2.1. **Tea- origin, production and importance**

People have been brewing tea made from the leaves of *Camellia sinensis* L. (O) kuntze (family *Theaceae*) for almost 50 centuries, far long back in the first millennium BC; while according to a legend even before 2000 BC. The original home or ‘the primary centre of origin’ of tea was South-East Asia, at the point of intersection between the 29° N (latitude) and 98° E (longitude) near the source of the Irrawaddy River at the confluence of North-East India, North Burma, South-West China and Tibet provinces (Mondal *et al.*, 2004). Tea thrives well within the latitude ranges between 45° N to 34° S, cutting across about 52 countries (Sana, 1989; Mondal *et al.*, 2004). Assam is the largest producer (1850 kilograms per ha) of tea in India, which is 53% of the total production (Baruah *et al.*, 2003). Tea cultivation is being practiced over centuries in indo-Burma mega-biodiversity hotspot of Assam and Dooars region of West Bengal.

**Figure 1. World map of tea production regions**

(Courtesy: FAOSTAT data, color-coded on a logarithmic scale)
The Chinese were the first to use tea as medicinal drink, later as beverage and now serving as morning drink for nearly 2/3 rd of the world population daily. About three billion kilograms of tea is produced and consumed yearly. The largest producers of tea are the People’s Republic of China, India, Kenya, Sri Lanka, and Turkey (Figure 1 and 2). India was the top producer of tea for nearly a century, but was replaced by China as the top tea producer in the 21st century. While India is the largest consumer of tea worldwide, the per-capita consumption of tea in India remains a modest 750 grams per person every year. In 2004, world tea production was recorded at 3.21 million tons annually; while in 2008, world tea production was reached over 4.73 million tons. Tea is consumed as part of daily diet to reduce the risk of cardiovascular disease and cancer (Tsubono et. al., 2002). This has lead to the increase of about 6.2% of world tea production, from 3,146,000 metric ton in year 2003 to 3,342,000 metric tone in year 2004 (FAO, 2007). According to Food and Agriculture Organization (FAO) of the United Nations as of January 2010, there is a downfall of tea production in India from the year 2004 to 2008 as it is recorded at 928,000 tones (26%) to 805,180 tones (17%) per annum; while an increment is seen in the production of tea in China from 1,047,345 tons (25%) to 1,257,384 tons (27%) per annum (Figure-2). The investigation of this downfall of tea production in this region of India is now becoming a very important issue.

Tea is the most popular beverage consumed all over the world because of its refreshing effects and known benefits to human health due to its
potential pharmacological properties such as antioxidative, antitumor and anticarcinogenic activities (Yamamoto et al., 1997). Tea is also a rich source of dietary metals such as manganese, zinc, iron, copper etc. Today, the relationship between tea and human health has become a subject of intensive studies throughout the world.

Organic farming is gradually replacing conventional farming due to increasing demand for organic food and growing environmental concerns (Hansen et al., 2001). The application of fertilizers to enhance soil fertility and crop productivity often negatively affects the biogeochemical cycle (Ma et al., 1990; Perrott et al., 1992; Steinshann et al., 2004). In the last few decades the rate of application of nitrogen, phosphorous and potassium (NPK) fertilizers in crop production is growing constantly (Adesmoyee and Kloeppper, 2009). Conventional farming using chemical fertilizer often shows negative impact such as soil erosion, leaching and run-off of nutrients, loss of organic matter, pollution of natural water, impairment of environment quality, and evaporation of green house gases especially nitrogen (N), leading to environmental pollution and health hazards (Tilman, 1998; Tilman et al., 2002; Gyaneshwar et al., 2002; Kennedy et al., 2004, Diepeningan et. al., 2006; Adesmoyee and Kloeppper, 2009). Therefore, it is now crucial to find out alternative ways of fertilizer management in crop cultivation. Organic farming is able to increase the level of total nitrogen, nitrate and available phosphorus in soil and preventing nutrients leaching (Hansen et al., 2001). It has turned the focus of the farmers, scientists and policy makers to look at the
integrated approach to nutrient management in order to make crop cultivation sustainable and less dependent on chemical fertilizers.

**Figure 2. Year wise production of tea in the world**

2004

2006

2008

Courtesy: Diagram is generated from the data recorded by the Food and Agriculture Organization (FAO) of the United Nations as of January 2010, Economic times (PTI), 2011.
2.2. Tea in waterlogged condition

Around 2,00,000 hectares of tea plantation in the NE region of India are suffering from waterlogged problem, which affects in its production both in terms of quality and quantity (Bordoloi, 1993). In tea, waterlogging occurs during the actively growing period of summer season when maximum damage happens to occur. The ability to flood tolerance varies within the plant species. The plants adopt themselves to flooding by modifying respiratory pathway to survive in aerobic to semi-aerobic conditions as a result of flooding (Crawford, 1972). Under this condition, the bushes turns sickly, plucking points are greatly reduced, stems are attacked by red rust, root remain swallow, twisted with enlargement of lenticles (Sharma and Swarup, 1988). Growth retarding hormones abscisic acid (ABA) increases 6-15 folds high within 12-18 hours of waterlogging. Metabolic activities of tea plant are reduced and after a certain stage the plant cannot be revived even if the water is drained out (Handique, 1997; Malik et al., 2001; 2002; Hazandy et al., 2009). It is also reported that under waterlogged condition Eh is decreased (Parent et al., 2008) and pH of the soil is increased towards neutrality with the dissolution of carbonate and bicarbonate during waterlogging (Lu et al., 2004; Parent et al., 2008). Some waterlogged soils become rich in Mn$^{2+}$ and Fe$^{2+}$, devoid of NO$_3^-$ and SO$_4^{2-}$, and anaerobic microbial metabolites may accumulate (Malik et al., 2002; Singh et al., 2010). In response of stress resistance, the accumulation of osmoprotectants,
like sugar molecules, proline, glycine-betaine etc. have been reported (Glick and Pasternak, 2003; Hazandy et al., 2009). No systematic study has so far been done for selection and characterization of tea tolerant or resistant to waterlogging. Selection and characterization of waterlogging tolerant cultivars will improve in its cultivation in NE India. Thus there is an urgent need to develop cultivars tolerant to waterlogged conditions and to develop and understand the physiological, biochemical and molecular characters of such plants which can be used in breeding program.

