REVIEW OF LITERATURE

The use of PGPR inoculants as biofertilizers provides a promising alternative to chemical fertilizers and pesticides (Kloepper, 1993, Kloepper and Adesemaye, 2009). The research on PGPR backs to 1950s when first study on PGPR was carried out. Since then, hundreds of different PGPR strains had been screened and evaluated in laboratory, greenhouse and field conditions all over the world (Zehnder et al., 2001). These microorganisms played important role in agriculture to promote the exchange of plant nutrients and reduce application of chemical fertilizers.

This type of beneficial plant-microbe interactions in the rhizosphere had influenced plant vigour and soil fertility by stimulating plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, improving soil structure and bioremediating the polluted soils by sequestering toxic heavy metal species and degrading xenobiotic compounds (like pesticides) (Bhattacharyya and Jha, 2012). Even, it was reported that with few exceptions, the sequestration of ferric iron by bacteria makes soils suppressive (Rovira, 1956) for some plant pathogens. One of such example was reported by Hornby, who suggested the antagonism due to soil microflora. It was the reason why the take-all fungus never shows its full capacity in normal soils (Hornby, 1983). A soil in which plants do not suffer from certain diseases or where disease severity is reduced, although a pathogen might be present. Such exceptional places exist and are known as natural suppressive soils (Haas and Défago, 2005). Further, suppressive soil can be considered as a promising hunting ground for antagonistic microorganisms, which resides in the rhizosphere.

2.1 RHIZOSPHERE

In 1904, Lorenz Hiltner the German agronomist and plant physiologist first coined the term "rhizosphere" to describe the plant-root interface, a word originating in part from the Greek word "rhiza", meaning root (Hiltner, 1904; Hartmann et al., 2008). Hiltner described the rhizosphere as the area around a plant root that is inhabited by a unique population of microorganisms influenced, by the chemicals released from plant roots.

The properties of the soil in close vicinity to plant roots, in rhizosphere are modified by a range of different processes occurring during plant growth, which in turn affect the
rhizospheric microbiota. Roots release polymerized sugar (that is, mucilage), low-molecular-mass compounds (that is, sugars, amino acids and organic acids), root border cells and dead root cap cells. These compounds (rhizodeposits) are used as carbon sources by soil microorganisms and represent approximately 25% of the carbon allocated to the roots in cereals and grasses (Jones et al., 2009). Rhizodeposits also contain secondary metabolites, such as antimicrobial compounds, nematicides and flavonoids (Oldroyd et al., 2013; Bais et al., 2006) which are involved in establishing symbiosis or in inhibiting pathogens and pests. Rhizosphere microflora can also directly or indirectly affect the composition and biomass of plant communities in natural ecosystems (Kardol et al., 2007; Schnitzer et al., 2011).

2.2 PLANT GROWTH PROMOTING RHIZOBACTERIA

Plant growth-promoting rhizobacteria (PGPR), are beneficial bacteria that colonize plant roots and enhance plant growth through a variety of mechanisms that include improvement of plant nutrition, production and regulation of phytohormones, and suppression of disease causing organisms (Ngoma et al., 2012). In last few decades, a large array of bacteria including species of Alcaligenes, Burkholderia, Aeromonas, Azotobacter, Arthrobacter, Gluconacetobacter, Pseudomonas, Serratia, Azoarcus, Azospirillum, Acinetobacter, Klebsiella, Bacillus, Enterobacter and Clostridium are considered as most important plant growth promoting rhizobacteria (PGPR) because they have beneficial effects on plants directly and indirectly by enhancing soil fertility (for example, increasing the amount of available nitrogen, and phosphorus and other plant nutrients); synthesizing several different phytohormones such as indole-3-acetic acid (IAA) that can enhance various stages of plant growth; suppressing soil-borne pathogens by the production of hydrogen cyanide, siderophores, antibiotics, and or competition for nutrients; and improving plant stress tolerance to drought, salinity, and metal toxicity. Moreover, some PGPR have the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyses ACC, the immediate precursor of ethylene in plants. In recent years, the concept of PGPR-mediated plant growth promotion is gaining worldwide importance and acceptance (Babalola, 2002; Albino et al., 2006; Gnanamanickam, 2006; Wang et al., 2006; Babalola and Akindolire, 2011; Kucerova et al., 2011). Biofertilization accounts for
approximately 65% of the nitrogen supplied to crops worldwide and 20 to 50% of the total soil organic phosphorus (Bloemberg et al., 2001; Richardson et al., 2001a). Beneficial effects of PGPRs have been reported by various workers worldwide on a wide range of crops including cereals, pulses, vegetables, oilseeds and plantation crops (Muthuraju and Jaysheela, 2005).

Now days, these bacteria are used to sustain agriculture as biofertilizers and biocontrol agents (Babalola, 2010a). Several different studies have depicted proteobacteria especially bacteria from family Pseudomonadaceae or Burkholderaceae as dominant members of rhizosphere microflora (Mendes et al., 2011; DeAngelis et al., 2009; Gomes et al., 2001; Sharma et al., 2005; Peiffer et al., 2013; Uroz et al., 2010).

Several organisms contribute to these processes, leading to countless interaction between plants, antagonists and mutualistic symbionts, both below ground and above ground (Bennett et al., 2007; Hol et al., 2010; Behie et al., 2012).

2.3 ROOT EXUDATES AND ITS REGULATION

The main functions of the ‘hidden’ part of the plant its root system, have traditionally been thought to be anchorage and absorption of water and ions, nutrient storage, and plant vegetative growth. However, roots secrete an enormous range of compounds into the surrounding soil (Lynch, 1987). In addition to the compounds that roots synthesize and accumulate (Flores et al., 1999; Bais et al., 2001), a remarkable diversity of micro- and macromolecular metabolites is also secreted into the rhizosphere as root exudates (Bais et al., 2001).

