2. REVIEW OF LITERATURE

2.1. PLANT GROWTH PROMOTING RHIZOBACTERIA AND THEIR BIODIVERSITY

The Rhizosphere representing the thin layer of soil surrounding plant roots and the soil occupied by the host supports large active groups of bacteria known as plant growth promoting rhizobacteria (PGPR). PGPR are known to rapidly colonize the rhizosphere and suppress soil borne pathogens at the root surface. These organisms can also be beneficial to the plant by stimulating growth (Bloemberg and Lugetenberg, 2001).

The beneficial effects of these rhizobacteria have been variously attributed to their ability to produce various compounds including phytohormones, organic acids, siderophores, fixation of atmospheric nitrogen, phosphate solubilization, antibiotics and some other unidentified mechanisms (Glick, 1995).

Motile rhizobacteria may colonize the rhizosphere more profusely than the non motile organisms resulting in better rhizosphere activity and nutrient transformation. Today researchers are able to repeatedly use them successfully in field experiments. Commercial applications of PGPR are being tested and are frequently successful however a better understanding of the microbial interactions that result in plant growth increases will greatly increase the success rate of field applications (Farzana et al., 2009).
PGPR are free living bacteria that are able to colonize plant roots and promote plant growth (Kloepper and Schroot, 1978). The improved plant growth and crop yield is attributed to disease suppression (Schroot and Ancock, 1982), iron chelation (Kloepper et al., 1988), antibiotic production (Weller, 1988), enhanced nutrient uptake (Lifshitz et al., 1987) and seedling emergence promotion (Klopper et al., 1989) and by plant phytohormone production.

Beneficial free living soil bacteria occurring in the plant rhizosphere are usually referred as plant growth promoting bacteria and abbreviated as PGPR (Kloepper et al., 1989). They are also referred as yield increasing bacteria, and abbreviated as YIB (Tang, 1994). PGPR play a major role in maintaining the soil ecosystem in the soil, rhizosphere, rhizoplane and phyllosphere that are ultimately beneficial to the plants.

Microorganisms which are mainly considered as PGPR include the members of the genera *Azotobacter, Azospirillum, Pseudomonas, Alcaligenes, Arthrobacter, Acinetobacter, Bacillus, Acetobacter, Achromobacter, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Rhizobium, Clostridium, Frankia, Streptomyces and Serratia* (Brown, 1974; Elmerich, 1984; Kloepper et al., 1988; Bashan and Levanony, 1990; Tang, 1994; Kloepper, 1997; Bashan and de Bashan, 2005).

Plants play an important role in selecting and enriching the types of bacteria in the rhizosphere by the influence of the constituents present in
their root exudates. The nature and concentrations of organic constituents of root exudates, and the corresponding ability of the bacteria to utilize these as sources of energy, determines the bacterial community development in the rhizosphere soil (Curl and Truelove, 1985). There is a continuum of bacterial presence in rhizosphere soil, rhizoplane and internal of the plant tissues (Hallmann et al., 1997).

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

Bacteria associated with plants can be either harmful or beneficial. PGPR may promote growth directly, by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderosphores that solubilize and sequester iron, or production of plant growth regulators, phytohormones (Klopper, 1997).

Some bacteria support plant growth indirectly by improving growth restricting conditions either via production of antagonistic substances or by inducing host resistance towards plant pathogens. Since associative
interactions of plant and microorganisms must have come into existence as a result of convolution; the use of either former or latter groups as bioinoculants forms one of the vital components for a long-term sustainable agriculture system (Tilak et al., 2005).

Arun et al. (2012) investigated the plant growth promoting isolates obtained on N free media in the rhizosphere of Cassia occidentalis, which have the potential to be used as inoculants for other crops. This provides a new dimension to the significance of weeds in agricultural ecosystems. The study opens up possibilities for utilization of this property of weeds in plant growth promotion, and subsequent of yield for agricultural crops.

