving growth restricting conditions either *via* production of antagonistic substances or by inducing host resistance towards plant pathogens. Since, associative interactions of plant and microorganisms must have come into existence as a result of convolution; the use of either former or latter groups as bioinoculants forms one of the vital components for a long - term sustainable agriculture system (Tilak et al., 2005; Usharani et al., 2013).

Rhizospheric bacterial communities have efficient systems for uptake and catabolism of organic compounds present in root exudates (Barraquio et al., 2000). Several bacteria have the ability to attach to the root surfaces (rhizoplane) making them to derive maximum benefit from root exudates. Few of them are more specialized, as they possess the ability to penetrate inside the root tissues (endophytes) and have direct access to organic compounds present in the apoplast. By occupying this privileged endophytic location, bacteria do not have to face competition from their counterparts as encountered in the rhizosphere or in soil (Kanchana et al., 2013a, b).

The use of PGPR offers an attractive way to replace chemical fertilizer, pesticides and supplements; most of the isolates result in a significant increase in plant height, root length and dry matter production of shoot and root of plants. PGPR help in the disease control in plants. Some PGPR, especially if they are inoculated on the seed before planting, are able to establish themselves on the crop roots. PGPR as a component in integrated management systems in which reduced rates of agrochemicals and cultural control practices are used as biocontrol agents. Such an integrated system could be used for transplanted vegetables to produce more vigorous transplants that would be tolerant to nematodes and other diseases for at least a few weeks after transplanting to the field (Kloepper et al., 2004).

PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large active groups of bacteria known as plant growth promoting rhizobacteria (PGPR). Plant growth promoting rhizobacteria are known to rapidly colonize the rhizosphere and suppress soil borne pathogens at the root surface (Rangarajan *et al*., 2003). These organisms can also be beneficial to the plant by stimulating growth (Bloemberg and Lugtenberg, 2001). Among these organisms, Fluorescent *Pseudomonas* are considered to be the most promising group of plant growth promoting rhizobacteria involved in biocontrol of plant diseases. They produce secondary metabolites such as antibiotics, phytohormones, volatile compound hydrogen cyanide and siderophores. Plant growth promoting ability of these bacteria is mainly because of the production of indole – 3 - acetic acid, siderophores and antibiotics.

The genera of PGPR include *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia*, *Bacillus, Paenibacillus* and some are members of the Enterobacteriaceae. Direct use of microorganisms to

promote plant growth and to control plant pests continues to be an area of rapidly expanding research. Rhizosphere colonization is one of the first steps in the pathogenesis of soil borne microorganisms. It is also crucial for the microbial inoculants used as biofertilizers, biocontrol agents, phytostimulators and bioremediators. *Pseudomonas* spp. is often used as model root colonizing bacteria (Lugtenberg et al., 2001).

The beneficial effects of these rhizobacteria have been variously attributed to their ability to produce various compounds including phytohormones, organic acids, siderophores, fixation of atmospheric nitrogen, phosphate solubilization, antibiotics and some other unidentified mechanisms (Glick and Ibid, 1995). Motile rhizobacteria may colonize the rhizosphere more profusely than the non - motile organisms resulting in better rhizosphere activity and nutrient transformation. They also eliminate deleterious rhizobacteria from the rhizosphere by niche exclusion thereby better plant growth. Induced systemic resistance has been reported to be one of the mechanisms by which PGPR control plant diseases through the manipulation of the host plant's physical and biochemical properties.

The recognition of plant growth promoting rhizobacteria (PGPR), a group of beneficial plant bacteria, as potentially useful for stimulating plant growth and increasing crop yields has evolved over the past several years to where today researchers are able to repeatedly use them successfully in field experiments. Increased growth and yields of potato, sugar beet, radish and sweet potato have been reported. Commercial applications of PGPR are being tested and are frequently successful. However, a better understanding of the microbial interactions that result in plant growth increases will greatly increase the success rate of field applications (Farzana et al., 2009).

PGPR, root - colonizing bacteria are known to influence plant growth by various direct or indirect mechanisms. Several chemical changes in soil are associated with PGPR. Plant growth promoting bacteria (PGPB) are reported to influence the growth, yield and nutrient uptake by an array of mechanisms. Some bacterial strains directly regulate plant physiology by mimicking synthesis of plant hormones, whereas others increase mineral and nitrogen availability in the soil as a way to augment growth.

OCCURRENCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

The rhizosphere is defined as the zone of soil in which the micro flora is influenced by the root (Hiltner, 1904). The mechanism by which PGPR species exert their beneficial effect on plants can be very diverse. PGPR produce plant hormones which promote root growth (Brown, 1972). The establishment of beneficial bacteria on roots systems *via* seed inoculation (that is, seed bacterization) has long been a major interest of agricultural researches (Brown, 1974). Ever since, the concept of the rhizosphere was first introduced, soil microbiologists have attempted to characterize and quantify microorganisms that inhabit this zone. Several methods including direct observation with light or electron microscopy have been used to demonstrate the rhizosphere effect.

BIOLOGICAL NITROGEN FIXATION BY PGPR ISOLATES

A number of bacterial species belonging to genera *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are associated with the plant rhizosphere and are able to exert a beneficial effect on plant growth (Tilak et al., 2005). The important role is played by plants in selecting and enriching the types of bacteria by the constituents of their root exudates. Thus, the bacterial community in the rhizosphere develops depending on the nature and concentrations of organic constituents of exudates and the corresponding ability of the bacteria to utilize these as sources of energy. There is a continuum of bacterial presence in soil rhizosphere, rhizoplane and internal of the plant tissues (Hallmann et al., 1997). Rhizospheric bacterial communities however have efficient systems for uptake and catabolism of organic compounds present in root exudates (Barraquiro et al., 2000).

