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Review

Plant growth promoting Rhizobacteria (PGPR): a review

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Soil microbial communities are often difficult to characterize, mainly because of their immense phenotypic and genotypic diversity. In the last ten years, a number of PGPR that have been identified has seen a great boost, mainly because the role of the rhizosphere as an ecological unit has gained importance in the functioning of the biosphere and also because mechanisms of action of PGPR have been deeply studied. A putative PGPR qualifies as PGPR when it is able to produce a positive effect on the plant upon inoculation, hence demonstrating good competitive skills over the existing rhizosphere communities. PGPR influence direct growth promotion of plants by fixing atmospheric nitrogen, Solubilizing insoluble phosphates, secreting hormones such as IAA, GAs, and Kinetins besides ACC (1-Aminocycloprapane-1-carboxylic acid) deaminase production, which helps in regulation of ethylene. Induced systemic resistance (ISR), antibiosis, competition for nutrients, parasitism, production of metabolites (hydrogen cyanide, siderophores) suppressive to deleterious rhizobacteria are some of the mechanism that indirectly benefit plant growth.

Keywords: Plant growth-promoting rhizobacteria bacteria / IAA / ACC deaminase / Phosphate solunilization / Rhizosphere / Diversity

INTRODUCTION

Soil bacteria are very important in biogeochemical cycles and have been used for crop production for decades. “Plant bacterial interactions” in the rhizosphere are the determinants of plant health and soil fertility. Interaction of plant growth promoting rhizobacteria (PGPR) with host plants is an intricate and interdependent relationship involving not only the two partners but other biotic and abiotic factors of the rhizosphere region (Figure 1) (Dutta and Podile 2010). “Plant growth-promoting rhizobacteria bacteria” are free-living soil bacteria that can either directly or indirectly facilitate rooting (Mayak et al.1999) and growth of plants (Glick 1995). In the last ten years, a number of PGPR that have been identified has seen a great boost, mainly because the role of the rhizosphere as an ecological unit has gained importance in the functioning of the biosphere and also because mechanisms of action of PGPR have been deeply studied.

A putative PGPR qualifies as PGPR when it is able to produce a positive effect on the plant upon inoculation, hence demonstrating good competitive skills over the existing rhizosphere communities. Generally, about 2–5% of rhizosphere bacteria are PGPR (Antoun and Prevost 2005). PGPR are the potential tools for sustainable agriculture and trend for the future. One of the mechanisms by which bacteria are adsorbed onto soil particles is by simple ion exchange and a soil is said to be naturally fertile when the soil organisms are releasing inorganic nutrients from the organic reserves at a rate sufficient to sustain rapid plant growth. These bacteria belong to the genera *Acetobacter, Acinetobacter, Alcaligenes, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Beijerinckia, Burkholderia, Derxia, Enterobacter, Gluconacetobacter, Herbaspirillum, Klebsiella, Ochrobactrum, Pantoae, Pseudomonas, Rhodococcus, Serratia, Stenotrophomonas* and *Zoogloea*

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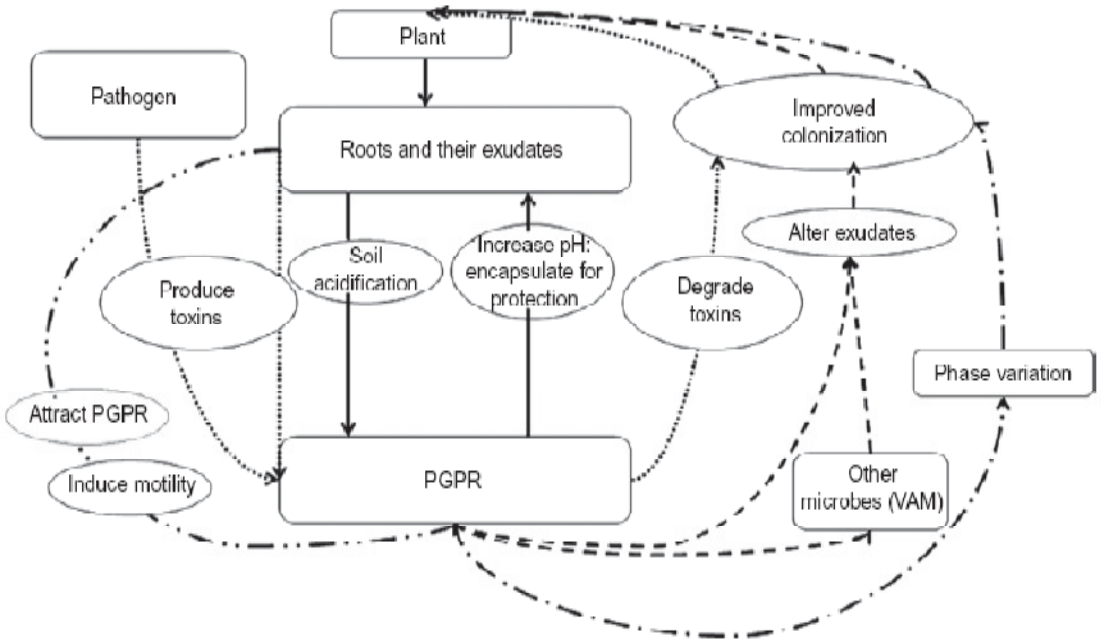

Figure 1: Interaction of plant root exudates, pathogens, PGPR, and other beneficial microbes in the rhizosphere. Adapted from Dutta and Podile (2010).

and have been subject of extensive research throughout the years (Babalola 2010).