The chemicals which are secreted by roots into the surrounding soils are generally called as root exudates. The exudates in the form of a wide range of chemical compounds modifies the chemical and physical properties of the soil and thus, regulates the structure of soil microflora in the immediate vicinity of root surface (Dakora and Phillips, 2002). In fact, some of the exudates act as repellants against different microorganisms while others act as attractants to lodge the microbes. The composition of these exudates is dependent upon the physiological status and species of plants and microorganisms (Kang et al., 2010).

Another study reported that these exudates also promote the plant-beneficial symbiotic interactions and inhibit the growth of the competing plant species (Nardi et al., 2000).
Plants depend on the ability of roots to communicate with microbes. Many bacteria and fungi are dependent on associations with plants that are often regulated by root exudates. A chemotactic response towards root-secreted organic and amino acids is the first step in root colonization by the bacteria (Zheng et al., 1996). Root exudates play an active and relatively well-documented role in the regulation of symbiotic and protective interactions with microbes (Hirsch et al., 2003; Buee et al., 2000; Neumann et al., 2002; Jones et al., 2003). The diversity of the microbial (bacterial and fungal) communities in soil is extraordinary, and 1 g of soil could contain more than 10 billion microorganisms belonging to thousands of different species (Roselló-Mora & Amann, 2001). Plant adaptation and survival in a given environment are primarily determined by the ability of an individual to acquire resources (Aerts, 1999).

The root system plays a big role in acquisition of resources in a natural heterogeneous soil environment (Lynch & Brown 2001). Root system architecture (RSA) changes in nutrient-rich patches of soil such as that found under conditions of high nitrate and phosphorus (Ho et al. 2005; Paterson et al. 2006). In addition, the release of organic compounds from roots is a key factor in internalizing acquired nutrients and in mediating plant–microbe interactions (Pierret et al. 2007). Therefore, modulating growth and root branching in regions of nutrient-rich patches may be expected to be coincident with increased root exudation that could affect the nutrient dynamics and microbial community (Paterson et al. 2006).

2.4 MACHANISMS INVOLVED IN PLANT GROWTH PROMOTION BY PGPR

PGPR is the most widely studied group of plant growth promoting bacteria. Beneficial mechanisms by which PGPR enhance plant growth and health are classified into direct and indirect (Ngoma et al., 2012). Plant growth promotion occurs by the alteration of the whole microbial community in rhizospheric region through the production of various substances by PGPR (Kloepper and Schroth, 1981). Generally, PGPR promote plant growth directly by either enhancing plant’s nutritional status facilitating resource acquisition (nitrogen, phosphorus and essential minerals) and stimulating systemic disease resistance mechanisms or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol
agents; chelation of available Fe in the rhizosphere, synthesis of extracellular enzymes that hydrolyze the fungal cellular wall and competition for niches within the rhizosphere (Zahir et al., 2004; Glick et al., 2007, Glick, 2012).

**Direct effect includes:** Phytostimulator and biofertilizer.

**Indirect effect includes:** Biopesticide or biocontrol agents.

### 2.4.1 DIRECT MECHANISM

Plant growth-promoting rhizobacteria are associated with many, if not all, plant species and are commonly present in many environments. Bacteria that colonize plant roots can be function as deleterious or beneficial rhizobacteria. Deleterious bacteria inhibit plant growth while beneficial bacteria PGPR promote the growth of plants through various mechanisms. Direct beneficial mechanisms can be demonstrated by root colonization, production of plant regulators, nitrogen fixation and increasing uptake of minerals (Ngoma et al., 2012).

**(a) Plant growth regulators**

The production of different phytohormones by PGPR is considered to be an important mechanism by which the bacteria promote plant growth, from seed germination to plant senescence (Vessey, 2003). The determination of endogenous concentrations of hormones is essential to elucidate the role of a particular hormone in any physiological process. It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites (Patten and Glick, 1996). The mechanisms by which PGPR enhance plant growth is through the production of various phytohormones such as indole-3-acetic acid (IAA), auxins, ethylene, cytokinin and gibberellin within the plant root zone (Gnanamanickam, 2006). These are known to function as coordinators of plant growth and development (for example, regulating the density and length of root hairs, thereby increasing the root surface zone which improves absorption of water and nutrients from the soil) (Gray and Smith, 2005). Among them, the most and well-studied are auxins and IAA (Gnanamanickam, 2006).

The plant growth regulator, IAA, is a natural auxin with vast physiological effects which play an important role in plant growth and development, including cell division, cell
elongation, cell differentiation, tropism, flower development, and plant vascular system patterning (Gravel et al., 2007). IAA is synthesized through L-tryptophan metabolism by plants and many soil microorganisms such as PGPR, fungus and algae. Root tissues are more sensitive to fluctuating concentrations of IAA than other plant tissues (Tanimoto, 2005). Several groups (Patten and Glick, 2002; Gravel et al., 2007) have supported this statement and demonstrated that the microorganisms commonly found in the rhizosphere of plants such as Pseudomonas spp. and Rhizobium spp. are often associated with their potential to stimulate plant growth by the production of IAA.

PGPR capable of degrading IAA might have a positive effect on plant growth. However, in a report on the utilization of IAA for growth by P. putida strain 1290, it was concluded that the strain has the potential to manipulate IAA concentrations in its interaction with plants and to stimulate plant growth as seed inoculant (Gravel et al., 2007). Furthermore, a study on the effect of P. putida through the production or degradation of IAA on tomato growth demonstrated that the bacteria had the potential to promote the reproductive growth of tomato plants. However, the synthesis of high quantities of IAA by PGPR has been shown to inhibit the growth of roots rather than promote it (Gravel et al., 2007).