2.1.1. Azospirillum

Azospirillum is a Gram negative common root and soil inhabitant in the tropics and so far five species have been described. Both A. brasilense and A. lipoferum are thoroughly studied among the members of Azospirilla and a number of morphological and physiological characters differentiate between both species (Tarrand et al., 1978). A. amazonense, an acid tolerant species isolated from the grasses and palm trees in Brazil (Magalhaes et al., 1983) and A. halopreferens was isolated from the root surface of Leptochloa fusca in Pakistan (Reinhold et al., 1985).

The occurrence of Azospirillum in the rhizosphere varied from 1 to 10 per cent of total rhizosphere population (Okon, 1985). The rhizosphere contains 100 times more Azospirilla than in the non-rhizosphere soils.
(De Coninck et al., 1988). *Azospirillum* has been isolated from different agro-climatic zones and in crops all over the world (Michiels et al., 1989). Sumner (1990) reported the occurrence of *Azospirillum* sp. in the rhizosphere of various field grown crops such as rice, wheat, maize, sorghum and pearlmillet collected from different locations. Presence of *Azospirillum* in the rhizosphere of *Cactus, Pachycreus, Pringlei* (Puente and Bashan, 1993) and Mediterranean herbaceous swards (Zaady et al., 1994). Kabir et al. (1995) categorized four species of *Azospirillum* based on the study made at molecular level using different oligonucleotide probes. Kavitha (2000) isolated *Azospirillum* species from wetland rice and reported that *Azospirillum* accounts for 18 per cent of the total heterotrophic population.

*Azospirillum* sp. are widely distributed soil nitrogen fixing bacteria that play an important role in the promotion of plant growth (Steenhoudt and Vanderleyden, 2000). Saleena et al. (2002) studied the diversity of *Azospirillum* strains isolated from rice plants grown in saline and non saline soils of coastal agricultural ecosystem and reported the predominance of *Azospirillum brasilense* and *Azospirillum lipoferum*.

Purushothaman (2002) isolated over 300 isolates of *Azospirillum* from the root tissues of cashew and characterized them. *Azospirillum* strains have no preferences for crop plants or weeds or for annual or perennial plants and can be successfully applied to plants that have no previous history
of *Azospirillum* in their roots. It appears that *Azospirillum* is not a plant specific bacterium and is a general root colonizer.

Rao and Charyulu (2003) reported that the rhizosphere of foxtail millet harbored a distinct population of diazotrophic, non-symbiotic bacteria and associative symbiont, *Azospirillum* spp.

Yasmin et al. (2004) isolated and characterized plant growth promoting bacteria (PGPB) occurring in four soils of Zanzibar, and Tanzania and evaluated their potential use as biofertilizers for rice. *Azospirillum* sp were isolated from the rhizosphere and rhizoplane of certain medicinal herbs *viz.*, Catharanthus roseus, Ocimum sanctum, Phyllanthus amarus, Coleus forskholii and Aloe vera (Geetha, 2003; Kalpana, 2005; Karthikeyan et al., 2007b; Sakthivel and Karthikeyan, 2012).

Usha and Kanimozhi (2011) isolated 10 strains of *Azospirillum*. Among them, four strains namely AZO- 3, AZO- 6, AZO- 8, and AZO- 10 were isolated from saline soil and the remaining six strains namely AZO- 1, AZO- 2, AZO- 4, AZO- 5, AZO-7, AZO- 9, were isolated from non saline soil.

Ilyas et al. (2012) reported that the total of eight strains of *Azospirillum* were isolated from rhizosphere and roots of maize plants grown in pots and it was observed that survival efficiency of *Azospirillum* from well watered plants was higher as compared to that of *Azospirillum* strain isolated from roots and rhizosphere samples of water stressed plants.
(having 8-12 % soil moisture). Inoculation of wheat with isolates to water stressed plants induces tolerance to water stress in plants. Isolate from water stressed conditions produced low concentration of indole acetic acid, gibberellic acid and trans zation riboside but higher concentration of abscisic acid. The isolated bacterial strains have technological implications for inoculants formulation and improved growth of cereal crops.