Rhizosphere and bacterial diversity

The rhizosphere is the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root. This zone is about 1 mm wide, but has no distinct edge. Rather, it is an area of intense biological and chemical activity influenced by compounds exuded by the root, and by microorganisms feeding on the compounds. Soil microbial communities are often difficult to characterize, mainly because of their immense phenotypic and genotypic diversity. Bacterial populations in upper layers of the soil can contain as many as 10^9 cells per gram of soil (Torsvik and Ovreas 2002). Most of these cells are unculturable.

The fraction of the cells making up soil microbial biomass that have been cultured and studied in detail is negligible and often comprises less than 5% of the total population (Borneman and Triplett 1997). The microbial population in and around the roots includes bacteria, fungi, yeasts and protozoa. Some are free living while others form symbiotic associations with various plants. Rhizosphere microbial populations could be regarded as a stable community around a particular plant species in a specific soil, or alternatively, as a succession of populations. The interaction between these microorganisms and the roots of the plant may be beneficial, harmful or neutral for the plant, and sometimes the effect of microorganisms may vary as a consequence of soil conditions (Singh and Varaprasad 2008). There are various approaches to studying microbial diversity, which can be broadly divided into (a) cultivation-based methods and (b) cultivation-independent methods (Ahmad et al. 2008). Both approaches have their own unique limitations and advantages.

Traditional methods to study microbial diversity have been based on cultivation and isolation. For this purpose, a wide variety of culture media have been designed to maximize the variety and populations of microorganisms. A Biolog based method applied for directly analyzing the potential activity of soil microbial communities, called community-level physiological profiling (CLPP), was used to study microbial diversity (Kennedy and Smith 1995). Molecular technology has helped to better understand microbial diversity. These molecular techniques include polymerase chain reaction (PCR) or real-time polymerase chain reaction (RT-PCR), which is used to target the specific DNA or RNA in soil. The 16S or 18S ribosomal RNA (rRNA) or their genes (rDNA) represent useful markers for prokaryotes and eukaryotes, respectively. The mixed PCR products can be used for (a) preparing clone libraries and (b) a range of microbial community fingerprinting techniques. Such clone libraries are useful to identify and characterize the dominant bacterial or fungal types in soil and thereby provide a picture of diversity. Moreover, a range of other techniques have been developed to fingerprint soil microbial communities, for example, Density gradient gel electrophoresis or temperature gradient gel electrophoresis amplified rDNA

restriction analysis (ARDRA) and ribosomal intergenic spacer length polymorphism (RISA) (Ranjard and Richaume 2001).

Jha et al. (2010) reported a good diversity index should encompass both Dominance indices (Example, Simpson index) and Information indices (Example, Shannon-wiener index). To comprehend such diversity it is advantageous to investigate the combined uses of species richness and diversity as well as to estimate the combinatorial effect of species richness and diversity in order to understand their role and distribution in their habitat.

Direct and indirect plant growth promotion

The term Plant Growth-Promoting Rhizobacteria (PGPR) was coined over three decades ago, they are non-pathogenic, strongly root colonizing bacteria on the surface of plant's roots which increase plant's yield by one or more mechanisms (Babalola 2010). Plant growth promoting rhizobacteria can affect plant growth by different direct and indirect mechanisms (Glick 1995). PGPR influence direct growth promotion of plants by fixing atmospheric nitrogen, Solubilizing insoluble phosphates, secreting hormones such as IAA, GAs, and Kinetins besides ACC (1-Aminocycloprapane-1-carboxylic acid) deaminase production (Glick et al. 1999), which helps in regulation of ethylene. Induced systemic resistance (ISR), antibiosis, competition for nutrients, parasitism, production of metabolites (hydrogen cyanide, siderophores) suppressive to deleterious rhizobacteria are some of the mechanism that indirectly benefit plant growth. According to Vessey (2003), numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in, on, or around plant tissues, and stimulate plant growth by a plethora of mechanisms are collectively known as PGPR. Gray and Smith (2005) have recently shown that the PGPR associations range in the degree of bacterial proximity to the root and intimacy of association. In general, these can be separated into extracellular (ePGPR), existing in the rhizosphere, on the rhizoplane, or in the spaces between cells of the root cortex, and intracellular (iPGPR), which exist inside root cells, generally in specialized nodular structures.

Free nitrogen fixing Plant growth promoting rhizobacteria

Rhizosphere associated N-fixing bacteria have increasingly been used in non-legume crop species such as Sugar beet, Sugar cane, Rice, Jatropha, Maize, and Wheat (Sahin et al. 2004). For example, experiments with *Bacillus* species indicated yield increases in cereals (Cakmakci et al. 2001) and maize (Pal 1998). Biological Nitrogen fixation can occur in bulk or rhizospheric soil. Fixed nitrogen can then is acquired through root uptake and contribute to the nitrogen account of the crop. The earliest large-scale experiments, exploiting PGPR potential to enhance crop productivity used N_2-fixing bacteria, with the implicit assumption that it was this activity that was producing the enhanced crop yields.