Evidently, IAA also acts as a reciprocal signaling molecule affecting gene expression in several microorganisms. Consequently, IAA plays a very important role in rhizobacteria-plant interactions (Spaepen and Vanderleyden, 2011). Moreover, down-regulation of IAA as signaling is associated with the plant defense mechanisms against a number of phyto-pathogenic bacteria as evidenced in enhanced susceptibility of plants to the bacterial pathogen by exogenous application of IAA or IAA produced by the pathogen (Spaepen and Vanderleyden, 2011). IAA has been implicated in virtually every aspect of plant growth and development, as well as defense responses. This diversity of function is reflected by the extraordinary complexity of IAA biosynthetic, transport and signaling pathways (Santner et al., 2009).

(b) Regulating plant ethylene level

Ethylene is a unique plant growth hormone found only in gaseous form and produced endogenously by almost all plants and also by PGPR (Babalola, 2010b). Ethylene
is involved in the regulation of numerous physiological processes in plants including seed dormancy, shoot and root growth differentiation, adventitious root formation, leaf and fruit abscission, induction of flowering and increased femaleness in dioecious plants, flower and leaf senescence, and fruit ripening (Babalola, 2010b). However, stress conditions such as wounding, drought, chilling temperature, exposure to chemicals and pathogen attack may induce the production of ethylene substantially with a net result of increasing root development (Gnanamanickam, 2006; Babalola, 2010b).

On the other hand, overproduction of this hormone has inhibitory effects on root development and may lead to abnormal growth of the plants. It is important to monitor the ethylene concentration in plant roots for normal growth and development of the plants (Saleem et al., 2007). To synthesize this hormone, plants need a precursor. Methionine has been identified as a biochemical and immediate precursor which is converted into ethylene via 1-aminocyclopropane-1-carboxylate (ACC) (Nazli et al., 2008). It has been discovered that some PGPR possess the enzyme ACC deaminase which can cleave ACC, the immediate precursor of ethylene in plants, to α-ketobutyrate and ammonia. The products of this hydrolysis are used by the ACC-degrading bacteria as nitrogen and carbon sources, and thereby, lower the level of ethylene in a developing seedling or stressed plant.

Bacteria such as Alcaligenes sp., Bacillus pumilus, Pseudomonas sp., Variovorax paradoxus, Azorhizobium caulinodans, Azospirillum spp., Gluconacetobacter diazotrophicus, Herbaspirillum spp. and Burkholderia vietnamiensis were identified by their ability to grow on minimal media containing ACC as the sole nitrogen source (Dobbelaere et al., 2003). Recently, expression of ACC deaminase activity was found in many strains of B. unamae and B. vietnamiensis, and the ACC deaminase gene (acdS) was also detected in these species as well as in B. phymatum, B. xenovorans and B. caribiensis. In general, a decreased level of ACC results in a lower level of endogenous ethylene, which eliminates the inhibitory effect of high ethylene concentrations (Shaharoona et al., 2006).

(c) Improvement of nutrient availability

Microorganisms having mechanisms that facilitate nutrient uptake or increase nutrient availability or stimulate plant growth are commonly referred to as biofertilizers.
(i) Nitrogen fixation

Nitrogen (N) is the most vital nutrient for plant growth and productivity. Although, there is about 78% N\textsubscript{2} in the atmosphere, which is unavailable to the growing plants. The atmospheric N\textsubscript{2} is converted into plant-utilizable forms by the process of biological N\textsubscript{2} fixation (BNF) which changes nitrogen to ammonia by nitrogen fixing microorganisms using a complex enzyme system known as nitrogenase (Kim and Rees, 1994). In fact, BNF accounts for approximately two-thirds of the nitrogen fixed globally, while the rest of the nitrogen is industrially synthesized by the Haber–Bosch process (Rubio and Ludden, 2008). Biological nitrogen fixation occurs, generally at mild temperatures, by nitrogen fixing microorganisms, which are widely distributed in nature (Raymond \textit{et al.}, 2004). Furthermore, BNF represents an economically beneficial and environmentally sound alternative to chemical fertilizers (Ladha \textit{et al.}, 1997).

Nitrogen fixing organisms are generally categorized as (a) symbiotic N\textsubscript{2} fixing bacteria including members of the family rhizobiaceae which forms symbiosis with leguminous plants (e.g. rhizobia) (Ahemad and Khan, 2012d; Zahran, 2001) and non-leguminous trees (e.g. Frankia) and (b) non-symbiotic (free living, associative and endophytes) nitrogen fixing forms such as cyanobacteria (Anabaena, Nostoc), Azospirillum, Azotobacter, Gluconoacetobacter diazotrophicus and Azocarbus etc. (Bhattacharyya and Jha, 2012). However, non-symbiotic nitrogen fixing bacteria provide only a small amount of the fixed nitrogen that the bacterially-associated host plant requires (Glick, 2012). PGPR that fix N\textsubscript{2} in non-leguminous plants are also called as diazotrophs capable of forming a nonobligate interaction with the host plants (Glick \textit{et al.}, 1999). The process of N\textsubscript{2} fixation is carried out by a complex enzyme, the nitrogenase complex (Kim and Rees, 1994). Structure of nitrogenase was elucidated by Dean and Jacobson (1992) as a two-component metalloenzyme consisting of (i) dinitrogenase reductase which is the iron protein and (ii) dinitrogenase which has a metal cofactor. Dinitrogenase reductase provides electrons with high reducing power while dinitrogenase uses these electrons to reduce N\textsubscript{2} to NH\textsubscript{3}.

Based on the metal cofactor three different N fixing systems have been identified (a) Mo-nitrogenase, (b) V-nitrogenase and (c) Fe-nitrogenase. Structurally, N\textsubscript{2}-fixing system varies among different bacterial genera. Most biological nitrogen fixation is carried
out by the activity of the molybdenum nitrogenase, which is found in all diazotrophs (Bishop and Jorerger, 1990).