2.2.1. The scenario in NE India- Robert Bruce and Charles Bruce were the first to discover the tea drinking practice in Assam among the ‘Shingpho’ tribe in 1830s. Since 1940, Assam Tea Corporation (ATC) started commercial production of tea and became the leading producer of tea in the world. Country’s tea production is declined by 1.3 percent to 966.4 million kgs in 2010 due to output drop in Assam, according to the tea board of India. The strong flavor of Assam tea, may be for the first time in history, has shown palpable deterioration with the production output also coming down significantly. Tea production in Assam has been considerably dropping with respect to the previous years due to various reasons. The state produced 564,000 tons of tea per annum in 2007, while it came down to 460,000 tons per annum in 2010 (Economic times, 2011, FAO). One major reason is soil waterlogging, which may arise due to various factors of climate changing like prolonged monsoon (Effect of waterlogging in tea gardens is shown in the Figure 3a, b and c). In addition, damage in water drainage systems in nearby
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areas of tea gardens due to human interference, the rising water level of the nearby rivers, seepage water, seasonal water-logging and run off water also contribute to this waterlogging. Prolonged monsoon also results in a damp weather which multiplies the pest attack and proliferation of fungal infections, making a dent in the tea quality and quantity. Aggressive pest control measures have not implemented in Assam considering the delicate and sensitive ecosystem that exist there (Economic times, 2011). The only solution to face these problems is the availability, screening and analyzing of the waterlogged resistant cultivars and also analyzing the soil characteristics.

2.3. Soil and tea nutrient status

Soil biochemical activities have been found to be very responsive in soil conservation and soil use pattern by human, such as non tillage, organic amendment, crop rotation and organic cultivation (Dick, 1994, 1997; Miller and Dick, 1995; Bergstrom et al., 1998). They catalyzes several important reactions necessary for the life process of micro-organisms in soil and stabilization of soil structure, the decomposition of organic wastes, organic matter formation and in nutrient cycling (Dick et al., 1992, 1994; Quilchano and Maranon, 2002; Singh et al., 2010). Nitrogen (N) is an important constituent of plant parts and plays a vital role in the physiology of the plant. Although applications of N can increase tea yields, it is recognized that the quality of the manufactured product is suppressed by large N rates (Cloughly
Potassium and magnesium are required in large quantities and they are both involved in almost all biological reactions. Potassium is the second major nutrient for tea after N and makes up 1.5-2% of the dry matter.
in tea leaves (Verma, 1993, 1997; Wu Xun et al., 1997). Magnesium occupies up to 0.30% of the leaves dry matter in the fresh leaves (Wu Xun et al., 1997). Potassium and magnesium deficiencies widely occur in the tea plantation regions mainly due to the higher precipitation and consequently higher leaching as well as the higher demands (Verma, 1997, Wu Xun et al., 1997). Zinc (Zn) is not readily absorbed by tea from soil and its deficiency is not readily corrected by ground application of zinc compound, but the foliar application is found to be very effective. Copper is an essential constituent of the enzyme polyphenol oxidase, which is vital for fermentation (Bonheure and Willson, 1992). The quality of tea depends on organic and inorganic composition of harvested shoots, which are changed into the substances, these are responsible for taste, flavor and colour of made tea. In this regard, balanced nutrition of tea is of particular importance to secure good harvested fresh leaves as a prerequisite for tea of superior quality (Wu Xun et al., 1997).

Soil micro-organisms can use mineral nutrients in soil as well as in decomposing substrates; thereby influencing soil microbial activity which affects on nutrient status of soil and vice-versa. The application of chemical fertilizers to ensure soil fertility and crop productivity often negatively affects the complex system of biogeochemical cycle (Tilman et al., 2002; Adesmoyee and Kloeper, 2009). This is due to low use efficiency of externally applied fertilizers by the plants, leaching, and evaporation to atmosphere (Tilman, 1998; Gyaneshwar et al., 2002; Kennedy et al., 2004). Chief reason for this problem is the low use efficiency of the externally
applied fertilizers by plants as well as their long term application. Since 1980s, the use of nitrogen in tea plantations has been increasing rapidly; though several researchers are being involved in the study on tea, research work on mineral nutrition is rare (Luczaj and Skrzydlewska, 2005). Therefore, emphasis must be given to the nutrient status of the tea gardens as well as their uptake by the plants and integrated nutrient management in their external application systems.

2.3.1. Phosphorus status-Phosphorus (P) is the second most essential macronutrient, after nitrogen (N) for plants and its deficiency is the most limiting factor for plant growth and development as the crop productivity is limited by P deficiency on 30-40%. Application of P fertilizer is usually the recommended treatment for enhancing soil P availability (Vance et al., 2003; Lambers et al., 2006). However, only less than 20% of P can be utilized by the plants and the 80-90% of applied P is fixed into the soil (Lambers et al., 2006). Due to the limited availability and resources of P fertilizers and their effects on environment, their extensive use can be a threat to the agriculture (Vance et al., 2003; Lambers et al., 2006). Many soils throughout the world are P-deficient because the free phosphorus concentration even in fertile soil is not higher than 10µM even at pH 6.5, where it is most soluble. As the tea garden waterlogged areas are moderately to highly acidic (Ma et al., 2000); and under the acidic or calcareous soil large amount of phosphorus is fixed in soils (Gyaneswar et al., 2002). Generally, acidification in tea garden soil is
determined due to age of tea plantation, origin of soil parent material and fertilizer application (Oh et al., 2006; Alekseeva et al., 2011). The problem of phosphorus deficiency is of particular concern for acid soil like tea garden soil, as tea prefers a low pH (4.5-5.5) soil (Zoysa et al., 1999); which cause unavailability of phosphorus due to binding to soil mineral surfaces and fixation into organic forms (Gyaneswar et al., 2002; Kochian et al., 2004). On the other hand, Rubio et al. (1997) reported that under waterlogged condition and during the anoxic state, soil P availability is higher and roots of waterlogged plants showed morphology more favourable to nutrient uptake and a higher physiological capacity to absorb P. Inorganic P in acidic soil is associated with Al and Fe compound, whereas in calcareous or alkaline soil calcium-phosphate is predominant (Gyaneswar et al., 2002). Phosphorus must be dissolved in the soil solution in order to be taken up by the plant roots. The dissolved forms of phosphorus in soil solution are in orthophosphates form ($H_2PO_2^-$ or $HPO_4^{2-}$, depending on soil pH). Once the plant roots remove P from soil solution, it is replenished by the residual P in the soil. Some microorganisms are reported to solubilize soil fixed P to soluble form and make it available for plant uptake are termed as phosphate solubilizing microorganisms (PSM) (Rodriguez and Fraga, 1999; Kuklinsky-Sobral et al., 2004; Hariprasad and Niranjana, 2009). The process, through which soil microbes transform organic forms of P or inorganic soil amendments into plantavailable P is called mineralization. The end products are orthophosphates and this form can be taken up by the plants. The soil solution can be
replenished from several pools of inorganic (mineral) P in the soil. These mineral P can also dissolve in the soil solution by a process called dissolution, when concentration of soluble P is diminished. P can detach from the soil particles, thereby supplying P to the soil solution via a process called desorption. Similarly, when concentration of P in the soil solution is too high, some of the dissolved P forms solid P minerals by a process called precipitation. Depending on the soil pH, precipitation can result in the formation of solid calcium phosphate minerals (high soil pH) or aluminum and iron phosphate minerals (low soil pH). Alternatively P can be removed from soil solution and attach to soil particles like clays or iron and aluminum-bearing minerals via a process called adsorption. Finally, solid rocks can be a source of P as they break down into soil over a long period of time by a process called weathering. But, when the rainfall or irrigation rate is higher than the soils ability to absorb water, as a result of runoff, soluble P is transported into water bodies like lakes, ponds, streams, rivers and bays. In addition, when then concentration of P in the soil exceeds the natural soil P holding capacity, P can be carried downward, or leached through the soil, called leaching. The phosphorus cycle is represented in the Figure 4 (Bolan et al., 2005). Once transported into water bodies or ground water, inorganic P can become a water pollutant.
Beside chemical fertilizers, amendments of other fertilizers, such as organic manure, compost extract and compost tea are also used in many parts of the world to enhance crop production (Adesmoye and Kloepper, 2009). PSB which are a class of plant growth promoting bacteria (PGPB), when used in the form of bio-inoculants is considered as eco-friendly and reduce possible adverse effect of chemical fertilizer. Their use has critical impact on soil functions that modulate metabolic processes involving specific soil properties and soil fertility and biogeochemical cycling of nutrients (Nannipieri et al., 2003; Wakelin and Ryder, 2004). As a result, the application of PSB conserves the soil, which is a limiting resource necessary for crop production.
2.3.2. Soil enzymes and physico-chemical properties- Soil microorganisms as one part can be sensitive biological markers and can be used to assess soil quality or degradation. There are many indicators of soil microbiological properties including microbial biomass content, microbial diversity and activity, and enzyme activity. Several studies have reported significant correlations among soil enzyme activities, microbial biomass and various soil properties (Burns, 1983; Nannipieri et al., 2003; Dick, 1994, 1997; Dick et al., 2000). The soil physicochemical and soil enzymes play key biochemical functions in the soil systems and such enzyme activities can be used as indices of microbial activity and soil fertility (Burns, 1983; Sinsabaugh et al., 1991; Nannipieri et al., 2003). They catalyze several important reactions necessary for the life processes of micro-organisms in soil and stabilization of soil structure, the decomposition of organic wastes, organic matter formation and in nutrient cycling (Dick et al., 1994).