One study in Russia to test the potential of a strain of *A. radiobacter*, isolated from the rhizosphere of rice (*Oryza sativa* L.), on winter wheat and spring barley appeared to give significant increases (5–30%) in yield in 2 out of 3 years. At the same time, it was estimated that the contribution of N_2 fixation to total N assimilation was between 23 and 32% (Bairamov et al. 2001). Bacteria such as *Enterobacter, Klebsiella, Burkholderia,* and *Stenotrophomonas,* have been gaining attention in the recent years, because of their association with important crops and potential to enhance the plant growth (Ramirez and Mellado 2005). N-fixing bacterial strains *Pseudomonas putida* RC06, *Paenibacillus polymyxa* RC05 and RC14, and *Bacillus* OSU-142 have great potential, and as formulations, they are used as biofertilizers for better yield and the quality of wheat, sugar beet, and spinach growth (Cakmakci et al. 2007). The N-fixing *Bacillus* strains and *A. brasilense* sp246 have a potential on plant growth activity of spring wheat and barley cultivation in organic and low-N input agriculture (Canbolat et al. 2006).

Mineral phosphate solubilization

Various soil microorganisms were reported to solubilize insoluble phosphorous complexes into solution and make it possible for its use by the plant (Tripura et al. 2005). The availability of phosphorus in many soils is in the range of 1 µmol l^{-1}, but plants require approximately 30 µmol l^{-1} to reach their maximum productivity. Most of the applied phosphatic fertilizers are also reprecipitated into insoluble mineral complexes and are not efficiently taken up by the plants. Certain group of higher plants evolved highly efficient mechanisms for absorbing phosphate even from very dilute solutions and achieves the maximum growth rates even with soil solution phosphate levels of 2 µmol l^{-}or less (Epstein 1972).

Microbial solubilization of inorganic phosphate compounds is of great economic importance in plant nutrition (Gaur 2002). Bacteria from genera such as *Achromobacter, Agrobacterium, Bacillus, Enterobacter, Erwinia, Escherichia, Flavobacterium, Mycobacterium, Pseudomonas* and *Serratia* are highly efficient in solubilizing unavailable complexed phosphate into available inorganic phosphate ion (Goldstein 2001).

Soil also contains a wide range of organic substrates, which can be a source of P for plant growth. To make this form of P available for plant nutrition, it must be hydrolyzed to inorganic P. Mineralization of most organic phosphorous compounds is carried out by means of

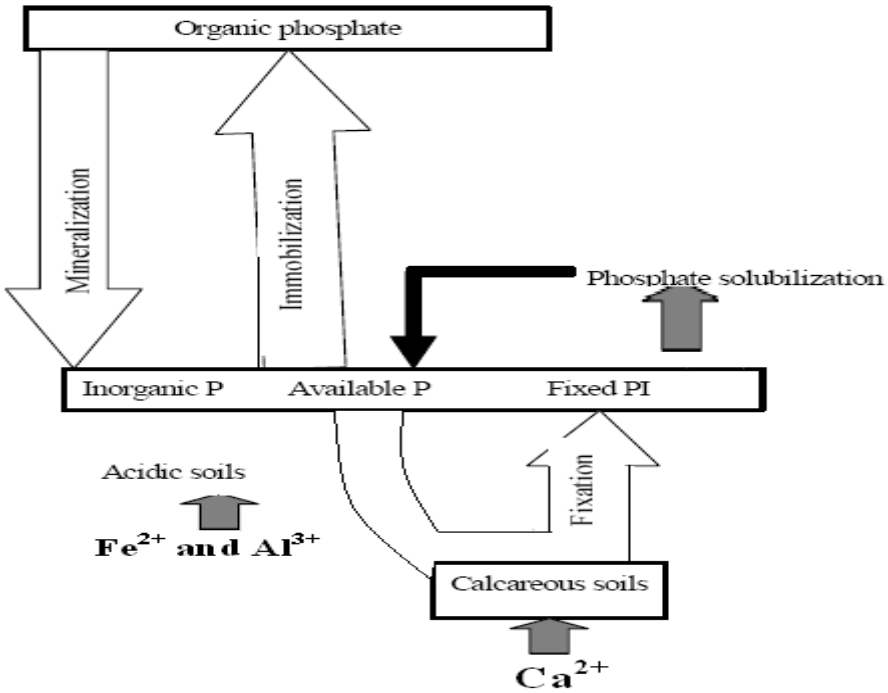

Figure 2: Mechanism for phosphorus channels in soil. Modified figure adapted from Bagyaraj et al. (2000).

enzymes like phosphatase, phytase, phosphonoacetate hydrolase, D-α-glycerophosphatase and C-P lyase (Hayat et al. 2010). Activity of various phosphatases in the rhizosphere of maize, barley, and wheat showed that phosphatase activity was considerable in the inner rhizosphere at acidic and neutral soil pH. Soil bacteria expressing a significant level of acid phosphatases include strains from the genus *Rhizobium, Enterobacter, Serratia, Citrobacter, Proteus and Klebsiella as well as Pseudomonas* and *Bacillus*. 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.

Mechanism for phosphate solubilization

Phosphate-solubilizing bacteria use different mechanism(s) to bring about the insoluble forms of the phosphate into soluble forms. Organic acids released by the micro-organisms act as good chelators of divalent cations of Ca^{2+} accompanying release of phosphates from insoluble phosphatic compounds. Organic acids may also form soluble complexes with metal ions associated with insoluble 'P', thus releasing the phosphate (Illmer and Schinner 1995). Many of the PSMs lower the pH of the medium either by H^+ extrusion (Illmer and Schinner 1995) or by secretion of organic acids such as acetic, lactic, malic, succinic, tartaric, gluconic, 2-ketogluconic, oxalic and citric acids.