(ii) Phosphorus solubilization

Phosphorus (P) is the second most important nutrient required by plant after nitrogen (Donahue et al., 1990). It exists in nature in variety of organic and inorganic forms. P availability is low in soils because of its fixation as insoluble phosphates of iron, aluminium and calcium. Most of the insoluble P forms are present as aluminum and iron phosphates in acid soils (Mullen, 2005), and calcium phosphates in alkaline soils (Goldstein and Krishnaraj, 2007). Indian soils are normally deficient in available phosphorus even though the bound component sufficiently abundant (Johri et al., 2003). Since deficiency of P is the most important chemical factor restricting plant growth, chemical phosphatic fertilizers are widely used to achieve optimum yields. Soluble forms of P fertilizer used are easily precipitated as insoluble forms, this leads to excessive and repeated application of P fertilizer to cropland. P supply through biological means is a viable alternative, phosphate solubilizing bacteria (PSB), phosphate solubilizing fungi (PSF) and actinomycetes that have been reported to be active in conversion of insoluble phosphate to soluble primary and secondary orthophosphate ions by many investigators (Chabot et al., 1993; Pal, 1998).

Although mechanism of phosphate solubilization is still not fully understood, several mechanisms have been implicated in the process. The production of organic acids seems to be the main mechanism (Illmer et al., 1995). These microorganisms secrete different types of organic acids e.g., carboxylic acid, gluconate, citrate, lactate and succinate (Deubel and Merbach, 2005) thus lowering the pH in the rhizosphere (He and Zhu, 1988) and consequently dissociate the bound forms of phosphate like Ca3 (PO4)2 in calcareous soils. Microorganisms are able to hydrolyze a wide range of organic P substrates when grown in culture and when added to soil different forms of organic P have been shown to be rapidly mineralized (Adams and Pate, 1992; Macklon et al., 1997; and Richardson, et al., 2005). The plant takes up several P forms but major part is absorbed in the forms of HPO4−2 or H2PO4−1. These microorganisms grow in media with tricalcium phosphate or similar insoluble materials as the only phosphate source and not only
assimilate the element but also solubilize quantities in excess of their nutritional demands there by making it available for plants (Chen et al., 2006).

On the other hand organic P can constitute between 30 and 50% of the total P of the soil, a high proportion of it corresponding to phytate (Borie et al., 1989; Turner et al., 2002). In this context there are bacteria capable of producing phytase enzymes for the mineralization of phytates (Lim et al., 2007; Jorquera et al., 2008b). Among the phytase producing rhizobacteria, species belonging to Bacillus, Burkholderia, Enterobacter, Pseudomonas, Serratia and Staphylococcus genera are the most common culturable bacteria (Richardson and Hadobas, 1997; Hussin et al., 2007; Shedova et al., 2008). Richardson and Hadobas (1997) isolated Pseudomonas spp. that utilized phytate from different soils in Australia.

The isolated strains exhibited a high phytase activity, releasing over 80% of the P content in the phytate. In a later study obtain the P from phytate, Richardson et al. (2001a) observed that the ability of pasture plants to acquire P from phytate was enhanced followed by inoculation with the specified Pseudomonas sp. strains. Similarly, Unno et al. (2005), isolated diverse bacteria with the ability to utilize phytate from the rhizosphere from white lupin (Lupinus albus). Almost all the isolates were classified as members of the Burkholderia genus and some of them significantly promoted the growth of the lupin (Jorquera et al. 2008a) isolated P solubilizing bacteria from the rhizospheres of five cultivated plants (Lolium perenne, Trifolium repens, Triticum aestivum, Avena sativa, Lupinus luteus), which presented more than one mechanism for utilizing insoluble forms of phosphorus. Moreover, all strains showed the capacity to produce P hydrolases. The major limitation today for use of these organisms is the lack of consistent effects in mobilizing P under field conditions. This is likely due to competition with the native microflora and environmental factors that either limit the population size or activity of the PGPR.

It is now clear from many studies that evaluation and ranking of P solubilizing bacteria under laboratory conditions do not necessarily correspond to the efficacy of the PGPR for enhancing plant P uptake under field conditions (Richardson, 2001b; Rengel, 2008). As with nitrogen fixing bacteria, the production of plant growth hormones that improve root surface area can have indirect effects on the ability to efficiently extract P.
from soil. Thus, it is likely that many so-called biofertilizers have dual action effects that are mediated by direct solubilization of inorganic P, mineralization of organic P, and stimulatory effects on plant root growth or mycorrhizae formation.

2.4.2 INDIRECT MECHANISMS OF ACTION

Indirect mechanisms of action involve the ability of PGPR to: (a) produce antibiotics; (b) successfully compete with pathogens for nutrients on the root; (c) induce systemic resistance by activating the plant defences (Lugtenberg and Kamilova, 2009); and (d) produce siderophores, lytic enzymes, cyanide and ammonia.