Several bacteria help to derive maximum benefit from root exudates by their ability to attach to the root surfaces. Since, associative interactions of plants and microorganisms must have come into existence as a result of co-evolution, the use of latter group as bioinoculants must be pre-adapted, so that it fits into a long term sustainable agricultural system. PGPR are commonly used as inoculants for improving the growth and yield of agricultural crops and offers an attractive way to replace chemical fertilizers, pesticides and supplements (Ashrafuzzaman et al., 2009).

The use of biofertilizer and bioenhancer such as N_2 fixing bacteria and beneficial microorganism can reduce chemical fertilizer applications and consequently lower production cost. Utilization of PGPR in order to increase the productivity may be a viable alternative to organic fertilizers which also helps in reducing the pollution and preserving the environment in the spirit of an ecological agriculture (Stefan et al., 2008). Thus, rhizospheric bacteria can be a promising source for plant growth promoting agent in agriculture (Chaiharn et al., 2005) and are commonly used as inoculants for improving the growth and yield of agricultural crops.

The use of PGPR isolates as inoculants biofertilizers is beneficial for rice cultivation as they enhance growth of

rice and by inducing other plant growth promoting traits. Applying the combined inoculation of PGPR as biofertilizer affects beneficially the yield and growth of chickpea in field conditions (Rokhzadi et al., 2008). Biological nitrogen fixation contributes 180 × 10^6 metric tons/year globally, out of which symbiotic associations' produces 80% and the rest comes from free-living or associative systems. The ability to reduce and derive 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, *viz*., *Rhizobium,* the obligate symbionts in leguminous plants and *Frankia* in non - leguminous trees, and non - symbiotic N_2 -fixing forms such as cyanobacteria, *Azospirillum*, *Azotobacter*, *Acetobacter diazotrophicus*, *Azoarcus,* etc.

Non - symbiotic nitrogen fixation has a great agronomic significance. One main limitation that it faces is the availability of carbon and energy source for the energy intensive nitrogen fixation process. However, this limitation can be compensated by moving closer to or inside the plants, *viz*., in diazotrophs present in rhizosphere, rhizoplane or those growing endophytically. Some important non - symbiotic nitrogen - fixing bacteria include *Azoarcus* sp., *Gluconacetobacter diazotrophicus*, *Herbaspirillium* sp., *Azotobacter* sp. (Vessey, 2003; Barriuso et al., 2008), *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum, Rhodopseudomonas* and *Xanthobacter* (Saxena and Tilak, 1998).

Bacillus species

Bacillus is the most abundant genus in the rhizosphere, and the PGPR activity of some of these strains has been known for many years, resulting in a broad knowledge of the mechanisms involved. There are a number of metabolites that are released by these strains (Charest et al., 2005), which strongly affect the environment by increasing nutrient availability of the plants. *Bacillus* species are naturally present in the immediate vicinity of plant roots.

Bacillus subtilis is able to maintain stable contact with higher plants and promote their growth. In a micro propagated plant system, bacterial inoculation at the beginning of the acclimatization phase can be observed from the perspective of the establishment of the soil microbiota rhizosphere. *Bacillus licheniformis* when inoculated on tomato and pepper shows considerable colonization and can be used as a biofertilizer without altering normal management in green houses (Garcia et al., 2004).

Bacillus species used as biofertilizers probably have direct effects on plant growth through the synthesis of plant growth hormones (Amer and Utkhede, 2007). Phosphate solubilizing *Bacillus* spp. stimulates plant growth through enhanced P nutrition increasing the uptake of N, P, potassium (K) and iron (Fe). Phosphorus biofertilizers could help increase the availability of phosphates accumulated in the soil and could enhance plant growth by increasing the efficiency of biological nitrogen fixation and the availability of iron (Fe) and zinc (Zn) through production of plant growth promoting substances.

Growth promotion and disease control by *Pseudomonas* and *Bacillus* are complex interrelated processes involving direct and indirect mechanisms that include synthesis of some metabolites (auxin, cytokinin and gibberellins), induction of 1 - amino cyclopropane – 1 -carbocylate (ACC) deaminase, production of siderophore, antibiotics, hydrogen cyanide (HCN), and volatile compounds. Others include mineral solubilization (e.g phosphorus), competition, and induced systemic resistance (Hamid Abbasdokht; Ahmad Gholam, 2010).

Jaizme-Vega et al. (2004) evaluated the effect of a rhizobacteria consortium of *Bacillus* spp. on the first developmental stages of two micropropagated bananas and concluded that this bacterial consortium can be described as a prospective way to increase plant health and survival rates in commercial nurseries. *Bacillus* is also found to have potential to increase the yield, growth and nutrition of raspberry plant under organic growing conditions (Orhan et al., 2006).

Bacillus megaterium is very consistent in improving different root parameters (rooting performance, root length and dry matter content of root) in mint (Kaymak et al., 2008). The *B. megaterium* var. *phosphaticum* and Potassium solubilizing bacteria *B. mucilaginosus* when inoculated in nutrient limited soil showed that rock materials and both bacterial strains consistently increased mineral availability, uptake and plant growth of pepper and cucumber, suggesting its potential use as fertilizer (Han et al., 2006; Supanjani et al., 2006). The *B. pumilus* can be used as a bioinoculant for biofertilizer production to increase the crop yield of wheat variety Orkhon in Mongolia (Hafeez et al., 2006).