The involvement of microorganisms in solubilization of inorganic phosphates was known as early as 1903 (Kucey et al. 1989). It is estimated that P solubilizing microorganisms may constitute 20 to 40% of the culturable population of soil microorganisms and that a significant proportion of these can be isolated from rhizosphere soil (Chabot et al. 1993). Most PSB are isolated from the rhizosphere of various plants and are known to be metabolically more dynamic than those isolated from sources other than rhizosphere.

These low levels of P are due to the high reactivity of soluble P with calcium (Ca), iron (Fe) or aluminium (Al), which leads to P precipitation (Figure 2). Inorganic P in acidic soils is associated with Al and Fe compounds, whereas calcium phosphates are the predominant form of inorganic phosphates in calcareous soils. Organic P may also make up a large fraction of soluble P, as much as 50% in soils with high organic matter content (Barber 1984). Phytate, a hexaphosphate salt of inositol, is the major form of P in organic matter, contributing between 50 and 80% of the total organic P (Alexander 1977). Although microorganisms are known to produce phytases that can hydrolyze phytate, phytate tends to accumulate in virgin soils because it is rendered insoluble as a result of forming complex molecules with Fe, Al and Ca (Alexander 1977). Phospholipids and nucleic acids form a

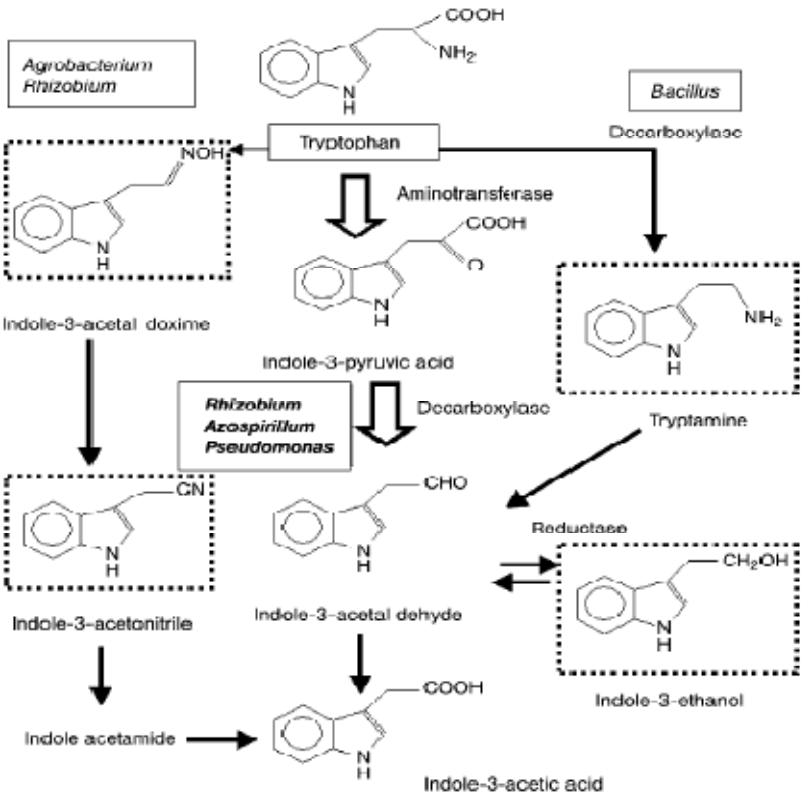

Figure 3: Tryptophan dependent auxin biosynthetic pathways in plants and microorganisms. Adapted from Beatriz et al. (2008).

mother pool of labile P in soil that is easily available to most of the organisms present (Molla and Chowdary 1984).

Maintenance of plant-available P in the soil is very imperative to avoid over exploitation of soil P which will lead to P deficiency and consequently, low plant yield. This maintenance is a function of the concentration of P in the labile pool and how readily it is released into the soil solution from the solid phase. This in turn depends on the P buffering capacity of the soil (Holford 1997) even though, P buffering capacity may not be directly related to P desorption ability of soils as observed by Raven and Hossner (1993). Phosphorus is released at a faster rate from the labile pool into the soil solution at lower buffering capacity. Holford (1997) reported 3 important soil components controlling the supply of P from the labile pool to replenish crop extraction. These include the amount of or concentration of P in the soil solution; the amount of P in the replenishment source that enters into equilibrium with the soil solution phase and P buffering capacity of the soil.

Phytohormone production

Plant growth and development involves a tight coordination of the spatial and temporal organization of cell division, cell expansion and cell differentiation. Orchestration of these events requires the exchange of signaling molecules between the root and shoot, which can be affected by both biotic and abiotic factors.

The interactions that occur between plants and their associated microorganisms have long been of interest, as knowledge of these processes could lead to the development of novel agricultural applications. Proposed molecules for plant-growth promotion by PGPR include bacterial synthesis of the plant hormones indole-3-acetic acid, cytokinin, and gibberellin and breakdown of plant produced ethylene by bacterial production of 1-aminocyclopropane- 1-carboxylate deaminase.

Auxins

Diverse bacterial species produce auxins as part of their metabolism including indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) or their precursors (Figure 3). Evidences indicating that IAA is a positive regulator of plant growth comes from the analysis of mutants that overproduce it, such as *super root* and *yucca*, which have long hypocotyls and increased numbers of lateral roots and root hairs and the positive effect of IAA application on growth of excised stems and hypocotyls and of auxin analogs in intact *Arabidopsis* seedlings.

Auxins synthesized by the plant and the microorganisms differ only in the biosynthetic pathway, depending on the plant and/or microorganism. More than 80% of soil bacteria in the rhizosphere are capable of producing auxins; thus, the potential of these microorganisms to affect the endogenous levels of this regulator and, therefore, its effects on plant growth are remarkable. Auxins principally affect plant roots (Salisbury 1994).