(a) Antibiotic production

The production of antibiotics is considered one of the most powerful and studied biocontrol tools that PGPR can use to combat proliferation of phytopathogens. Antibiotics constitute a wide and heterogeneous group of low molecular weight chemical organic compounds that are produced by a wide variety of microorganisms (Raaijmakers et al., 2002). Under laboratory conditions many different types of antibiotics produced by PGPR have shown to be effective against phytopathogenic agents (Bowen and Rovira, 1999). Antibiotic production is considered as one of the most important trait of bacteria. In the past years, many different types of antibiotics produced by PGPR including butyrolactones, zwittermycin A, kanosamine, oligomycin A, oomycin A, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin, viscosinamide, xanthobaccin, and 2,4-diacetyl phloroglucinol (2,4-DAPG) have been shown to be effective against phytopathogenic agents (Whipps, 2001). Among them, 2, 4-DAPG is one of the most efficient antibiotics in the control of plant pathogens and can be produced by various strains of Pseudomonas (Fernando et al., 2006). The 2,4-DAPG has a wide spectrum of properties in that it is antifungal (Loper and Gross, 2007; Rezzonico, et al., 2007), antibacterial (Velusamy et al., 2006) and antihelmintic (Cronin et al., 1997). In soils, it suppresses the growth of the wheat pathogenic fungus Gaeumannomyces graminis var. tritici, (Raaijmakers et al., 1999) reported a production of 0.62 ng 2,4-DAPG per 105-107 CFU g-1 root by P. fluorescens, strain Q2-87.
(b) Hydrogen cyanide (HCN) Production

HCN is a volatile, secondary metabolite that suppresses the development of microorganisms and that also affects negatively the growth and development of plants (Siddiqui et al., 2006). HCN is a powerful inhibitor of many metal enzymes, especially copper containing cytochrome C oxidases. HCN is formed from glycine through the action of HCN synthetase enzyme, which is associated with the plasma membrane of certain rhizobacteria (reviewed by Blumer and Haas, 2000). Various studies attribute a disease protective effect to HCN, e.g. in the suppression of “root-knot” and black rot in tomato and tobacco root caused by the nematodes Meloidogyne javanica and Thielaviopsis basicota, respectively (Voisard et al., 1989; Siddiqui et al., 2006). The subterranean termite Odontotermes obesus, an important pest in agricultural and forestry crops in India, is also controlled by HCN (Devi et al., 2007). However, there are investigations reporting harmful effects on plants, inhibition of energy metabolism of potato root cells (Bakker and Schippers, 1987), and reduced root growth in lettuce (Alström and Burns, 1989). Likewise, HCN produced by Pseudomonas in the rhizosphere inhibits the primary growth of roots in Arabidopsis due to the suppression of an auxin responsive gene (Rudrappa et al., 2008).

(c) Siderophore production

Iron is an essential element to virtually all forms of life and plays an important role in different physiological processes such as respiration, photosynthesis, DNA synthesis and defence against reactive oxygen species (Dellagi et al., 2009). However, its availability is extremely limited by very low solubility of ferric hydroxide complexes at neutral pH (Wensing et al., 2010). To survive in such an environment, plant- associated PGPR have different strategies of obtaining iron from the soil, which include the synthesis of siderophores which are selective ferric ion chelators. These low molecular weight compounds are secreted in response to iron deficiency (Dellagi et al., 2009; Wensing et al., 2010). Siderophore producing PGPR can prevent the proliferation of pathogenic microorganisms by sequestering Fe3+ in the area around the root (Siddiqui, 2006). Fe depletion in the rhizosphere does not affect the plant, as the low Fe concentrations occur at microsites of high microbial activity during establishment of the pathogen. Many plants can use various bacterial siderophores as iron sources, although the total concentrations are
probably too low to contribute substantially to plant iron uptake. Plants also utilize their own mechanisms to acquire iron; dicots via a root membrane reductase protein that converts insoluble Fe3+ into the more soluble Fe2+ ion, or in the case of monocots by production of phytosiderophores (Crowley, 2006).

Carrillo-Castañeda et al. (2002) reported positive effects on alfalfa plantlet growth after the inoculation of siderophore producing Pseudomonas, Rhizobium and Azospirillum grown in iron limited cultures. The inoculated alfalfa seeds increased their germination as well as the root and stem dry weight. Nevertheless, as with other PGPR, the growth promotion that occurred may be due to other mechanisms or combinations of mechanisms that increase nutrient availability, suppress pathogens, or affect root growth via hormone production.

(d) Induced systemic resistance

Interaction of some bacteria with the plant roots can result in plants resistant to some pathogenic bacteria, fungi, and viruses. This phenomenon is called induced systemic resistance (ISR). ISR shares many properties with innate immunity in humans (Lugtenberg and Leveau, 2007). When plants are growing, their roots enter quickly into a symbiosis with diverse microorganisms. This symbiosis may play the role of beneficial (aid in the uptake of water and minerals, such as phosphate, and protection of biotic and abiotic stress) or pathogenic agents in the development of plants (Gnanamanickam, 2006). In case of pathogenic bacteria, the immune response of the plant is characterized by the production of salicylic acid, which in revenge, induces a set of genes encoding pathogenesis-related proteins in the plant (Gnanamanickam, 2006).

Some strains of PGPR were shown to act as inducing agents in different plants by producing salicylic acid which is responsible for the induction of induced systemic resistance in plants. Induced systemic resistance was observed first with Pseudomonas sp. strain WCS417r against Fusarium wilt of carnations and by selected rhizobacteria against the fungus Colletotrichum orbiculare in cucumber (Compant et al., 2005). Available reports showed that in rice, seed-treatment followed by root-dipping and a foliar spray with P. fluorescens strains Pf1 and FP7 induce systemic resistance against the sheath blight pathogen, R. solani. PGPR can also induce systemic protection against bacterial diseases.
Seed treated with *P. fluorescens* strain 97 protected beans against halo blight disease caused by *P. syringae pv. phaseolicola* (Gnanamanickam, 2006). ISR was discovered by the findings that resistance can be induced by the rhizobacterium *Pseudomonas* sp. strain WCS417r against *Fusarium* wilt of carnation (Van Peer *et al*., 1991) and by selected rhizobacteria against the fungus *Colletotrichum orbiculare* in cucumber (Wei *et al*., 1991). ISR is dependent on jasmonic acid and ethylene signaling in the plant (Van Loon *et al*., 2007).