Pseudomonas species

Pseudomonas sp. is ubiquitous bacteria in agricultural soils and has many traits that make them well suited as PGPR. The most effective strains of *Pseudomonas* have been Fluorescent *Pseudomonas* spp. Considerable research is underway globally to exploit the potential of one group of bacteria that belong to fluorescent *Pseudomonas* (FLPs). FLPs help in the maintenance of soil health and are metabolically and functionally most diverse (Lata et al., 2002). The presence of *Pseudomonas fluorescence* inoculant in the combination of microbial fertilizer plays an effective role in stimulating

yield and growth traits of chickpea. Isolates of FLPs from roots, shoots and rhizosphere soil of sugarcane provides significant increases in fresh and dry masses (Mehnaz et al., 2009). Field trials of a *Pseudomonas* strain lead to a great increase in yield of legumes (Johri, 2001).

Specific strains of the *Pseudomonas fluorescens* group have recently been used as seed inoculants on crop plants to promote growth and increase yields. These *Pseudomonas*, termed PGPR, rapidly colonize plant roots of potato, sugar beet and radish and cause statistically significant yield increased upto 44% in field tests. The occurrence and activity of soil microorganisms are affected by a variety of environmental factors (e.g. soil type, nutrient abundance, pH, moisture content) as well as plant related factors (species, age). So, while working on two winter wheat cultivars it was found that the genus *Pseudomonas* show higher counts, thus the population size of bacteria of the genus *Pseudomonas* depends on the development phase of wheat plants (Wachowska et al., 2006).

Pseudomonas spp. are important plant growth promoting rhizobacteria (PGPR) used as biofertilizers and are able to enhance crop yield by direct and indirect mechanisms (Walsh et al., 2001). Several researchers have shown that fluorescent *Pseudomonas* is abundant in the rhizosphere of different crops (Kumar and Sugitha, 2004). Effectively, they produce a variety of biologically active substances among which growth promoting compounds represent a keen interest (Rodriguez, 2006).

The strains of *Pseudomonas* are able to solublize phosphorous in soil and increase its availability to plants (Sundara et al., 2002). Some strains of *Pseudomonas* produce chelating agents called siderophores with high affinity for iron absorption. Microbial siderophores can enhance plant growth through increasing iron solubility in the plant rhizosphere. Such products are also able to alleviate the unfavorable effects of pathogens on plant growth.

Plant growth promoting rhizobacterial strains belonging to fluorescent *Pseudomonas* were isolated from the rhizosphere of rice among 30 strains, that were confirmed as *Pseudomonas fluorescens*. These *P. fluorescens* strains were characterized by PCR-RAPD analysis and biochemical methods. Ten exhibited strong antifungal activity against *Pyricularia oryzae* mainly through the production of antifungal metabolites. Chanway et al. (1989) reported that 32 bacterial strains representing *Pseudomonas putida, Pseudomonas fluorescens* and *Serratia* sp. were isolated from soil and were seen to colonize soya been roots in laboratory, green house and field assays when applied as seed inoculants. The colony forming units (CFU) was ranged from 1 - 9 to 6.1 CFU/g of root.

Reddy et al. (2007) obtained thirty isolates of *P. fluorescens* from rice rhizosphere and were tested for antifungal activity against *Magnaporthe grisea, Dreschelaria oryzae*, *Rhizoctonia solani* and *Sarocladium oryzae* that are known to attack rice plants. One *P. fluorescens* isolate effectively inhibited the mycelial growth in all these fungi in dual culture tests (62 - 85%). The antifungal compounds were extracted with equal volume of ethyl acetate. The antifungal compounds from *Pseudomonas fluorescens* at 5% completely inhibited the pathogens. The antifungal compounds were tentatively identified on thin layer chromatography (TLC) at Rf 0.22, 0.35, 0.42 and 0.51. These compounds were individually purified by Column chromatography and retested for antifungal activity.

Egamberdieva (2010) analyzed the plant growth promoting bacteria for their growth-stimulating effects on two wheat cultivars. The investigations were carried out in pot experiments using calcarous soil. The results showed that bacterial strains *Pseudomonas* sp. and *P. fluorescens* were able to colonize the rhizosphere of both wheat cultivars. Their plant growth-stimulating abilities were affected by wheat cultivars. The bacterial strains *Pseudomonas* sp. and *P. fluorescens* significantly stimulated the shoot and root length and dry weight of wheat.

Maleki et al. (2010) isolated 144 bacteria from cucumber rhizosphere and screened as potential biological control agents against *Phytophthora drechsleri,* causal agent of cucumber root rot, *in vitro* and greenhouse condition. On the basis of dual culture assays, eight isolates were selected for root colonization, PGPR and greenhouse studies. Among these isolates, isolate CV6 exhibited the highest colonization on the roots and significantly promoted plant growth under *in vitro* condition.

Ramette et al. (2006) revealed that *Pseudomonas* have plant growth promoting properties. Isolated strains showed high ability of IAA production, phosphate solubilization and siderophore production, while genotyping analysis showed that *Pseudomonas* isolated from the rhizosphere of rice are genetically diverse. Nevertheless, the strains were distributed into 11 genotypes, including five groups of fluorescent *Pseudomonas*.