Among the soil enzymes, phosphatase is a broad group of enzymes that catalyze the hydrolysis of both esters and anhydrides of phosphoric acids, the hydrolysis of organic phosphate to inorganic phosphate (Schimdt and Laskowski, 1961; Cookson, 2002). The release and availability of this enzyme depends on the soil pH. Phosphatase enzymes break down organic P compound releasing essential plant nutrient P. The rate of synthesis, release and stability of alkaline and acid phosphatase have crucial role in P cycle in soil (Eivazi and Tabatabai, 1977). The dehydrogenase enzyme is also known as the respiratory chain enzyme play the major role in the process of cellular
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energy generation. Dehydrogenase activity is commonly used as an indicator of biological activity in soils and it is known to oxidize soil organic matter by transferring protons and electrons from substrate to acceptors, which is a part of respiration pathways of soil micro-organisms and are closely related to the type of soil: air: water conditions (Doelman and Haanstra, 1979; Kandeler et al., 1996; Glinski and Stepniewski, 1985). In addition, dehydrogenase activity has been used as an indicator of microbiological activity in arid and agricultural soil (Beyer et al., 1992; Garcia et al., 1994a). Several studies have been revealed correlations of dehydrogenase enzyme activities with metabolic parameters such as number of microbial cells, soil respiration, ATP concentration, C/N turnover and organic matter content. Urease is the ubiquitous cell free exoenzyme in the nature, produced by the plants, animals and micro-organisms which can be used as an indicator of soil fertility (Guettes et al., 2002). Urease enzyme is responsible for the hydrolysis of urea fertilizer applied to the soil into NH₃ and CO₂ and gives the information on the rate of degradation of N-containing compound (Andrew et al., 1989; Byrnes and Amberger, 1989; Cookson, 2002, Schimdt and Lawoski, 1961). Due to this role, urease activities in soils have received a lot of attention since it was first reported by Rotini (1935).

Innumerable works are on these soil enzyme activities having been found to be very responsive to human activities and a detail account of it have been given by various workers (Andrew et al., 1989; Beyer et al., 1992; Byrnes and Amberger, 1989; Garcia et al., 1994b). All the physical, chemical,
biological and biochemical properties of soil are very much important for its behavior (Arshad and Coen, 1992; Parr et al., 1992) and also they are useful for detecting the deterioration of soil quality that are most closely related to nutrient cycles, including soil respiration, microbial biomass, nitrogen mineralization capacity and activities of soil enzymes (Visser and Parkinson, 1992). Studies on soil enzyme activity of tea garden soil in relation to nutrient management are scarce.

2.4. Micro-organisms in the tea rhizosphere

Studies have evidently proved that the rhizospheric micro flora includes both deleterious and beneficial elements which have the potential to influence both plant growth and crop yields significantly (Kleopper, 1994; Glick, 1995; Rodriguez and Fraga, 1999; Dekaboruah and Dileep kumar, 2002a & b; Adesmoye and Kloepper, 2009). Rhizospheric micro-organisms were classified as beneficial (symbiotic) or harmful (pathogenic) or having no effect on the plant. The tea rhizosphere also provides a site for studying microbial interactions under natural conditions in a specific environment, due to the perennial nature of tea (Pandey and Palni, 2004). The rhizosphere of young tea plants and other perennial plants of different ages stimulates different microbial growth (Pandey and Palni, 2004). The work of Baby et al. (2001) evidences higher microbial dynamics in the rhizosphere of younger tea plantations than that of the older ones.
2.4.1. Plant growth promoting rhizobacteria (PGPR)- A considerable number of bacterial species, mostly those associated with the plant rhizosphere are able to exert a beneficial effect upon plant growth. They can be used as biofertilizer or control agents for agricultural improvement; which are broadly grouped as PGPR (Plant Growth Promoting Rhizobacteria) (Klepper, 1994; Glick, 1995; Rodriguez and Fraga, 1999; Deka Boruah and Dileep kumar, 2002a & b). Kloepper and Schroth (1978) has coined the term PGPR, for rhizosphere associated free-living bacteria. Subsequently, the term more precisely was used to only those bacteria which are having direct application on plant growth promotion (Barthakur and Bezbaruah, 1997; Bezbaruah et al., 1996a & b; Dileep kumar and Bezbaruah 1996; Dileep kumar 1996, 1998; Dileep kumar et al., 2001; Rodriguez and Fraga, 1999). Researchers hypothesized that the use of rhizobacterial strains that have beneficial effect on plant growth and development can be employed for efficient use of externally applied fertilizers (Vessey, 2003; Adesmoye and Kloeppe, 2009; Duarah et al., 2011). Initially, though the application of PGPR was concentrated on biological control (Suslow et al., 1982; Glick, 1995; Hariprasad and Niranjana, 2009); but recently the use of PGPR has been diverted to exploit them for nutrient use efficiency in crop plants (Adesmoye and Kloeppe, 2009). The mechanism by which PGPR can exert a positive effect on plant growth can be of two types: direct and indirect (Glick, 1995). Indirect growth promotion is the decrease or prevention of deleterious effect of pathogenic micro-organisms, mostly due to the synthesis of
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antibiotics (Sivan and Chet, 1992) or siderophores (Leong, 1986) by the bacteria; while the direct growth promotion can be through the synthesis of phytohormones (Xie et al., 1996), N2 fixation (Christiansen-Weneger, 1992), reduction of membrane potential of the roots, synthesis of some enzymes that modulate the level of plant hormones, as well as the solubilization of inorganic phosphate and mineralization of organic phosphate, which makes phosphorous available to the plants (Rodriguez and Fraga, 1999; Rodriguez et al., 2006; Hariprasad and Niranjana, 2009; Duarah et al., 2011). Among these bacteria, free living Pseudomonas sp. (produces antibiotics, indole acetic acid, siderophores, phosphorous solubilizing activity etc.), Bacillus sp. (production of antibiotics, phytohormones, phosphorous solubilizing activity), Proteus, Methylobacterium, Azospirillum are some of the potential PGPR strains.