**(e) Lytic Enzymes**

Diverse microorganisms secrete and excrete other metabolites that can interfere with pathogen growth and/or activities. Many microorganisms produce and release lytic enzymes that can hydrolyze a wide variety of polymeric compounds, including chitin, proteins, cellulose, hemicellulose, and DNA. Expression and secretion of these enzymes by different microbes can sometimes result in the suppression of plant pathogen activities directly. For example, control of *Sclerotium rolfsii* by *Serratia marcescens* appeared to be mediated by chitinase expression (Ordentlich *et al*., 1988; Compant *et al*., 2005). Lytic enzymes can reduce different polymeric substances such as chitin, proteins, cellulose, hemicellulose and DNA (Vivekananthan *et al*., 2004). Chitinase produced by *S. plymuthica* C48 inhibited spore germination and germ-tube elongation in *B. cinerea*; but *Serratia marcescens* was considered to produce extracellular chitinases which act as antagonists against *Sclerotium rolfsii* (Frankowski *et al*., 2001). It was demonstrated that extracellular chitinase and laminarinase synthesized by *P. stutzeri* lyse mycelia of *F. solani* (Compant *et al*., 2005). Bacterial species like *Bacillus* have been proved to control the fungal diseases. Recent reports showed that they are capable of lysing chitin, which is a major constituent of the fungal cell wall. In addition these bacteria have the ability to disintegrate proteins (proteolytic activity) which plays a key role in the nitrogen cycle (Praveen *et al*., 2012).

Since, our study includes strains of two important PGPR genus *Burkholderia* and *Acinetobacter*. We would like to give a brief account for their PGPR traits.
2.5 Burkholderia spp.

Bacteria belonging to the genus *Burkholderia* are very common in soil, water and are associated with plants (McArthur *et al*., 1988) have a wide natural diversity, not only in taxonomy, but also in ecological features. Some species are pathogenic to plants or to mammals and others are widespread in the environment. Over the past two decades, research on *Burkholderia* species has been steadily expanding. Members of the genus *Burkholderia* are very abundant, occupying diverse ecological niches (Estrada-de los Santos *et al*., 2001; Coenye & Vandamme, 2003), including soil (van Elsas *et al*., 2002; Salles *et al*., 2002, 2004; Janssen, 2006) and hospital (Coenye & Vandamme, 2003) environments. Many members of the genus can cause infections in humans and animals (Coenye & Vandamme, 2003; Valvano *et al*., 2005, 2006). Nevertheless, in recent years, a growing number of *Burkholderia* strains and species have also been reported as plant-associated bacteria. Indeed, *Burkholderia* spp. can be free-living in the rhizosphere as well as epiphytic and endophytic, including obligate endosymbionts and phytopathogen (Coenye & Vandamme, 2003; Janssen, 2006). Some *Burkholderia* species also have agricultural benefits. Some improve crop production (Trân Van *et al*., 2000) or fix nitrogen (Gillis *et al*., 1995) and control plant disease by inhibiting the growth of bacterial and fungal phytopathogens (Aoki *et al*., 1993).

*Burkholderia* spp. in general, were reported as a dominant component of the bacterial community in several soil ecosystems and as such could be a useful indicator of microbial community shifts resulting from agricultural practices (tillage, cropping system, addition of organic matter) (Hallmann *et al*., 1999; Mazzola, 1999; Nusslein and Tiedje, 1999). Members of *Burkholderia* genus had been shown as important (PGPR) owing to their biofertilizer, biocontrol and bioremediation activities and thus, had an overall positive effect on plant health. The genus Burkholderia is a large group of bacteria composed of more than 70 species living in diverse habitats. Many of these have recently been discovered to be associated with plants either as diazotrophs or plant growth-promoting bacteria (Gyaneshwar *et al*., 2002). The genus *Burkholderia* includes at least 43 gram-negative bacilli species with exceptional metabolic versatility (Compant *et al*., 2008).

Two factors that could be attributed to the ecological versatility of the members of this genus includes: huge coding capacity of their large multi-replicon genomes (6-9 Mb)
allowed them to be metabolically robust and, an array of insertion sequences in their genomes which promoted genomic plasticity and general adaptability (Lessie et al., 1996). The G+C content of Burkholderia spp. genome was reported 64% to 68.3% by Yabuuchi et al. (1992). Similar to other PGPR strains, B. vietnamensis, B. ubonensis, B. kururiensis and B. pyrrocinia had been shown to confer PGPR traits by mechanisms including p-sol., IAA production, atmospheric N2 fixation, siderophore production and antagonism against plant pathogenic bacteria and fungi (Madhaiyan et al., 2008; Santos et al., 2001; Parke and Gurian-Sherman, 2001).

Recently, it had been found that B. phytofirmans PsJN exerts phenotypic effects throughout the whole life cycle of the plant via modulating the transcriptional profiles of inoculated plants (Poupin et al., 2013). But the bacterial genes responsible for these activities are still not known. Further, genome sequencing of Burkholderia sp. strain KJ006 revealed that it contains accD gene encoding ACC deaminase, the pqq operon for pyrroloquinoline quinone biosynthesis, the nif gene cluster and genes for biocontrol activities (Kwak et al., 2012).

Burkholderiales, isolated from wide array of sources such as soil, rhizosphere, sediment or sludge, had also received attention from bioremediation field and their genomic analysis revealed the presence catabolic genes especially oxygenases that were reported to involved in the cleavage of several priority aromatic pollutants (Kwak et al., 2012; Perez-Pantoja et al., 2012). Though, there was reported a great phenotypic variation among the B. cepacia complex (Bcc) strains, even in the maize rhizosphere, genetic fingerprinting of individual isolates using analysis of random amplified polymorphic DNA (RAPD) patterns indicated that, of the 83 isolates collected, there were 68 distinct haplotypes (Di Cello et al., 1997).