PRODUCTION OF PLANT GROWTH PROMOTING SUBSTANCES BY PGPR ISOLATES

Plant hormones are chemical messengers that affect a plants ability to respond to its environment. Hormones are organic compounds that are effective at very low concentration; they are usually synthesized in one part of the plant and are transported to another location. They interact with specific target tissues to cause physiological responses, such as growth or fruit ripening. Each response is often the result of two or more hormones acting together. Because hormones stimulate or inhibit plant growth, many botanists also refer to them as plant growth regulators. Botanists recognize five major groups

of hormones: auxins, gibberellins, ethylene, cytokinins and abscisic acid.

Saranraj et al. (2013) collected the paddy rhizosphere soil sample from ten different locations in Cuddalore district of Tamil Nadu. The population of bacteria, fungi and actinomycetes in the rhizosphere soil sample of paddy was estimated by Serial dilution and Pour plating method. The total bacterial population ranged from 15.8 to 24.3 × 10^6 cfu g-^{1} of soil and the highest population of 24.3 × 10^6 cfu g-^{1} was observed in soil of Sivapuri. The total fungal and actinomycetes population were ranged between 8.9 to 13.3 × 10^3 cfu g^{-1} and 10.5 to 19.3 × 10^5 cfu g^{-1} of soil respectively. The occurrence of *Pseudomonas fluorescens* also examined and the population ranged between 7.71 × 10^6 cfu g^{-1} and 7.21 x 10^6 cfu g^{-1} of soil. The *P. fluorescens* was isolated and characterized by gram staining, motility test, plating on King's B medium and bio-chemical tests. The ten *P. fluorescens* isolates obtained from the rhizosphere of paddy were tested for their efficiency of IAA and siderophore production. The maximum IAA production was recorded by the isolate PF-8. The minimum production of IAA was found in PF - 4 isolates. The isolate *P. fluorescens* (PS-8) showed maximum siderophore production and the least siderophore production was showed by the *P. fluorescens* isolate PS- 4.

Indole - 3- Acetic acid

Indole – 3 - acetic acid (IAA) is a member of the auxin family of phytohormones that influence many cellular functions in plants and therefore are important regulators of plant growth and development. In addition to production in plant tissues, IAA synthesis is widespread among plant-associated bacteria (Patten and Glick, 1996) and provides bacteria with a mechanism to influence plant growth (Patten and Glick, 2002).

Indole – 3 - acetic acid is the member of the group of phytohormones and is generally considered the most important native Auxin (Ashrafuzzaman et al., 2009). It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic responses, cellular responses such as cell expansion, division and differentiation and gene regulation. Diverse bacterial species possess the ability to produce the auxin phytohormone IAA. Different biosynthesis pathways have been identified and redundancy for IAA biosynthesis is widespread among plant - associated bacteria. Interactions between IAA - producing bacteria and plants lead to diverse outcomes on the plant side, varying from pathogenesis to phytostimulation.

The isolates producing IAA have stimulatory effect on the plant growth. When the crop is inoculated with the isolates capable of IAA production significantly increases the plant growth by the N, P, K, Ca and Mg uptake of sweet potato cultivar (Farzana and Radizah, 2005). There is a significant increase in rooting and root dry matter of cuttings of eucalypts when grown on IAA producing rhizobacteria inoculated substrate. Some rhizobacterial isolates stimulates the rhizogenesis and plant growth, maximizing yield of rooted cuttings in clonal nurseries (Teixeria et al., 2007). When cucumber, tomato and pepper are inoculated with different strains of PGPR which produce IAA, there is a significant increase in the growth of the vegetables (Kidoglu et al., 2007).

The IAA of microbial origin plays a major role in promotion of orchid germination, at least when the bacterial strains are in tight association with the seeds. *Azospirillum brasilense* strain Az39 and *Brayrhizobium japonicum* strain E109 both are able to excrete IAA into the culture medium, at a concentration sufficient to produce morphological and physiological changes in young seed tissues of Corn (*Zea mays* L.) and Soybean (*Glycine max* L.) and are responsible for their early growth promotion (Cassana et al., 2009). The use of PGPR isolates is beneficial for rice cultivation as they enhance the growth of rice by inducing IAA production.

Some microorganisms produce auxins in the presence of a suitable precursor such as L - tryptophan. The tryptophan increases the production of IAA in *Bacillus amyloliquefaciens*. Tien et al. (1979) showed that *Azospirillum* is able to produce auxins when exposed to tryptophan. Plants inoculated with the rhizobia together with Ag^+ ion and L - tryptophan (Trp), give the highest root dry weight, and significantly increase the uptake of N, P and K compared to non - inoculated control plants.

Beyeler et al. (1999) explained that the biocontrol strain CHA0 of *P. fluorescens* produces small amounts of indole-3-acetic acid *via* the tryptophan side chain oxidase and the tryptophan transaminase pathways. A recombinant plasmid (pME3468) expressing the tryptophan monooxygenase pathway was introduced into strain CHA0; this resulted in elevated synthesis of indole-3-acetic acid *in vitro*, especially after addition of L - tryptophan. In natural soil, strain CHA0/pME3468 increased fresh root weight of cucumber by 17 to 36%, compared to the effect of strain CHA0; root colonization was about 10^6 cells per g of root. However, both strains gave similar protection of cucumber against *Pythium ultimum*.