2.1.2. Azotobacter

Azotobacter is a Gram negative, free living aerobic heterotrophic bacteria capable of fixing atmospheric nitrogen and has been observed in sugarcane, corn, oat and soybean rhizosphere (Rennie et al., 1981). Azotobacter species although mesophilic in nature, capable of showing thermotolerance up to 40°C (Rajkumar and Lakshmanan, 1995; Behl et al., 2006).

The cells are large, ovoid, 1.5-2.0 μm or more in diameter, pleomorphic and occur in single or in pairs or in irregular clumps and sometimes in chains of varying length. It consists of seven species viz., A. armeniacus, A. beijerinckii, A. chroococcum, A. nigricans, A. paspali, A. agilis and A. vinelandii. Phytohormones produced by A. chroococcum strain isolated from Pantoea agglomerans was reported by Narula et al. (2006).

A. chroococcum in Indian soils rarely exceeded $10^4$ to $10^5$ g$^{-1}$ soil and is highly influenced by the antagonistic action of soil microflora and organic matter content of soil (Kannaiyan, 2000a). Azotobacter indicum was
capable of reducing nitrate to nitrite and to nitric oxide and nitrous oxide under anaerobic conditions (Furina et al., 2002).

*Azotobacter* sp was isolated from the rhizosphere of certain medicinal herbs *viz.*, *P. amarus, C. roseus, O. sanctum, Coleus forskholii, Aloe vera* (Geetha, 2003).

Sachin (2009) reported *A. chroococcum* was successfully isolated from the rhizosphere soil of seven weeks old bamboo plants in forest area of Allahabad.

Karthikeyan and Sakthivel (2011) isolated and characterized the *Azotobacter chroococcum* from the rhizoplane of *Eucalyptus camaldulensis*. This isolate produces significant quantities of IAA, which is inoculated with *E. camaldulensis* cuttings produces higher growth than synthetic rooting hormone indole butyric acid (IBA) treated cuttings. The stem cuttings of *E. camaldulensis* responded positively to *Azotobacter chroococcum* inoculation through increased root proliferation and growth.

A total of twenty five endophytic bacteria were isolated from different parts of mungbean plants such as leaves and roots and four isolates which have shown better response for PGPB marking as FR, FL, NR and NL. Further, this four isolates analyzed for 16S rDNA sequences for genus identified as isolate FR belongs to genus *Azotobacter* sp., isolates FL
belongs to genus *Azotobacter vinelandii* and isolates NR and NL belongs to *Azotobacter chroococcum*, respectively (Aung et al., 2011).

Irum Naz et al. (2012) isolated and characterized *Azotobacter vinelandii* strain-Khsrl from roots of the weed, *Chrysopogon atheri* (golden beard grass weed). The isolate was capable of producing phytohormones: indole-3- acetic acid, gibberelic acid, trans-zation riboside and abscisic acid in culture supernatant, stimulated growth of *Zea mays* L. seedlings and augmented proline content of roots and shoots both under normal and NaCl stressed conditions.

2.1.3. *Pseudomonas*

*Pseudomonas striata, P. cissicola, P. fluorescens, P. pinophilum, P. putida, P. syringae, P. aeruginosa, P. putrefaciens* and *P. stutzeri* have been isolated from rhizosphere of *Brassica*, chickpea, maize, soybean and other crops, and few species from desert soils and Antarctica lake (Bardiya and Gaur, 1974; Kole and Hajra, 1997; Gupta et al., 1998).

Molecular characterization and diverse analysis of *Pseudomonas* include PCR-based typing methods, RAPD - PCR and DNA sequence based characterization, which are broadly applicable for typing bacterial species (Samiyappan et al., 2006).

Geels and Schippers (1983) and Weststeijn (1990) isolated fluorescent *Pseudomonas* from potato tubers using modified King’s ‘B’
medium supplemented with cyclohexamide, chloramphenicol, antimycin and para hydroxyquinoline.