2.4.2. Phosphate solubilizers as PGPR- Among the different PGPR, the phosphate solubilizing bacteria (PSB) on plant growth and development, their application in nutrient management and fertilizer use efficiency was studied by several researchers (Rodriguez and Fraga, 1999; Piex et al., 2001; Hariprasad and Niranjana, 2009; Kim et al., 2009). PSB in different ecological niches are being used in p-management in a sustainable crop production (Rodriguez and Fraga, 1999; Hariprasad and Niranjana, 2009). They are known to solubilize insoluble phosphates and make it available for plant uptake through different mechanisms by secreting some acid phosphatase, phytase enzyme, organic acid and growth regulators such as
IAA and GA (Rodriguez and Fraga, 1999; Vessey, 2003; Kuklinsky-Sobral et al., 2004; Hariprasad and Niranjana, 2009). PSB are involved in plant-microbe interaction, thereby helping plant growth and development and also induce disease resistance against some plant pathogens (Glick et al., 2007; Poonguzhali et al., 2007; Xie et al., 1996; Patten and Glick, 2002; Richardson, 2001). The solubilization of P can also enhances the nitrogen (N) fixation, also increases the availability of other nutrients through the production of phytohormones.

2.4.2.1. Pseudomonads- The aerobic pseudomonads contribute a large and quite diverse array of bacteria. Among them the most extensively studied subgroup of aerobic Pseudomonas consists of the fluorescent pseudomonads primarily characterized by their ability to produce water soluble yellow green fluorescent pigment, pyoverdin (Tripathy et al., 2007; Palleroni, 1984; Sing and Arora, 2004). The genus Pseudomonas, in particular, has tremendous importance due to its widespread application in agriculture and biotechnology (Deka Boruah and Dileep kumar, 2002a & b; Pallorani, 1992; Weller, 1998; Trivedy et al., 2008). Pseudomonads have the capacity to produce a wide array of compounds antagonistic to plant pathogenic bacteria and fungi, consequently possess the ability to control soil-borne plant diseases and also promote plant growth (Deka Boruah and Dileep kumar, 2002a & b; Negi et al., 2005). P-solubilization by pseudomonas through the secretion of some organic acids which can act as chelator has also been reported (Kim et al., 1998; Gyaneswar et al., 2002; Chen et al., 2006). Considering the
significance of this array of bacteria in agricultural sectors, extensive studies have been made to isolate and characterize *Pseudomonas* species from various ecological niches.

**2.5. Plant growth promoting characters of PSB**

2.5.1. **Solubilization of nitrogen, phosphorus and other essential elements**- Biological nitrogen fixation, P-solubilization, improvement of other nutrient uptake by plants and phytohormone production are some examples of mechanisms that can directly influence plant growth. Plant N uptake through symbiotic N fixation and non-legume biological fixation have been reported by several workers (Dobbelaere *et al*., 2001; Vessey, 2003; Kennedy *et al*., 2004; Wu *et al*., 2005; Shaharoona *et al*., 2008). Phosphorus biofertilizers in the form of micro-organisms can help in increasing the availability of accumulated phosphates for plant growth by solubilization (Subba Rao, 1982; Goldstein, 1986; Kucey *et al*., 1989). In addition, micro-organisms involve in P-solubilization as well as better scavenging of soluble P can enhance plant growth by increasing the efficiency of biological nitrogen fixation, also enhancing the availability of other trace elements such as Al, Fe, Zn etc. (Kucey *et al*., 1989; Rodriguez and Fraga, 1999; Hariprasad and Niranjana, 2009). It was reported by Mc Dermott (1999) that every aspect of the process of formation of the N₂-fixing nodule is limited by the availability of P. Legumes like Alfalfa (Al-Niemi *et al*., 1997; Deng *et al*., 1998), clover,
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common bean, cow pea (Cassman et al., 1981) and Pigeon pea (Itoh, 1987) show a positive response to P supplementation. However, there are only a few reports of P solubilization by Rhizobium (Chabot et al., 1996; Halder et al., 1991; 1992). The fundamental works done on microbial P solubilization are found to be less, although it is known that P is the most limiting factor for N₂ fixation by Rhizobium-legume symbiosis (Gyaneswar et al., 2002).

2.5.2. Production of Organic acids- Several researchers reported that the PSB, specially some P solubilizing pseudomonas solubilize fixed P to the soluble form through the secretion of some organic acids, which can act as chelator (Kim et al., 1998; Gyaneswar et al., 2002; Chen et al., 2006). The ability of PSB to solubilize P-complexes has been attributed to their ability to reduce pH of the surroundings, either by releasing organic acids or protons. Organic acids such as citrate, lactate, succinate etc. secreted by PSB contribute P solubilization, which can either directly dissolve the mineral phosphates as a result of anion exchange or can chelate both Fe and Al ions associated with phosphate (Bajpai and Rao, 1971; Hariprasad and Niranjana, 2009). Finally insoluble P is converted into soluble monobasic (H₂PO₄⁻) and dibasic (HPO₄²⁻) ions, a process referred to as mineral phosphate solubilization. The role of organic acids produced by phosphate solubilizing micro-organism (PSM) in P solubilization is due to the lowering of pH, chelation of cations and by formation of soluble complexes with metal ions (Ca, Al, Fe) associated with insoluble P and resulting in release of P (Omar, 1998; Rodriguez and Fraga, 1999; Hariprasad and Niranjana, 2009). The
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organic acids produced by PSB, which is the major mechanism for P solubilization includes monocarboxylic (acetic, formic) monocarboxylic hydroxyl (lactic, glyconic), dicarboxylic hydroxyl (maleic, malic), tricarboxylic hydroxy acid (citric) and monocarboxylic, ketoglucenic, dicarboxylic (oxalo acetic, tartaric and succinic acids) (Bajpai and Rao, 1971; Lal et al., 2002; Vikram et al., 2007).