Shino et al. (2002) investigated the IAA biosynthesis in strain *P. fluorescens* HP72. After several repeated subcultures, the spontaneous IAA low - producing mutant HP72LI was isolated. The IAA low production of the strain HP72LI was due to the low tryptophan side chain oxidase (TSO) activity. Colonization of strain HP72 on the bent grass root induced root growth reduction, while strain HP72LI did not induce such growth reduction. The colonization ability of strain HP72 on the bent grass root is higher than that of strain HP72LI. However, as for biocontrol ability, a significant difference in both strains was not detected.

Khakipour et al. (2008) evaluated the auxin productivity potential in studied *Pseudomonas* strains through

chromatography, using HPLC devise; comparing the methods used and appointing IAA synthesize method by the studied strains in the applied cultivars. In fact, a variety of auxins like indole-3-acetic acid (IAA), indole-3-pyruvic acid, indole-3-butyric acid and indole lactic acid; cytokinins and gibberellins are detected, with auxin production being quantitatively most important. *Azospirillum brasilense* strain SM has the potential to be a competent rhizospheric bacterium as it triggers the IAA accumulation under nutrient stresses, likely environmental fluctuations and long - term batch cultures and beneficially influences the growth of sorghum.

Prassana Battu and Reddy (2009) isolated twenty *P. fluorescens* strains from rice growing soil samples and characterized. One of the *P. fluorescens* isolated and identified from the dual culture test. It was fermented for secondary metabolite in a small scale and extracted with ethyl acetate. The isolated metabolite tested against rice fungal pathogens. The structure of the compound was elucidated by high resolution NMR spectroscopy.

Karnwal (2009) obtained 30 fluorescent *Pseudomonas* isolates from different plant rhizosphere and were characterized on the basis of biochemical tests and plant growth promoting activities. *P. fluorescens* and *Pseudomonas aeruginosa* showed the best plant growth promoting activity. These isolates were tested for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan at 50, 100, 200 and 500 µg/ml. For both strains, indole production increased with increases in tryptophan concentration *P. aeruginosa* was less effective in production of indole acetic acid than *P. fluorescens*. Inoculation of rice seeds with *P. fluorescens* and *P. aeruginosa* showed a good level of indole acetic acid compared to uninoculated seeds.

Sivasakthivelan and Saranraj et al. (2013) analyzed the biocontrol strain *P. fluorescens* Psd for indole-3-acetic acid (IAA) biosynthesis and studied the effect of its consequent manipulation on its plant-growth-promoting (PGP) potential. While the indole pyruvic acid (IPyA) pathway commonly associated with PGP bacteria was lacking, the indole acetamide (IAM) pathway generally observed in phytopathogens was expressed in strain Psd. Over expression of IAM pathway genes *iaaM-iaaH*, from *Pseudomonas syringae* subsp. *savastanoi* drastically increased IAA levels and showed a detrimental effect on sorghum root development.

Siderophore production

Iron is an essential growth element for all living organisms. The scarcity of bioavailable iron in soil habitats and on plant surfaces foments a furious competition (Whipps, 2001). Under iron limiting conditions PGPB produce low molecular weight compounds called siderophores to competitively acquire ferric ion. Siderophores (Greek: "iron carrier") are small, high - affinity iron chelating compounds secreted by microorganisms such as bacteria, fungi and grasses (Miller and Marvin, 2009). Microbes release siderophores to scavenge iron from these mineral phases by formation of soluble Fe^{3+} complexes that can be taken up by active transport mechanisms. Many siderophores are non - ribosomal peptides (Miethke and Maraheil, 2007), although several are biosynthesized independently.

Siderophores are also important for some pathogenic bacteria for their acquisition of iron. Siderophores are amongst the strongest binders to Fe^{3+} known, with enterobactin being one of the strongest of these (Raymond et al., 2003). Distribution of siderophore producing isolates according to amplified ribosomal DNA restriction analysis (ARDRA) groups, reveals that most of the isolates belong to Gram negative bacteria corresponding to the *Pseudomonas* and *Enterobacter* genera and *Bacillus* and *Rhodococcus* genera are the Gram positive bacteria found to produce siderophores (Tian et al., 2009).

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. Some PGPB strains go one - step further and draw iron from heterologous siderophores produced by cohabiting microorganisms. *Pseudomonas* sp. have the capacity to utilize siderophores produced by diverse species of bacteria and fungi and *Pseudomonas putida* can utilize the heterologous siderophores produced by rhizosphere microorganisms to enhance the level of iron available to it in the natural habitat (Loper and Henkels, 1999). The two strains of *P. fluorescens* along with *Pseudomonas putida* produce maximum yield of hydroxamate type of siderophore in the modified succinic acid medium (SM).

Soil bacteria isolates including *Azotobacter vinelandii* and *Bacillus cereus* produces siderophores and they can be used as efficient PGPR to increase the yield of the crop (Husen, 2003). *Bacillus megaterium* from rhizosphere is able produce siderophore and thus it helps in the plant growth promotion and reduction of disease intensity. Specific strains of the *P. fluorescens* group have recently been used as seed inoculants on crop plants to promote growth and increase yields of various crops. These results prompted Kloepper et al. (1980) to investigate the mechanism by which plant growth was enhanced.