**Bcc application in agricultural soils**

Although the Bcc is indigenous to agricultural soils, augmentation with specific strains of Bcc was reported to protect seeds and seedlings from early invading pathogens. Typically, it was accomplished by applying $10^6$ to $10^8$ cfu Bcc per seed at planting, but soil drenches and drip irrigation delivery systems had also been used. The temporary boost in the Bcc population in the infection court during the initial hours and days after planting
could provide protection similar to that provided by fungicides (Hebbar et al., 1992; Heungens and Parke, 2001).

The members of the Bcc were reported to live as plant commensals in intimate association with roots. They were apparently among the most predominant culturable bacteria in the rhizosphere of plants, commonly present at $10^3$ to $10^5$ cfu g⁻¹ roots (King and Parke, 1996; Tsuchiya et al., 1995). The strain of *Burkholderia* spp. had been found to be associated with sugarcane (Paungfoo-Lonhienne et al., 2014), *Mimosa flocculosa* (effective in fixing nitrogen) (Zuleta et al., 2014), *Lebeckia ambigua* (Reeve et al., 2013) and from the rhizosphere (Zhao et al., 2014). Similarly, Groenhagen et al. (2013) isolated *Burkholderia ambifaria* from the rhizosphere of pea and maize.

Moreover, members of the Gramineae like eastern gama grass was reported to support Bcc population up to $10^5$ cfu g⁻¹ root (Brejda et al., 1994). Similar results had been found in perennial ryegrass (Nijhuis et al., 1993) and maize ($10^5$ to $10^6$ cfu g⁻¹ root) (Nacamulli et al., 1997), where Bcc could comprise 0.6 to 3.6% of the total culturable bacteria in the rhizosphere (Di Cello et al., 1997). Bcc bacteria, along with *B. graminis*, are abundant in the rhizosphere of wheat (Viallard et al., 1998) and *B. vietnamiensis* may be present on rice roots at populations of $10^7$ to $10^{10}$ cfu g⁻¹ fresh roots (Fisher et al., 1993). Some strains of Bcc were not confined to the external rhizosphere surfaces of roots but can also colonize internal root tissue (Hebbar et al., 1992; Nacamulli et al., 1997).

Some endophytic strains were capable of associative N₂-fixation, which may account for their remarkable plant growth-promoting activities. Potentially novel N₂-fixing species of *Burkholderia* had been reported as phloem and metaxylem colonizing endophytes of tropical and temperate Gramineae. Their agricultural use could potentially boost production of the important world food crops (rice, wheat, maize) under N-limiting conditions. Van et al. (2000) demonstrated that in field studies on rice inoculated with the root colonizing and endophytic strain *B. vietnamiensis* TVV74, resulted in 13-22% increase in grain yield. Further, the production of antibiotic by Bcc strain AMMDR1 was reported to reduce the diversity of the rhizosphere microflora and may contribute to its success as a root colonist (Kang et al., 1998).

Moreover, some of pectic enzymes produced by many *B. cepacia* strains could enable them to invade plant mucilage and intercellular spaces in the epidermis or root
cortex (Hebbar et al., 1992; Yohalem and Lorbeer, 1997). Even, Bcc-like bacteria had recently been detected within spores and root-invading hyphae of mycorrhizal fungi (Perotto and Bonfante, 1997), possibly aiding their survival and providing a mechanism for delivery into the plant root. Additional phenotypes that contributed to root colonization by other rhizosphere bacteria that may apply to Bcc include cell surface properties (Lipopolysaccharides (LPS) and pili that enhance adhesion to plant root cells (Mitter et al., 2013; Vesper, 1987) and retention by soil particles (DeFlaun et al., 1999).

Bcc strains were found to be able to control soilborne plant diseases caused by fungi and oomycetes. Some of the most studied examples included biocontrol of damping-off diseases caused by Pythium spp. (Bowers and Parke, 1993; Heungens and Parke, 2000; King and Parke, 1993), Rhizoctonia solani (Kang et al., 1998) and Fusarium spp. (Bevivino et al., 1998; Hebbar et al., 1992). Martin and Loper (1999) suggested that damping-off diseases are prime targets for biological control efforts because of their wide host range, worldwide distribution, and economic importance, and because they offer an alternative to seed treatment with fungicides that can cause deleterious health effects.

Further, these damping-off diseases were also reported as logical targets of biological control because of the short period of plant susceptibility (hours or days) and thus protection is required for only a short time. The highly localized site of infection was reported to allow placement of the biocontrol agent directly on the plant part requiring protection. However, biocontrol must be enacted very quickly to prevent infection. The production of antibiotics like cepacin, cepaciamide, xylocandins (Deepacidines), pseudanes (Dquinolinones), phenylpyrroles and phenazine play important role in plant disease suppression (Parke and Gurian-Sherman, 2001).

Pyrrolnitrin produced by strains of Bcc may contribute to suppression of Rhizoctonia solani, Fusarium spp. and other fungi sensitive to this antibiotic. Even in the absence of disease, strains of Bcc were reported to enhance the growth or yield of maize (Bevivino et al., 1998), wheat (Germida and Walley, 1996) and rice (Van et al., 2000). In the case of B. vietnamiensis, which fixes atmospheric N₂, enhanced the plant growth, which may be due to increased nitrogen availability to the host (Van et al., 2000).
2.6 *Acinetobacter* spp.