2.5.3. Production of phytase- Phytic acid (myo-inositol hexakis dihydrogen phosphanate) and mixed cation salts of phytic acid, designated as phytate, are a group of organic phosphorus compounds found widely in nature. Phytate are strongly complexed in soils, representing an important class of soil organic phosphorus and are sources of P for plant uptake (Hayes et al., 2000; Borie and Rubio, 2003; Turner et al., 2003). It is also an abundant plant constituent comprising 1-5% (w/w) of edible legumes, cereals, oil seeds, pollens and nuts (Cheryan, 1980). The use of phytate as P source may help in lesser use of inorganic P fertilizer in agro-pastorial ecosystem. In general, phosphatases are not able to hydrolyze the phytate. Phytase (myo-inositol hexakisphosphate phosphohydrolase), also known as phytate degrading enzymes, hydrolyse phytate to myo-inositol and phosphate in a stepwise manner (Konietzny and Greiner, 2002). Some micro-organisms are reported to produce phytase enzyme (Kim et al., 1998; Greiner and Farouk, 2007). The possible role of microbial phytase produced by PGPR in supporting plant growth under P limitation has not been investigated, but its purification and
characterization from a few PGPR is done for improving utilization of P from phytate (Kim et al., 1998; Konietzny and Greiner, 2002; Greiner and Farouk, 2007).

2.5.4. Production of plant growth hormones- Among all the mechanisms by which PSB stimulate plant growth, the production of plant growth regulators is found as an important one. Growth regulators such as auxins (indole-3-acetic acid, IAA) and gibberelic acid, GA3) regulate plant growth and influence a range of developmental process in plants including stem elongation, germination, dormancy, sex expression and fruit senescence etc. (Elezar and Escamilla, 2001; Gelmi et al., 2002). Plant cells synthesize IAA from the precursor tryptophan. Chemically, it can be synthesized by the reaction of indole with glycolic acid in the presence of base at 250 °C (Figure 5).

Figure 5. Synthesis of indole-acetic acid

![Indole-acetic acid synthesis](image)

Condition for indole acetic acid synthesis

The growth regulators are reported to be produced by the plants itself and also are found to be produced by micro-organisms, specially by the phosphate solubilizing bacteria found in the rhizosphere regions and thereby they
promote plant growth and control phytopathogens through secretion of some organic acids or phytohormones. The indole ring contained in L-tryptophan reacts with the acids produced by the PSB, form IAA or somehow the more complex structure GA₃ (Khalid et al., 2004; Kuklinsky-Sobral et al., 2004).

Some strains of *Pseudomonas, Enterobacter, Staphylococcus, Azotobacter* and *Azospirillum* produce plant growth regulators such as ethylene, auxins or cytokinins and have, therefore, been considered as useful for improving plant growth and yield (Patten and Glick, 2002).

### 2.5.5. Production of secondary metabolites

Siderophore is defined as low molecular weight, ferric ion chelating agent secreted by bacteria and fungi growing under low iron stress. Siderophores generally have three functional or iron chelating groups; each generally possesses two atoms of oxygen or less commonly two nitrogen atoms. They provide the plant growth promoting activity and suppression of root diseases (Meyer and Abdallah, 1978, Bakkar *et al.* 1987; Homma *et al.*, 1989; Keel *et al.*, 1990). Siderophores enhance the acquisition of iron in an iron deficient environment (Neilands 1979; Kloeper *et al.*, 1980) thereby making iron available to plants. Some PGPR produce siderophores and there is evidence that a number of plants can absorb bacterial Fe³⁺ siderophore complexes (Wang *et al.* 1993; Duijff *et al.*, 1994). *Pseudomonas fluorescence* is mostly reported to produce a variety of secondary metabolites including siderophores and antibiotics which enhance plant growth and/or suppress many phytopathogens. Siderophores such as pyoverdins, pyochelin as well as antibiotics exhibit
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plant growth promoting activity accompanied with suppression of root
diseases and pest control (Kloepper et al., 1980; Kandan et al., 2002; Deka
Boruah and dileep Kumar, 2002a & b).

Production of antibiotics in several strains of fluorescent pseudomonads
has been also recognized as a major factor in suppression of pathogens
(Dowling and O’Gara, 1994). Pyocyanine is a blue pigment produced by
Pseudomonas aeruginosa, which is a redox active phenazine compound. It
kills mammalian and bacterial cells through the generation of reactive oxygen
intermediates (Hassett et al., 1992). A number of disease-suppressive
antibiotic compounds have been characterized like phenazines, pyrrole-type
antibiotics, pyo-comounds, indole derivatives etc. The antibiotics
pyoluteorin, pyrrolnitrin, phenazine-1-carboxylic acid (PCA) and 2,4-di-
diacetylphloroglucinol (2,4-D) are currently the major focus of research in
biological control (Dowling and O’Gara, 1994; Battu and Reddy, 2009).

2.5.6. PGPR as Disease Control Agents- Biological control of plant
pathogens and deleterious microbes, through the production of antibiotics,
lytic enzymes, hydrogen cyanide and siderophores or through competition for
nutrients and space can improve plant health and growth (Antoun and
Kloeper, 2001). Bacterial antagonisms have been widely exploited towards
the management of plant diseases (Haas and Defago, 2005). A great diversity
of rhizospheric microorganisms have been described, characterized, and in
many cases tested for activity as biocontrol agents against soil borne
phytopathogens. Such microorganisms can produce substances that may limit the damage caused by phytopathogens like siderophores, antibiotics and a variety of enzymes. These microorganisms can also function as competitors of pathogens for colonization sites and nutrients (Dwivedi and Johri, 2003). Fluorescent pseudomonads have been widely used for disease control (Deka Boruah and Dileep Kumar, 2002b). Two major mechanisms have been proposed to explain the suppressive and antagonistic effects of fluorescent pseudomonads. In general, most microorganisms, including bacteria and fungi excrete molecules under Fe-starvation conditions, known as siderophores, which can trap traces of iron and form stable complexes. *Fluorescent pseudomonads* inhibit phytopathogens by producing secondary metabolites with antibiotic activity like phenazines, pyrroles, acetylphloroglucinols and cyanides (Haas and Defago, 2005). Viewing of the importance of the siderophores and antibiotics, several research works were carried out the isolation and characterization of these molecules (Deka Boruah and dileep Kumar, 2002b; Djibaoui and Bensoltane, 2005; Miranda et al., 2007).

**2.5.6.1. Tea phytopathogens and their control**- Being a monocultural crop, tea provides a stable microclimate for a number of pests and diseases and so a large number of pathogenic organisms are available in this ecological niche. Perennial habit of tea plant, peculiar cultural conditions, and warm humid climate of tea growing areas are highly conductive for the induction of diseases (Hijra, 2001). The crop loss in tea due to pests and diseases was
recorded around 10-20% in southern India by Sathyanarayana and Baruah (1983). Most of the tea diseases are of fungal origin. Chen and Chen (1989) described nearly 400 tea pathogens. More than 300 species of fungi are reported that affect different parts of the tea plant. Being a foliage crop, leaf diseases are more concern as it leads to direct crop loss and quality deterioration of the final produce (Baby et al., 1998). Blister blight, grey blight, leaf blight, leaf spot, leaf rot and leaf rust are common leaf diseases of tea bush. The major stem diseases are collar canker and thorny stem blight; while the major root diseases are charcoal stump rot, brown root rot, red root rot, black root rot, violet root rot and diplodia disease (Baby et al., 2001; Ponmurugan et al., 2007).