A previous study indicated that PGPR increase plant growth by antagonism to potentially deleterious rhizoplane fungi and bacteria, but the nature of this antagonism was not determined. They presented evidence that PGPR exert their plant growth promoting activity by depriving native microflora of iron. PGPR produces extracellular siderophores which efficiently complex environmental iron, making it less available to certain native microflora. The siderophores production by

Bacillus and *Pseudomonas* when assessed both in the presence and in absence of technical grade of herbicides show that the metabolic activities of plant growth promoting rhizobacteria decline following herbicides application (Munees and Mohammad, 2009).

Siderophores are low molecular weight (<10 kD) iron binding compounds synthesized by microbes in large quantity under iron limited conditions. Siderophores chelate the ferric ions with a high specific activity and serve as vehicles for the transport of iron (Fe^{3+}) into the microbial cell. Most of the siderophores have either hydroxamate, catechol or carboxylate ligands (Hofte, 1993).

Djibaoui and Bensoltane (2005) tested the ability of *Pseudomonas* to grow and to produce siderophores is dependent on the iron content and the type of carbon sources in the medium. Under conditions of low - iron concentration the *Pseudomonas* isolates studied produced yellow - green fluorescent iron - binding peptide siderophores and the biosynthesis of this siderophores was affected by several different environmental parameters. Four basal media, supplemented with different concentration of iron were employed to study the effect of iron and different organic carbon sources on siderophore production in *P. fluorescens*. The highest siderophores concentration was obtained in succinate medium. Ferric iron increased the growth yield and completely repressed siderophores production above 200 g/l, but had a positive effect below 160 g/l.

Urszula (2006) tested the ability of six strains belonging to the genus *Pseudomonas* isolated from the rhizosphere of wheat to produce pyoverdin. The studied strains demonstrated a varied level of production of the siderophore, depending on the culture conditions. The highest level of pyoverdin was determined after 72 h of growth at 20 - 25°C in iron - free medium supplemented with succinate. The synthesis of pyoverdin by all the strains studied was strongly repressed by the addition of iron ions (III) to the growth medium. Calcium, cadmium and magnesium ions stimulated the synthesis of the siderophore examined, whereas zinc and lead ions partially decreased its level. Enrichment of the growth medium in cobalt ions completely inhibited the synthesis of siderophores as well as growth of the bacteria.

Phosphate solubilization

Phosphorous is one of the major nutrient second only to nitrogen in requirement for plants. Most of the phosphorous in soil is present in the form of insoluble phosphates and cannot be utilize by plants (Pradhan and Sukla, 2006). The ability of bacteria to solublize mineral phosphates has been of interest to agricultural microbiologists as it can enhance the availability of phosphorous for plant growth PGPR has been show to solublize precipitated phosphates and enhance phosphate availability to rice that represent a possible mechanism of plant growth promotion under field condition (Verma et al., 2001).

The improvement of soil fertility is one of the most common strategies to increase agricultural production. The biological nitrogen fixation is very important in enhancing the soil fertility. In addition to biological nitrogen fixation, Phosphate solubilization is equally important. Phosphorus (P) is major essential macronutrients for biological growth and development. Microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants. The ability of some microorganisms to convert insoluble phosphorus (P) to an accessible form, like orthophosphate, is an important trait in a PGPB for increasing plant yields (Rodriguez et al., 2006). The rhizospheric phosphate utilizing bacteria could be a promising source for plant growth promoting agent in agriculture.

The use of phosphate solubilizing bacteria as inoculants increases the phosphorous uptake by plants (Chen et al., 2006). Among the heterogeneous and naturally abundant microbes inhabiting the rhizosphere, the Phosphate solubilizing microorganisms (PSM) including bacteria have provided an alternative biotechnological solution in sustainable agriculture to meet the phosphorous demands of plants. These organisms in addition to providing phosphorous to plants also facilitate plant growth by other mechanisms.

Current developments in our understanding of the functional diversity, rhizosphere colonizing ability, mode of actions and judicious application are likely to facilitate their use as reliable components in the management of sustainable agricultural systems (Zaidi et al., 2009). PSM include largely bacteria and fungi. The most efficient PSM belong to genera *Bacillus, Rhizobium* and *Pseudomonas* among bacteria, and *Aspergillus* and *Penicillium* among fungi. Within rhizobia, two species nodulating chickpea, *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum*, are known as good phosphate solubilizers (Rivas et al., 2006). However, it is known that every aspect of the process of nodule formation is limited by the availability of phosphorous.

PGPR AS BIOCONTROL AGENT

PGPR are indigenous to soil and the plant rhizosphere and play a major role in the biocontrol of plant pathogens. They can suppress a broad spectrum of bacterial, fungal and nematode diseases. PGPR can also provide protection against viral diseases. The use of PGPR has become a common practice in many regions of the world. Although, significant control of plant pathogens has been demonstrated by PGPR in laboratory and greenhouse studies, results in the field have been inconsistent.

Recent progress in our understanding of their diversity, colonizing ability and mechanism of action, formulation and application should facilitate their development as

reliable biocontrol agents against plant pathogens. Some of these rhizobacteria may also be used in integrated pest management programmes. Greater application of PGPR is possible in agriculture for biocontrol of plant pathogens and biofertilization (Siddiqui, 2006). The bacterial strains isolated from *Lolium perenne* rhizosphere are capable of acting as plant growth promoting bacteria and as biocontrol agents as they show various plant growth promoting activities (Shoebitz et al., 2007).