Those released by rhizobacteria mainly affect the root system, increasing its size and weight, branching number and the surface area in contact with soil. All these changes lead to an increase in its ability to probe the soil for nutrient exchange, therefore improving plant nutrition and growth capacity (Gutiérrez Mañero et al. 1996). Another important result of inoculation with auxin-producing bacteria is the formation of adventitious roots, which derive from the stem. The auxins induce the stem tissues to redifferentiate as root tissue. All the above effects can vary considerably depending on the auxin concentration that reaches the root system, including an excess that could be inhibitory.

Gibberellins

There is little information regarding microorganisms that produce gibberellins, although it is known that symbiotic bacteria existing within nodules in leguminous plants to fix nitrogen (rhizobia) are able to produce gibberellins, auxins and cytokinins in very low concentrations when the plant is forming the nodule and there is a high cell duplication rate (Atzorn et al. 1988). However, the production of gibberellins by PGPR is rare, with only two species being documented that produce gibberellins, *Bacillus pumilus* and *Bacillus licheniformis*.

These bacteria were isolated from the rhizosphere of *A. glutinosa* and have shown a capacity to produce large quantities of gibberellins GA_1, GA_3, GA_4 and GA_{20} *in vitro*. These types of hormones are the largest group of plant regulators, including more than 100 different molecules with various degrees of biological activity. The common structure of these diterpenic growth regulators is a skeleton of 19–20 carbon atoms, and there is a clear relationship between structure and biological effect. The reason for the pronounced effect of gibberellins is that these hormones can be translocated from the roots to the aerial parts of the plant.

The effects in the aerial part are notable and more so when the bacteria also produce auxins that stimulate the root system, enhancing the nutrient supply to the sink generated in the aerial part. The first report of gibberellin characterization in bacteria using physico-chemical methods was by Atzorn et al. (1988), who demonstrated the presence of GA_1, GA_3, GA_4 and GA_{20} in gnotobiotic cultures of *Rhizobium meliloti*. Apart from *Azospirillum* sp. and *Rhizobium* sp., production of gibberellin-like substances has also been claimed in numerous bacterial genera, although the techniques used (TLC, bioassays, HPLC-UV) are of poor resolution and/or reliability. Using unequivocal physico-chemical methods, such as GC-MS, production of gibberellins has been confirmed in *Acetobacter diazotrophicus, Herbaspirillum seropedicae* and *Bacillus* sps. . In fungi, the general pathway is similar to that of higher plants, although the genes and enzymes involved differ.

Cytokinins

Cytokinins are purine derivatives that promote and maintain plant cell division in cultures and are also involved in various differentiation processes including shoot formation, primary root growth and callus formation. Plants continuously use cytokinins to maintain the pools of totipotent stem cells in their shoot and root meristems (Leibfried et al. 2005). Endogenous cytokinin overproduction in transgenic plants causes pleiotropic phenotypic alterations including cytokinin-auxotrophic growth of calli *in vitro* (Howell et al. 2003).

Analysis of cytokinin-overproducing and cytokinin-deficient mutants has confirmed a stimulatory role for these compounds in the regulation of cell division activity in the shoot meristem and young leaves (Frank et al. 2002). Auxins and cytokinins interact in the control of many important developmental processes in plants, particularly in apical dominance, and root and shoot development. The balance between auxin and cytokinin is a key regulator of in vitro organogenesis. Exposing callus cultures to a high auxin to cytokinin ratio results in root formation, whereas a low ratio of these hormones promotes shoot development. Many experiments have demonstrated the existence of synergistic, antagonistic or additive interactions between auxins and cytokinins, suggesting complex signal interactions involved in the modulation of root and shoot architecture.

Exopolysaccharide (EPS) production

Exopolysaccharides are carbohydrate polymers that are secreted by a wide variety on plant growth promoting rhizobacteria. Thay can remain associated with the cell wall to form a bound capsule layer or they can be released in to cells surrounding as extracellular slime (Glick et al. 1999). EPS have vital roles in a variety of processes such as formation of biofilm (Bhaskar and Bhosle 2005), protection of bacterial cell from desication (Pal et al. 1999), for maintaining primary cellular functions and antibacterial activity against predators, gelling ability, pollutant degradation kinetics (Fusconi and Godinho 2002), bioremediation activity and plasma substituting capacity (Allison, 1998).

The synthesis of EPS may be related to stress. In *E.coli*, LonS, an ATP dependent enzyme known to

eliminate stress denatured protein (the lon gene belongs to the *E. coli* heat shock regulon) controls the activity of regulatory proteins like ResA, a positive transcriptional regulator of the synthesis of proteins encoded by cellular genes (Stewart et al. 1997). EPS production is reported in strains like *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Streptococcus mutans* (Vimala and Lalithakumari 2003). Swarming is an ubiquitous occurrence among beneficial bacteria belonging to different genera such as *Vibrio, Pseudomonas, Serratia, Escherichia, Bacillus and Azospirillium*.

These swarming require the presence of a capsular exopolysaccharide. For example swarming may be involved in the ability of *Proteus mirabilis* to colonise the urinary tract (Gygi et al. 1995). These polysaccharides play important roles in many biological processes, and they can function as the virulence determinants in the pathogens (Peng et al. 2008). The production of EPS is a very common trait among bacteria and is probably a critical determinant for achieving successful colonization of any surface. In addition exopolysaccharides may be involved in cell aggregation and their synthesis may increase the chances of survival for the bacteria under desiccation or nutrient deprived conditions and helps in nitrogen fixation by preventing high oxygen tention (Glick et al. 1999).