While concerning the rhizosphere, i.e. the main source of the pathogens or the fungus, the both primary and secondary root diseases are threat to the tea gardens (Mishra, 2006). Unlike foliar or stem diseases, the root diseases can lead to death of bushes and hence cause serious concern. Most often the symptoms are noticed after the death of bushes. Since the pathogens are soil borne in nature, the treatment is aimed at curing the ‘sick soil’ before replanting the area. The most common primary root diseases of tea plants in the plains of North East India are, red root (Poria hypolateritia), charcoal stump rot (Ustulina zonata) brown root (Fomes noxius, Fomes lamaeensis) and violet root rot (Sphaerostilbe repens), which can spread by means of direct contact or from left over diseased woody material as well as also can be spread by air borne spores. The diseased materials may also be
carried by rain run-off water to healthy teas. Adoption of proper cultural practices is a vital point for control of primary root diseases as no effective chemicals are available at the moment to control these pathogens. Secondary root diseases of fungal origin are also common in tea plants. Among them in waterlogged tea gardens violet root rot and brown root rot were found to be common (Figure 6a and b).

A number of bacteria showed affective antagonism under gnotobiotic conditions against major root pathogens of tea. They were *Trichoderma* spp (Baby and Chandra Mouli, 1996; Hazarika *et al.*, 2000; Sarmah *et al.*, 2005), *Gliocladium virens* (Baby and Chandra Mouli, 1996; Hazarika *et al.*, 2000), *Bacillus pumilis, Bacillus megaterium, Serratia marcescens* and *Ochrobactrum anthropi* (Chakraborty and Chakraborty, 2005), strains of fluorescent *Pseudomonas* (Hazarika *et al.*, 2000, Dileep Kumar *et al.*, 2005) and non-fluorescent *Pseudomonas* (Dileep Kumar *et al.*, 2005), *Bacillus subtilis* (Hazarika *et al.*, 2000) and strains of *actinomycetes* (Sarmah, 2005). Field experiments on red root disease showed that biocontrol agents like *T. Harzianum* and *Gliocladium virens* were effective in controlling the disease in new planting area as there was no casualty in young plants (Baby *et al.*, 2004). In north-east India, biocontrol of brown root and charcoal stump rot was effective with *Trichoderma* (Barthakur *et al.*, 2002; Sarmah *et al.*, 2005). Application of bioformulations of bacterial antagonists like *Psuedomonas fluorescens* and *Bacillus subtilis* was also equally effective to *Trichoderma* in controlling primary root diseases of tea (Premkumar and Baby, 2005).
Hazarika et al. (2000), used spore suspensions of antagonistic microbes like *T. harzianum*, *T. viride*, *G. virens*, *B. subtilis* and *P. fluorescens* in a induced disease resistance study on potted tea plants with *Ustulina zonata*. Though chemical control measures are effective in controlling tea diseases, in concern on environmental safety, pesticide residues in tea, and escalating cost of fungicides; biological controls is used as an alternative to chemical fungicides (Baby et al., 2005). The success of biocontrol agents under field conditions depends on certain factors like carrier materials and organic matter status in the soil (Barthakur et al., 2002; Ajay et al., 2004, 2005).

**Figure 6. Photographs showing diseased tea root affected by root borne fungus**

a) Brown root rot  

b) Violet root rot

Red arrow mark shows the infected area by the respective fungus (Source: a- Gingia tea estate; b- Nyagogra tea estate)
2.6. Mechanism of P solubilization

There are two components of P in soil viz., organic and inorganic forms; a large proportion is present in insoluble forms which is not available for plant uptake. Inorganic forms occur in soil as insoluble mineral complexes or some of them appear from the externally used chemical fertilizers. The organic matter is also an important reservoir for immobilized P that accounts for 20-80% of soil P (Rodriguez and Fraga, 1999; Richardson, 2001). Another form of organic P remains in soil as phytate, which are found in complex form (Hayes et al., 2000; Borie and Rubio, 2003; Turner et al., 2003). The principal mechanism for the solubilization of mineral phosphate is the action of organic acids synthesized by some soil micro-organism by the electron transfer between the oxidation stage from phosphine (-3) to phosphate (+5); while the solubilization of organic phosphate, i.e. the mineralization of organic phosphorus is carried out by the action of some phosphatase enzymes (Rodriguez and Fraga, 1999; Bagyaraj et al., 2000; Rodriguez et al., 2006; Chen et al., 2006). The mechanism is well described through the Figure 7.

Organic P usually accounts for 30% to 65% of total P in soils and must be converted to inorganic or low-molecular weight organic acids before they can be assimilated by plants. The common forms of P are inositol phosphatases, phosphoesters, phosphodiesters (phospholipids and nucleic acids), and phosphotriesters. A large part of the organic P is remain is the soil as inositol phosphatases (phytate), accounting for half or more of organic P.
Figure 7. Mechanism of phosphorus solubilization by PSB

**Mineral phosphate solubilization**
- Organic acid acidifies microbial cell
- \( e^- \) transfer from phosphine(-3) to phosphate (+5)
- Release of Pi from mineral phosphate by proton substitution

**Organic phosphate solubilization**
- Phosphatase
- Dephosphorylating reaction
- Hydrolysis of phosphoester bond
- Cleaving of C-P bonds.

**Genetics**
- Mps\(^+\) phenotype \( \rightarrow \) PQQ synthase (cofactor of GD synthesis)
- Oxidation of glc/ production of gluconic acid \( \uparrow \) \( H^+ \) generation
- Decrease of pH \( \rightarrow \) Mineral phosphate solubilization

- Pho regulation \( \rightarrow \) Pho B binds with Pho box
- (PhoA gene) \( \rightarrow \) Alk/ acid phosphatase
- Hydrolysis of phosphodiester bond \( \rightarrow \) C-P cleavage
- Org. phosphate solubilization

Schematic representation of P-solubilization

In soils and are the most important in plant nutrition (Rodriguez and Fraga 1999). Phosphatases refer to an enzyme that can hydrolyze phosphate esters and anhydrides. These include phosphoprotein phosphatases, phosphodiesterases, diadenosine tetraphosphatases, exonucleases, 5'-
nucleotidases, phytases, alkaline and acid phosphatases, phosphomonoesterases, etc. Phosphatases are sometimes described as phosphomonoesterase and found in some of the PGPR including genus *Bacillus* (Idriss *et al.* 2002), *Pseudomonas*, and *Rhizobium*, as reviewed by Rodriguez and Fraga (1999). Molecular biology tools have been used to elucidate plant-microbe interactions in P metabolism (Chen *et al.* 2006). Though molecular biology techniques are an advantageous approach for obtaining and characterizing improved PGPR strains that are involved in p-solubilization; release of genetically modified organism is controversial (Rodriguez and Fraga 1999; Rodriguez *et al.*, 2006)