A major group of rhizobacteria with potential for biological control is the *Pseudomonades* (Kremer and Kennedy, 1996). *Pseudomonas* sp. is ubiquitous bacteria in agricultural soils. Tremendous progress has been made in characterizing the process of root colonization by *Pseudomonas*, the biotic and abiotic factors affecting colonization, bacterial traits and genes contributing to rhizosphere competence and the mechanisms of pathogen suppression (Weller, 2007). *Pseudomonas* possesses many traits that make them well suited as biocontrol and growth promoting agents. These include the ability to (i) grow rapidly *in vitro* and to be mass produced; (ii) rapidly utilize seed and root exudates; (iii) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant; (iv) produce a wide spectrum of bioactive metabolites (that is, antibiotics, siderophores, volatiles and growth promoting substances); (v) compete aggressively with other microorganisms; and (vi) adapt to environmental stresses.

The major weakness of *Pseudomonas* as biocontrol agents is their inability to produce resting spores, which complicates formulation of the bacteria for commercial use. Fluorescent *Pseudomonas* spp. has been studied for decades for their plant growth promoting effects through effective suppression of soil borne plant diseases. Among various biocontrol agents, Fluorescent *Pseudomonas*, equipped with multiple mechanisms for biocontrol of phytopathogens and plant growth promotion, are being used widely (Banasco et al., 1998) as they produce a wide variety of antibiotics, chitinolytic enzymes, growth promoting hormones, siderophores, HCN and catalase, and can solublize phosphorous (Seong and Shin, 1996). *P. fluorescens* MSP-393, a plant growth - promoting rhizobacteria is an efficient biocontrol agent in rice grown in saline soils of coastal ecosystems (Paul et al., 2006).

Bacillus subtilis is also used as a biocontrol agent. This prevalent inhabitant of soil is widely recognized as a powerful biocontrol agent. In addition, due to its broad host range, its ability to form endospores and produce different biologically active compounds with a broad spectrum of activity, *B. subtilis* as well as other *Bacilli* are potentially useful as biocontrol agents (Nagorska et al., 2007). *Bacillus megaterium* from tea rhizosphere is able to solublize phosphate, produce IAA, siderophore and antifungal metabolite and thus it helps in the plant growth promotion and reduction of disease intensity (Chakraborty et al., 2006). Two strains (*Bacillus thuringiensis* and *Bacillus sphaericus*) have the ability to solublize inorganic phosphates and help in the control of the lepidopteron pests.

Antagonistic activity of pgpr isolates against phytopathogens

PGPR improve plant growth by preventing the proliferation of phytopathogens and thereby support plant growth. Some PGPR synthesize antifungal antibiotics, e.g. *P. fluorescens* produces 2, 4-diacetyl phloroglucinol which inhibits growth of phytopathogenic fungi. Certain PGPR degrade fusaric acid produced by *Fusarium* sp. causative agent of wilt and thus prevents the pathogenesis (Nowak et al., 1994).

Some PGPR can also produce enzymes that can lyse fungal cells. For example, *Pseudomonas stutzeri* produces extracellular chitinase and laminarinase which lyses the mycelia of *Fusarium solani*. In recent years, 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, root - rot of pea, root - rot of wheat, damping - off of sugar beet and as the prospects of genetically manipulating the producer organisms to improve the efficacy of these biocontrol agents. A concern was shown 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 (Kumar et al., 2002).

Gaur et al. (2004) confirmed that DAPG producing *Pseudomonas* 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. Fluorescent *Pseudomonas* exhibit strong antifungal activity against *Pyricularia oryzae* and *Rhizoctonia solani* mainly through the production of antifungal metabolites. One of the isolate of a *fluorescent Pseudomonas* spp. EM85 is found to be strongly antagonistic to *Rhizoctonia solani*, a causal agent of damping-off of cotton. The *P. oryzihabitans* and *X. nematophila* strains produce secondary metabolites and suppress *Pythium* and *Rhizoctonia* species which also causes damping - off of cotton.

Fluorescent *Pseudomonas* also exhibits strong antifungal activity against *Rhizoctonia bataticola* and *Fusarium oxysporum* found in rice and sugarcane rhizosphere, mainly through the production of antifungal metabolites (Kumar et al., 2004). *Xanthomonas oryzae* and *Rhizoctonia solani* – the bacterial leaf blight (BB) and sheath blight (ShB) pathogens of rice (*Oryza sativa*) are

suppressed by indigenous *Pseudomonas* strains isolated from rhizosphere of rice cultivated in the coastal agri - ecosystem under both natural and saline soil conditions (Reddy et al., 2008). Isolates of *P. fluorescens* from rice rhizosphere are also shown to exhibit strong antifungal activity against *Pyricularia oryzae* and *Rhizoctonia solani* mainly through the production of antifungal metabolites. 50 - 60% of fluorescent *Pseudomonas* recovered from the rhizosphere and endorhizosphere of wheat grown in Indo-gangetic plains are antagonistic towards *Helminthosporium sativum* (Gaur et al., 2004).

Zadeh et al. (2008) worked to show the antagonistic potential of non - pathogenic rhizosphere isolates of fluorescent *Pseudomonas* in the biocontrol of *Pseudomonas savastanoi* which is the causative agent of Olive knot disease. *Pseudomonas corrugata*, a form that grows at 4°C under laboratory conditions, produces antifungals such as diacetylphloroglucinol and phenazine compounds. *P. fluorescens* CHA0 suppresses black root rot of tobacco, a disease caused by the fungus *Thielaviopsis basicola* and contributes in the biocontrol of *Meloidogyne javanica*, the root-knot nematode, *in situ* (Siddiqui et al., 2005). In addition, certain soils from Morens, Switzerland, are naturally suppressive to *Thielaviopsis basicola* - mediated black root rot of tobacco, and fluorescent *Pseudomonas* populations producing the biocontrol compounds (Ramettee et al., 2006).