Siderophore production

Siderophore is low molecular weight compounds (400–1,500 Dalton) preferentially chelate iron (Fe^{+++}) and transport it into the cell across the cell membrane. 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 of the bacterium. Once inside the cell, the iron is released and is then available to support the microbial growth. Iron is an important micronutrient used by bacteria and it is essential for their metabolism. In the soil, it is unavailable for direct assimilation by microorganisms because ferric iron (Fe^{3+}), which predominates in nature, is only sparingly soluble and too low in concentration to support microbial growth. To survive, soil microorganisms synthesize and secrete this low-molecular iron binding compound. The siderophores bind most of the Fe^{+3} in the rhizosphere and effectively prevent the proliferation of fungal pathogens by depriving them of available iron (Kloepper et al. 1980). Suppression of the pathogens arises because iron deficiency causes growth inhibition, decrease in nucleic acid synthesis inhibition of sporulation, and causes changes in cell morphology (Mathiyazhagan et al. 2004).

After reduction and release of iron into periplasm and then to cytoplasm, ferrous is susceptible to oxidation. To prevent this ferrous ion from entering into the cell cytoplasm is readily trapped into carrier molecule and maintains in its new reduced form. Alternatively this reduced ferrous form may be directly transferred into metabolic activities before it get oxidized. Because iron is essential for survival and growth, bacteria that are better adapted to obtain iron can compete better. Competition for iron occurs on two levels; competition for ferric iron and competition for the iron siderophores complex. The former is dependent on the properties of the siderophores while the latter is a function of the outer membrane ferric siderophore receptor.

In earlier literature iron chelators like ferrichromes, ferrioxamines etc were termed as sideramines and sideromycins which are today collectively called siderophores. These molecules are also known as ionophores. Generally they are categorized into two groups 1) Hydroxamates 2) Catecholates. Winkelman and Dreschel (1997) have classified bacterial siderophores into 5 types. 1) Catecholates, 2) Hydroxamates, 3) Peptide siderophores, 4) Mycobactin, and 5) Citrate hydroxamates. Hydroxamates contain three secondary hydroxamate group. Each hydroxamate group provided 2 oxygen atoms which form a bidentate ligand with iron.

Therefore each siderophores forms a hexadentate octahedral complex with Fe^{+2}. Catecholates are chemically derivatives of 2, 3, dihydroxy benzoic acid. Each catecholate provided two oxygen atoms for chelation with iron so that a hexadentate octahedral complex is formed (Chincholkar et al. 2000). A myriad of environmental factors can also modulate the siderophore synthesis, pH, iron level and forms of iron ions, presence of trace elements, and an adequate supply of C, N, and P (Duffy and Defago 1999). Microbial siderophores vary widely in overall structure but most contain hydroxamate and catechol groups, which are involved in chelating the ferric ion (Neilands 1995). Initially, the siderophore binds to ferric iron in the external environment.

The iron-siderophore complex is then recognized by the corresponding outer membrane receptor protein. Binding of the ferric-siderophore complex induces considerable conformational changes, perhaps signaling to initiate TonB interaction. Using energy presumably provided by the TonB complex (proton motive force), the ferric-siderophore complex is actively transported into the periplasm. Once in the periplasm, the ironsiderophore complex is bound to a periplasmic binding protein that transports the complex to the ABC-type transporter in the cytoplasmic membrane, which transports the complex into the cytoplasm utilizing energy from the hydrolysis of ATP (Figure 4). Iron is released from the siderophore by either reduction via ferric reductases, or by chemical modification or breakdown of ferric siderophore complexes by acetylation and esterases, respectively (Neilands et al. 1987). Indirectly rhizobacteria helps plant growth by releasing biocontrol agents for protecting plants against phytopathogens (Jha and Saraf, 2011)