### 2.7. Need of the bacterial diversity study

Soil is a dynamic, living matrix, an essential part of the terrestrial ecosystem and a critical resource for agricultural production and a food reservoir, which maintains most of life processes (Lynch, 1990; Pinton *et al.*, 2001). The diversity and community structure in the rhizosphere is influenced by both; plant and soil type (Latour *et al.*, 1996). Microbial diversity describes complexity and variability at different levels of biological organization (Rosello-Mora and Amann, 2001). Soil comprises a variety of microhabitats and the microorganisms adapt to microhabitats and live together in consortia with more or less sharp boundaries, interacting with each other and with other parts of the soil biota (Rosello-Mora and Amann, 2001; Ranjard and...
Richaume, 2001; Sessitsch et al., 2001). Presently, the use of molecular approaches to study the microbial diversity has improved on the knowledge on population structure in a microbial inhabitant (Hugenholtz et al., 1998). Considering the importance of phosphate solubilizing bacteria on plant growth and development, the diversity and screening of P solubilizing bacteria in different niches has been reported so far by many workers (Hariprasad and Niranjana, 2009; Ponmurugon and Gopi, 2006a and b; Baby et al., 2001; Piex et al., 2001; Chabot et al., 1996; Kucey et al., 1989). Through 16S rRNA sequencing technique, a complete microbial community composition can be described and can indicate possible nutritional requirements as well as physiological properties of many ecosystems (Torsvik and Overeas, 2002; Polz et al., 1994; Distel et al., 1991). For successful functioning of introduced microbial bio-inoculants and their influence on soil health, exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behaviour in soil habitats (Hill et al., 2000). The era of molecular microbial ecology has uncovered only a part of novel microbiota, most of which is based on DNA and rRNA analysis (Torsvik and Overeas, 2002) and the molecular methods used globally for diversity assessment of different cropping systems include, phospholipid fatty acid (PLFA) analysis (Miethling et al., 2000; Chelius and Triplett, 2001) terminal- restriction fragment length polymorphism (T-RFLP) (Dunbar et al., 2000), single-strand conformation polymorphism (SSCP) (Zumstein et al., 2000; Schmalenberger and Tebbe, 2002), and
denaturing/temperature gradient gel electrophoresis (DGGE) with the plant rhizosphere belonging to genera that are able to exert a beneficial effect on plant growth. The RAPD technique was developed also as an efficient tool to analyze phylogenetic relationships among and within closely related species (Williams et al., 1990). Major molecular techniques include PCR (Polymerase chain reaction), RAPD (randomly amplified polymorphic DNA), RFLP (restriction fragment length polymorphism), AFLP (amplified fragment length polymorphism), SSR (single sequence repeats) and 16S-rRNA gene sequencing. RAPD is the most reliable, rapid and practical method (Mehmood et al., 2008) used for phylogenetic relationships among and within closely related species (Williams et al., 1990).

### 2.8. Prospect of PGPR in fertilizer management

Despite growing information on the effects of biofertilizers on nutrient uptake, there is a need for a continuous discussion of emerging scientific data and reevaluation of methodologies. Although the population has been growing and available land for agriculture has been shrinking, intensive agriculture that involves heavy and continuous use of fertilizers has ensured high crop productivity (Tilman, 1998). But the intensive and extensive use of fertilizer causes environmental pollution due to the low efficiency in the uptake of fertilizer, which is a major factor that aggravates the negative environmental effects (Barlog and Grzebisz, 2004). Over 50% of the applied
N can be lost from agricultural systems as N\textsubscript{2}, trace gases, or leached nitrate and the impacts are usually long term and global in scope (Tilman, 1998, Kennedy et al., 2004). Similarly, phosphorous (P) fertilization the second essential macronutrients, also precipitated even more than 90\% and later causes P pollution (Rodriguez and Fraga, 1999, Sharpley et al., 2003, Gyaneshwar et al., 2002).

Canbolat et al. (2006) was conducted the study with barley seedlings and concluded that microbial inoculants impact on plant nutrient uptake with respect to time or the stage of growth of the plant. Furthermore, they reported the increases in N and P content of plant dry matter with each inoculated Bacillus strain compared with the control. Somewhat similar study was conducted by Elcoka et al. (2008), where chickpea inoculated with strains of Rhizobium, N\textsubscript{2}-fixing Bacillus subtilis OSU-142, and P solubilizing B. megaterium M-3 in comparison with mineral fertilizer application was applied in “controlled environments” and in the field. The authors showed that single, double, and triple inoculations significantly increased all parameters measured (including N content), with equal or higher proportion compared to treatments with N, P, and NP fertilizers in controlled experiments. Egamberdiyeva (2007) indicated that PGPR as bacterial inoculates had better stimulating effect on the growth and nutrient (N, P, and K) uptake of maize in nutrient-deficient soil than loamy sand. This is contrary to the common assumption that the usefulness of PGPR is limited under nutrient deficient conditions (Khan, 2005). Use of PGPR as biofertilizer may
help in plant growth in sustainable agriculture, which are eco-friendly and reduces the deleterious effects of using the chemical fertilizer. In this study, the effort was given for the management of nutrient use efficiency through the application of PSB in order to make the applied nutrients more available to the plant.

2.8.1. Substitution of chemical fertilizers by microbial inoculants- A bacterial inoculant is a formulation containing one or more beneficial bacterial strains (or species) in an easy-to-use and economical carrier material, either in organic, inorganic, or synthesized from defined molecules. The term ‘Biofertilizer”, is a misleading but widely used term meaning “bacterial inoculant”. Usually it refers to preparations of microorganism(s) that may be a partial or complete substitute for chemical fertilization (like rhizobial inoculants), which are eco-friendly and reduces the deleterious effects of the chemical fertilizer (Bashan, 1998). Usually it refers to preparations of microorganism(s) that may be a partial or complete substitute for chemical fertilization (like rhizobial inoculants), which are eco-friendly and reduces the deleterious effects of the chemical fertilizer. According to Vessey et al. (2003), microbial inoculants are the microbes which when applied to plants consistently promote plant growth, maintain the plant health and yield enhancement by enhanced physiological activity and nutrient uptake of the introduced plants. Wu et al. (2005) reported that microbial inoculants increased the growth and nutritional assimilation of total N, P, and K in maize and improved soil properties. Study of Shaharoona et al. (2008)
with inoculation of *Pseudomonas fluorescence* (strain ACC50) and *P. fluorescence* biotype F (strain ACC73) in pot and field showed increased use efficiency of N and P at all tested NPK fertilizer doses in wheat. Furthermore, Amir *et al.* (2005) reported enhanced uptake of N and P in oil palm seedlings in Malaysia, following PGPR inoculation in the nursery. Aseri *et al.* (2008) conducted experiments in the field in India and assessed the effectiveness of PGPR (*Azotobacter chroococcum* and *Azotobacter brasilence*) on the growth, nutrient uptake and biomass production of pomegranate (*Punica granatum* L.). Adesemoye *et al.* (2008) reported that inoculation with mixed strains were more consistent than single strain inoculations. Though many studies reported enough on the mode of action of PGPR on plant growth but there are many data to be accounted to correlate the claim of PGPR in nutrient management.