Pseudomonas shows biocontrol potential against phytopathogenic fungi *in vivo* and *in vitro* conditions from chickpea rhizosphere. *Pseudomonas putida* has potential for the biocontrol of root - rot disease complex of chickpea by showing antifungal activity against *Macrophomina phaseolina*. It has also been shown that anaerobic regulator ANR - mediated cyanogenesis contributes to the suppression of black root rot (Saraf et al., 2008).

Pseudomonas strains acts as the effective candidates in suppressing *Pseudomonas capsici* in all seasons of plant growth as fluorescent *Pseudomonas* antagonizes all the reproductive phases of the *Phytophthora capsici*, the causal organism of foot rot disease (Paul and Sarma, 2006). Some metabolites produced by *Pseudomonas aeruginosa* Sha8 produces toxic volatile compound which reduces the growth of both *Fusarium oxysporum* and *Helmithosporium* sp. while, *Aspergillus niger* is not affected (Hassanein et al., 2009). *Bacillus luciferensis* strain KJ2C12 reduces *Phytophthora blight* of pepper by protecting infection courts through enhanced effective root colonization with protease production and an increase of soil microbial activity. Lima bean (*Phaseolus lunatus* L.) plants release hydrogen cyanide (HCN) in response to damage caused by natural enemies, thereby directly defending plant tissue. The bacteria *P. fluorescens* CHA0 shows biocontrol against the ciliated protozoa *Tetrahymena pyriformis* which feeds on it (Kim et al., 2009).

The nutritional superiority of more vigorous AM plants has been proposed to be a mechanism in reduction of root diseases. Wild rhizobial cultural filtrates and AM plants are found to have a significant antagonistic effect against soil born pathogenic fungi and therefore enhance the plant resistance to diseases. Siderophore mediated antagonism by *Acinetobacter calcoaceticus* was observed against common phytopathogens *viz*., *Aspergillus flavus*, *Aspergillus niger, Colletotrichum capsicum* and *Fusarium oxysporum* (Jousset et al., 2009).

Soil application of bacterial PSMs manages the wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Khan et al., 2007). Inoculation of pepper with the phosphate solubilizing bacteria significantly reduces the *Phytophthora blight* or crown blight of peppers and increases the yield compared to untreated controls. *Azotobacter* isolates, *Pseudomonas* and *Bacillus* showed broad spectrum antifungal activity on Mueller Hinton medium against *Aspergillus*, one or more species of *Fusarium* and *Rhizoctonia bataticola* (Akgul and Mirik, 2008).

Wafaa and Haggag (2007) investigated the effect of *Paenibacillus polymyxa* (syn. *Bacillus polymyxa*) which produces an exopolysaccharide (EPS) on control of *Aspergillus niger*. In an *in vitro* assay, two strains of *Paenibacillus polymyxa* were tested against *Aspergillus niger*. Both strains showed inhibitory effect against *Aspergillus niger.* When these strains of *Paenibacillus polymyxa* were applied to seed and sowed in soil infested with *Aspergillus niger*, they significantly suppressed crown rot disease development and decreased survival of the *Aspergillus niger* pathogen. Over a period of 60 days, the population of bacteria was greatly increased. The bacterium colonized plant roots and were able to migrate downward with the root as it elongated.

Carissimi et al. (2009) isolated the bacteria strains with antifungal activity against *Bipolaris sorokiniana* to evaluate the best growth conditions for the antifungal production; and to test its action *in vivo*. The bacterial strains were pre-screened against four *Bipolaris sorokiniana* isolates on plates containing Sabouraud maltose agar. The isolate that showed the best result was grown on different culture media, cells were filtrated and the filtrates were tested against *Bipolaris sorokiniana* on plates with PDA medium. The *in vivo* test was done on wheat seeds, infected with *Bipolaris sorokiniana* isolate on a chamber with controlled temperature. *Bacillus* was chosen among the 86 bacterial isolates tested against the phytopathogen. The filtrate from *Bacillus* grown on tryptic casein soy broth (TSB) and straw culture media showed a similar degree of inhibition against the phytopathogen, the same result was not observed with malt extract broth media.

Reddy and Rao (2009) isolated plant growth promoting rhizobacterial strains belonging to fluorescent *Pseudomonas* from the rhizosphere of rice. Among 30

strains that were confirmed as *P. fluorescens*, this *P. fluorescens* strain was characterized by PCR-RAPD analysis and biochemical methods. Ten exhibited strong antifungal activity against *Pyricularia oryzae* and *Rhizoctonia solani* mainly through the production of antifungal metabolites.

Ningthoujam et al. (2009) screened for activity against some major rice fungal pathogens such as *Curvularia oryzae*, *Pyricularia oryzae* and *Fusarium oxysporum* showed potent antagonistic activities in dual culture assay. Among 33 indigenous actinomycetes isolates, LSCH-10C isolated from Loktak lake sediment on chitin agar, was found most promising to be developed as biocontrol agent (BCA) for rice. The nature of the activity in terms of fungitoxic or fungistatic nature was also determined. This report presents the preliminary results of these bio-control actinomycetes. Some of the strains have been selected for further studies towards application as rice BCAs.

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