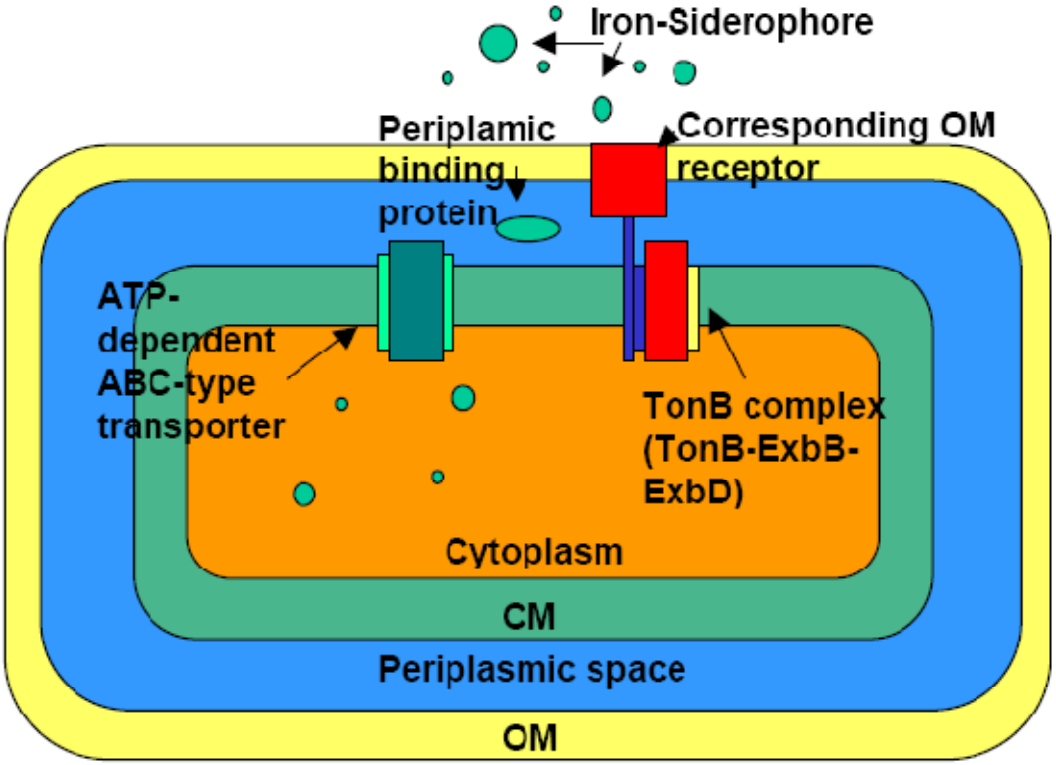

Figure 4: General Siderophore-Mediated Iron Transport in a Gram-Negative Cell. Adapted from Clark (2004).

The capacity to utilize siderophores is important for the growth of bacteria in the rhizosphere (Jurkevitch et al. 1992) and on the plant surface. Specific siderophore producing *Pseudomonas* species strains rapidly colonized roots of several crops and resulted in increased yield (Schroth and Hancock 1982). Enhanced plant growth caused by *Pseudomonas* strains was often accompanied by the reduction in pathogen populations on the roots. There is convincing evidence to support a direct role of siderophore mediated iron competition in the biocontrol activity exhibited by such isolates.

The antagonism depends on the amount of iron available in the medium, siderophores produced by a biocontrol agent and sensitivity of target pathogens. Siderophore-producing rhizobacteria improve plant health at various levels: they improve iron nutrition, inhibit growth of other microorganisms with release of their antibiotic molecule and hinder the growth of pathogens by limiting the iron available for the pathogen, generally fungi, which are unable to absorb the iron siderophore complex.

HCN and Ammonia production

Hydrogen cyanide is formed during the early stationary growth phase. It does not take part in growth, energy storage or primary metabolism, but is generally considered to be a secondary metabolite that has an ecological role and confers a selective advantage on the producer strains (Vining 1990). The production of HCN was a more common trait of *Pseudomonas* (88.89%) (Ahmad et al. 2008). Cyanide occurs in solution as free cyanide, which includes the cyanide anion (CN^-) and the non-dissociated HCN. Cyanide is a phytotoxic agent capable of inhibiting enzymes involved in major metabolic processes and is considered one of the typical features of deleterious rhizobacterial isolates (Bakker and Schippers 1987). Nevertheless, at present its applications in areas of biocontrol methods are increasing (Devi et al. 2007). Some cyanogenic rhizobacteria are typically host specific and associated with the roots of their host plants. Therefore, HCN produced in the rhizosphere of seedlings by selected rhizobacteria is a potential and environmentally compatible mechanism for biologically controlling weeds and minimizing deleterious effects on the growth of desired plants (Kremer and Souissi 2001).

Ammonia production is related with the nitrogen fixation and mostly observed in the leguminous rhizobacteria. Important biochemical reactions of biological nitrogen fixation occur mainly through symbiotic association of nitrogen fixing microorganisms with legumes that converts atmospheric elemental nitrogen (N_2) into ammonia (NH_3). A range of plant growth promoting

rhizobacteria participate in interaction with C3 and C4 plants (e.g., rice, wheat, maize, sugarcane, Jatropha and cotton), and significantly increase their vegetative growth and grain yield (Kennedy et al. 2004). *Azospirillum* species are aerobic heterotrophs that fix N_2 under microaerobic conditions and grow extensively in the rhizosphere of gramineous plants (Kennedy et al. 2004). ^{15}N tracer techniques showed that *Azospirillum brasilense* and *Azospirillum lipoferum* contributed 7–12 % of wheat plant N by biological nitrogen fixation (Malik et al. 2002). Inoculation with Azospirillum brasilense significantly increases N contents of cotton up to 0.91 mg plant^{-1} (Fayez and Daw 1987). Inoculation with *Azospirillum* also significantly increased N content of sugarcane leaves in greenhouse experiments (Muthukumarasamy et al. 1999) which reflects the production of ammonia by *Azospirillum.*

Production of 1-aminocyclopropane-1-carboxylase

Plant growth promoting rhizobacteria (PGPR) contain a vital enzyme, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (EC 4.1.99.4), which regulates ethylene production by metabolizing ACC (an intermediate precursor of ethylene biosynthesis in higher plants) into a-ketobutyrate and ammonia (Shaharoona et al. 2007). The microbial enzyme 1-aminocyclopropane-1-carboxylate deaminase cleaves ACC irreversibly, this being the immediate precursor of ethylene in plants (Saraf et al. 2010).

This enzyme facilitates plant growth as a consequence of the fact that it sequesters and cleaves plant produced ACC, thereby lowering the level of ethylene in the plant. In turn, decreased ethylene levels allow the plant to be more resistant to a wide variety of environmental stresses, all of which induce the plant to increase its endogenous level of ethylene; stress ethylene exacerbates the effects of various environmental stresses.