### 2.8.2. PGPR in N\textsubscript{2} management-

The most studied and longest exploited PGPR are the rhizobacteria for their ability to fix N\textsubscript{2} in their legume hosts. Commercial rhizobacterial inoculants for use in cultivation of legume crops were first introduced in the year 1890 (Fred *et al.*, 1932) and reviewed the importance of rhizobium (Hansen, 1994, Gualtieri and Bisseling, 2000, Sessitsch *et al.*, 2001; McInnes *et al.*, 2004). Plant N uptake through symbiotic N fixation and non-legume biological fixation/non-associative uptake have been reported widely in several studies (Kennedy *et al.*, 2004; Dobbelaere *et al.*, 2001; Vessey, 2003; Wu *et al.*, 2005; Shaharoona *et al.*, 2008). As proposed by Bhattacharjee *et al.* (2008) that with progressive
understanding of the interactions between nitrogen-fixing bacteria and cereal crops, the world is closer to the dream of developing an eco-friendly nutrient source for cereal crops. Understanding of the key factors governing microbial ecology of the rhizosphere is highly needed but has yet to be fully achieved.

2.8.3. PGPR in Phosphorous management- Though the soil is a large reservoir of P, it is also the one of the most deficient minerals in terms of agricultural crop productions (Stevenson and Cole, 1999). The major portion of soil P is found in insoluble forms and hence it can’t be taken up by plants. Plants can absorb P only in two soluble forms, the monobasic (H$_2$PO$_4^-$) and the dibasic (HPO$_4^{2-}$) ions (Glass, 1989). Hence, a farmer shall have to apply a large amount of fertilizers (Ohno et al., 2005). Consequently, a significant part of the P may constitute an environmental problem in the future. Soil microorganisms are responsible for transforming immobilized soil P into plant-available forms by conversion of insoluble forms of P to plant available forms (Gerretsen, 1948, Kim et al., 1998, Rodriguez and Fraga, 1999, Richardson, 2001; Rodriguez et al., 2006; Hariprasad and Niranjana, 2009). Research is underway to determine the potential for exploitation of P solubilizing microorganisms to increase plant growth on soils with high immobilized P. Phosphorous solubilization by PSB to make it available for plants uptake was revealed in several studies, include Azotobacter chropoccum in wheat (Kumar and Narula, 1999); Bacillus sp. (Pal, 1998), Enterobacter agglomerans in tomato (Kim et al., 1998); Pseudomonas chlororaphis and P. putida in Soybean; Pseudomonas poae, P. trtivalis, and
Review of literature

*Rhizobium radibacter* in Chinese cabbage (Poonguzhali *et al.*, 2008). It was also reported that phosphate solubilizing bacteria are common in rhizosphere (Nautiyal *et al.*, 2000). But not all the phosphorus solubilizing bacteria are responsible for plant growth and phosphorus management but other factors also influence plant growth (Vessey, 2003).

2.8.4. PGPR in management of other elements- Inoculation with PGPR have been shown to influence the uptake of other essential macro- and micro-nutrients in addition to N and P (Peix *et al.*, 2001; Khan, 2005; Wu *et al.*, 2005; Adesemoye *et al.*, 2008). In a review, Khan (2005) observed that inoculation with PGPR such as *Pseudomonas* and *Acinetobacter* strains had resulted in enhanced uptake of Fe, Zn, Mg, Ca, K, and P by crop plants. In a study with strains of *Mesorhizobium mediterraneum* inoculated onto chickpea and barley, the nutrient content of K, Ca, and Mg in addition to P and N significantly increased in both plants (Peix *et al.*, 2001). Increased uptake of macro and micronutrient were recorded for seed inoculated plants with PGPR *Methylobacterium*, *Azospirillum* and also with the co-inoculation in red pepper, rice and tomato (Kim *et al.*, 2009; Madhayan *et al.*, 2009). Kohler *et al.* (2008) also demonstrated the effects of PGPR (*Pseudomonas mendocina*) on uptake of N, P, Fe, Ca, and Mn in lettuce (*Lactuca sativa* L. cv. Tafalla) under three different levels of water stress. Han and Lee (2005) reported an increased uptake of P and K when soil was fertilized with rock P and K and co-inoculated with P solubilizing bacteria *B. megaterium* and K solubilizing bacteria *B. mucilaginosus*. Sheng and He (2006) reported improved uptake of
K through the inoculation of PGPR B. edaphicus strains NBT produces organic acids (citric, oxalic, tartaric, succinic, and α-ketogluconic) lead to chelation of metals and mobilization of K from K-containing minerals. Sulfur (S) and Fe uptake have been achieved also through sulfur-oxidizing bacterial inoculant and siderophore-producing bacteria, respectively (Anandham et al., 2009). They suggested that the increased uptake of Fe, P, and K was associated with higher N rates but higher N was a result of mechanisms other than biological N\textsubscript{2} fixation.

2.8.5. PGPR on fertilizer use efficiency- The current proposition towards solving agro-environmental problems is integrated nutrient management (INM). By INM, it is not meant to substitute the application of fertilizers as a whole but, minimization of fertilizers applied without affecting the production. In other words, through INM, farmers may stop the over usage of fertilizers. The INM system promotes low chemical input but improved nutrient-use efficiency by combining natural and manmade sources of plant nutrients in an efficient and environmentally prudent manner (Adesmoye and Kloeppe, 2009). Most of the findings conclude that increase plant N content might have resulted from increased fertilizer utilization efficiency in an INM system (Adesmoye et al., 2008; Madhaiyan et al., 2009; Kim et al., 2009).

The amount of fertilizer applied to plants is usually large; the part of the applied fertilizer taken up by plants is usually small, ranging between 10% to 40% depending on soil type, fertilizer type, and plant; and the part of the applied fertilizer that is lost could be in the range of 60% to 90% of the
original amount of fertilizer or manure applied (Gyneshwar et al., 2002; Barlog and Grzebisz, 2004; Kennedy et al., 2004; Tilman et al., 2002; Adesmoye and Kloeper, 2009). In such a situation, the question to the scientific community as raised by that whether it is possible to reverse the trend of using high percentage of applied fertilizer and applying large amounts of fertilizers, by supplementing reduced fertilizer with PGPR inoculantion, maintaining plant growth and high yield comparative to the use of full recommended fertilizer rates. In such a case investigation on use of PGPR to maintain higher nutrient uptake is inevitable.