*Acinetobacter* is a genus of Gram-negative bacteria belonging to the wider class of Gamma proteobacteria. Members of the genus *Acinetobacter* are commonly found in the environment (Dhakephalkar and Chopade, 1994). In recent years, members of the genus *Acinetobacter* have been isolated from the rhizosphere of different plants (Kuklinsky-Sobra et al., 2004; Roberts *et al*., 2005; Li *et al*., 2008). *Acinetobacter* spp. strains play an important role in plant growth promotion (Huddedar *et al*., 2002; Chopade *et al*., 2008; Indiragandhi, 2008; Sachdev *et al*., 2010), as certain strains of this genus are known to be involved in phytostimulation based on the production of plant-growth-promoting hormones (Huddedar *et al*., 2002; Chopade *et al*., 2008), solubilization of phosphate (Gulati *et al*., 2009; Peix *et al*., 2009), and production of siderophores (Indiragandhi, 2008; Sachdev *et al*., 2010, Sarode *et al*., 2009). Meanwhile, other *Acinetobacter* strains exhibit indirect PGPR activity via the growth suppression of phytopathogenic fungi, such as *Cryphonectria parasitica*, *Phytophthora capsici*, and *Rhizoctonia solani* (Huddedar *et al*., 2002; Liu *et al*., 2007), and potential biocontrol properties against pathogenic bacteria like the *Ralstonia solanacearum* related to the wilt of tomatoes (Xue *et al*., 2008).

There have been several reports on the presence of *Acinetobacter* sp. in the rhizosphere of wheat, a major crop plant. Kleeberger *et al*., (1983) have isolated *Acinetobacter* sp. during their studies on the characterization of the Gram negative micro-flora associated with the wheat plant. *Acinetobacter* strains have also been reported along with other bacterial strains from saline soil samples where this crop has been cultivated (Egamberdieva *et al*., 2008). In our laboratory, *Acinetobacter* sp. isolated from the wheat rhizosphere was used for the production of indole acetic acid (Huddedar *et al*., 2002; Chopade *et al*., 2008). Thus *Acinetobacter* strains have been reported from the rhizosphere of wheat, and in some cases their properties benefiting plant growth have also been investigated.

2.7 PGPR traits are affected by xenobiotics

Pesticides in soils continue to be studied more than any other environmental contaminant, because they are used widely to control pests that affect agricultural crops
and pests in the home, yards, and gardens. There is also increasing interest in their transformation products (TPs), because they can be present at higher levels in soil than the parent pesticide itself. Generally, pesticide TPs could show lower toxicity to biota than the parent compounds (Nawab et al., 2003). The extensive and indiscriminate application of agrichemicals including various fungicides of different chemical families in agronomical practices adversely affect the beneficial microorganisms like PGPR and their physiological activities important to soil fertility (Moorman, 1989; Wani et al., 2005; Srinivas et al., 2008) and ultimately influence the plant growth (Ahemad and Khan, 2009, 2011).

In general, degradation of pesticides in soil is facilitated by both biotic and abiotic factors including chemical, sunlight and microbial agents and among these factors biodegradation is the most commonly used method for converting synthetic chemicals into inorganic products (Alexander, 1999; Bassey and Grigson, 2011). Pesticides are degraded by chemical and microbiological processes. Chemical degradation occurs through reactions such as photolysis, hydrolysis, oxidation and reduction (Bavcon et al., 2003) Biological degradation takes place when soil microorganisms consume or break down pesticides (Nawab et al., 2003). These microorganisms are mainly distributed in the top centimeters of the surface layer of the soil, where the organic matter acts as food supply (Navarro et al., 2003) The extent of degradation ranges from formation of metabolites (TPs) to decomposition in inorganic products (Kale et al., 2001). The tolerance or resistance against pesticides including fungicides is a complex process of microorganisms regulated at both physiological and genetic level (Herman et al., 2005).

Previously, many studies have reported in which pesticides were applied at the recommended and the higher doses to investigate the effects on PGP activities of PGPR. Four technical grade insecticides, fipronil, pyriproxyfen, imidacloprid and thiamethoxam were applied at the recommended and the higher doses to investigate their effects on plant growth-promoting activities of phosphate-solubilizing Klebsiella sp. strain PS19, isolated from mustard rhizosphere. All tested insecticides displayed a concentration-dependent inhibition in plant growth promoting traits, like, inorganic phosphate solubilization, biosynthesis of phytohormones and siderophores, of rhizobacterial strain PS19. For example, the phosphate-solubilizing activity of Klebsiella sp. PS 19 was reduced maximally by 95%, at 3900 μg l⁻¹ pyriproxyfen over control (Ahemad and Khan, 2011).
At the recommended rate, the magnitude of toxicity of insecticides to plant growth promoting traits was less severe compared to the higher doses. Ahemad and Khan 2011, observed that impact of the highest dose rate of insecticides on 2,3-dihydroxybenzoic acid (DHBA) was almost equal to those observed for SA. Thiamethoxam decreased the indole acetic acid (IAA) synthesis maximally by 86% whereas fipronil had least toxicity and reduced it by 67% relative to the control. Among the experimental insecticides, pyriproxyfen at 3900 μg l⁻¹ in general, had the greatest toxic effects for plant growth promoting activities of the test strain.

Changpeng et al., 2009, investigated the microbial response to 2,4-D butyl ester (herbicide) application at different concentrations (CK, 10, 100 and 1000 μg g⁻¹) in the soils with two fertility levels. Culturable microorganisms were affected by the herbicide in both soils, particularly at the higher concentration. After treating soil with 100 μg g⁻¹ herbicide, culturable bacteria and actinomycetes were significantly higher, compared to other treatments. Treatment of soil with 1000 μg g⁻¹ 2,4-D butyl ester caused a decline in culturable microbial counts, with the exception of fungal numbers, which increased over the incubation time. The higher stress level was in the treatments with high concentrations of herbicide (1000 μg g⁻¹) for both soils. Pesticides display non-target effects on active microbial populations that serve important ecosystem functions, thereby emphasizing the need to critically investigate and validate the use of bio-pesticides in agriculture before accepting them as safe alternatives to chemical pesticides (Gupta et al., 2013; Ahemad and Khan, 2012)