The ACC deaminase-containing soil bacteria decrease a significant portion of the physiological damage to plants following environmental stresses including phytopathogen infection, exposure to extremes of temperature, high salt, flooding, drought, exposure to metals and organic contaminants, and insect predation. For many plants a burst of ethylene is required to break seed dormancy but, following germination, a sustained high level of ethylene can be inhibitory to root elongation. PGPB that contain the enzyme ACC deaminase, when bound to a plant root or to the seed coat of a developing seedling, may act as a mechanism for insuring that the ethylene level within the plant's tissues does not become elevated to the point where root (or shoot) growth is impaired. By facilitating the formation of longer roots and shoots, these bacteria may enhance the survival of some seedlings, especially during the first few days after the seeds are planted. The bacterial enzyme activity is localized only in the cytoplasm (Jacobson et al. 1994). ACC deaminase activity has been induced in both *Pseudomonas* sp. strain ACP and *P. putida* GR12-2 by ACC, at levels as low as 100 nM (Jacobson et al. 1994) both bacterial strains were grown on a rich medium and then switched to a minimal medium containing ACC as its sole nitrogen source. The rate of induction, similar for the enzyme from the two bacterial sources was relatively slow, complete induction required 8 to 10 hour. Enzyme activity increased only approximately 10 fold over the basal level of activity, even when the concentration of ACC increased up to 10,000-fold. Pyridoxal phosphate is a tightly bound cofactor of ACC deaminase in the amount of approximately 3 mol of enzyme-bound pyridoxal phosphate per mole of enzyme, or 1 mol per subunit (Honma 1985). ACC deaminase enzymatic activity is quantified by monitoring the production of either ammonia or α-ketobutyrate, the products of ACC hydrolysis (Honma and Shimomura 1978). However, at present, monitoring the amount of α-ketobutyrate is more widely used by researchers. The presence of ACC deaminase was also verified by FTIR (Fourier Transform Infrared) spectra. FT-IR spectra clearly shows the peak at 1683 cm^{-1} which shows that ketonic group is present (–C=O). Whereas 3452 cm^{-1} peak shows that the presences of amino group (–NH2) (Jha et al. 2012)

To date, ACC deaminase has been detected only in microorganisms; and no microorganism is known to synthesize ethylene via ACC (Fukuda et al. 1993). However, there is strong evidence that the fungus, *Penicillium citrinum*, produces ACC from SAM via ACC synthase, one of the enzymes of plant ethylene biosynthesis, and degrades the ACC by ACC deaminase. It appears that the ACC, which accumulates in the intracellular spaces of this fungus, can induce ACC deaminase (Jia et al. 2006). In addition, throughout the many years that plants and microorganisms have been associated with each other, some plants may have obtained microbial ACC deaminase genes. However, at the present time, there are no reports of ACC deaminase activity occurring naturally in plants.

PGPR that contain the enzyme ACC deaminase, when bound to the seed coat of a developing seedling, act as a mechanism for ensuring that the ethylene level does not become elevated to the point where initial root growth is impaired. By facilitating the formation of longer roots, these bacteria may enhance the survival of some seedlings, especially during the first few days after the seeds are planted. In addition, plants that are treated with ACC deaminase-containing PGPR are dramatically more resistant to the deleterious effects of stress ethylene that is synthesized as a consequence of stressful conditions such as flooding (Grichko and Glick 2001), heavy metals (Grichko et al. 2000), the presence of phytopathogens (Wang et al. 2000), and drought and high salt. In each of these cases the ACC deaminase-containing PGPR

markedly lowered the level of ACC in the stressed plants thereby limiting the amount of stress ethylene synthesis and hence the damage to the plant. These bacteria are beneficial to plant growth since in the natural environment plants are often subjected to ethylene producing stresses. However, it should be emphasized that ACC deaminase-containing PGPR facilitate plant growth to a much greater extent with plants that are ethylene sensitive such as canola, peppers and tomatoes. It is expected that this activity will be useful in both agricultural and horticultural settings, as well as in environmental cleaning (phytoremediation) protocols.

Conclusion and Future Prospects

Direct interactions occurring between members of different microbial types often result in the promotion of key processes benefiting plant growth and health. Syntrophic relationships between different organisms have been demonstrated in several microbial ecosystems. Therefore, mixed inoculants (combination of microorganisms) that interact synergistically are currently being devised, which yield better and quick results (Bashan 1998). Recently, a microbial consortium for plant growth promotion was suggested (Seneviratne 2003).

It has been suggested that development of plant growth promoting consortium (PGPC), could be a feasible strategy for increased activity and better viability of plant growth promoting rhizobacteria (PGPR). When these strains are made into an inoculum consortium, each of the constituent strains of the consortium not only out competes with the others for rhizospheric establishments, but complement functionally for plant growth promotion (Shenoy and Kalagudi 2003). Co-inoculation of some Rhizobacteria resulted in enhanced nodulation and plant growth.

A variety of rhizosphere microorganisms, including *Bacillus*, *Pseudomonas, Acinetobacter, Azospirillum,and Enterobacter* species, are commonly found in the rhizosphere of leguminous and nonleguminous crops. The three isolates *B. brevis* (MS1), *B. licheniformis* (MS3), *A. calcoaceticus* (MS5) have the ability to produce IAA, solubilize inorganic P, and produce ACC deaminase and siderophores. They enhanced the growth of *Jatropha. curcas* in individual trials. Plant growth was further improved maximally when the three were applied together (Jha and Saraf 2012). By virtue of their rapid colonization of the rhizosphere and stimulation of plant growth, there is currently considerable interest in exploiting these rhizosphere bacteria to improve crop production.

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