Gamiel and Katan (1993) obtained strains of *P. putida* from tomato roots using modified King’s ‘B’ medium incorporated with cyclohexamide, ampicillin, chloramphenicol and pentachloro benzene. Glick *et al.* (1995) demonstrated a novel procedure for rapid isolation of plant growth promoting *Pseudomonas* using 1-aminocyclopropane-1-carboxylate (ACC) as the sole source of nitrogen. The *Pseudomonas* strain designated as RRLJ 134 was isolated from the top layer of sandy loam having a pH of 5.0 in a tea plantation in the Dooars region (Kumar and Bezbaruah, 1996).

Benchabane (2004) isolated about 500 fluorescent strains of *Pseudomonas* from the rhizosphere of different plants namely Tomato, Potato, Corn and Vine and suggested that the plant and the soil type play a considerable role in the distribution and the taxonomic diversity of fluorescent *Pseudomonas*.

Inoculation of Canola seedling with *Pseudomonas putida* GR12-2 has been found to increase the root and shoot dry weight of canola under cold and salinity stress conditions (Glick *et al.*, 1997). Cheng *et al.* (2007) reported that the inoculation of ACD (ACC-deaminase) producing *Pseudomonas putida* strain could protect the plant under salinity stress. Glick *et al.* (1994) reported that *Pseudomonas putida* contain an enzyme, ACC-deaminase which hydrolysed ACC to ammonia and α-ketobutyrate.
This process eventually led to decreased level of ACC, and thereby reduced the level of endogenous ethylene. Thus the potential inhibitory effect of increased ethylene concentration could be eliminated (Yuhashi et al., 2000).

Karthikeyan and Deiveekasundaram (2006) isolated *Pseudomonas* sp. from the rhizosphere of *C. roseus, C. forskholii, Aloe vera* and *O. sanctum*.

*Pseudomonas* sp. are direct promotion plant growth entails either production of the phytohormones such as auxin, cytokinins, gibberellins and 1-aminocyclo propane-1-carboxylic acid (ACC) deaminase affecting the root morphogenesis and increasing absorptive through asymbiotic N\textsubscript{2} fixation and phosphate solublization (Gyaneshwar et al., 2002; Glick et al., 2007).

A PGPR *Pseudomonas fluorescens* B16 isolated from the roots of germinaceous plants has been shown to colonize the roots of various plants, and to increase the height, flower number, fruit number and total fruit weight of tomato plants (Minorsky, 2008).

Maleki et al. (2010) was isolated *Pseudomonas fluorescens* strain Cv6 from cucumber rhizosphere soil in Vermanin. This strain Cv6 was shown to have broad spectrum *in vitro* antibiotic activity against eleven additional plant pathogens.

Djuric et al. (2011) reported that the occurrence of different fluorescent *Pseudomonas* spp., indigenous soil bacteria in maize rhizosphere. The isolate *Pseudomonas fluorescens* strain PS2, with antagonistic activities against *Curvularia lunata* and *Fusarium equisetii* and
with functional properties distinctive for PGPR may represent precious biological alternative for harmful pesticides and chemical fertilizers application in agriculture field due to crucial role of rhizobacteria to plant health maintenance and soil fertility.

Kapoor et al. (2012) reported that thirty strains of fluorescent *Pseudomonas* were isolated from the rhizosphere soil of Apple and Pear plants of their normal sites and replant sites.

Noori and Saud (2012) reported that the twenty strains of *Pseudomonas* isolated from the rhizospheric soils of paddy areas in Malaysia and were screened for their plant growth promoting activity. All the twenty tested isolates of *Pseudomonas* were positive for the production of siderophore and HCN, out of twenty antagonist bacteria strains, fifteen starins (75 %) showed positive for the production of plant growth promoting hormone and IAA.

2.1.4. *Bacillus*

*Bacillus* is gram positive, free living aerobic heterotrophic bacteria occurring in the rhizosphere of several crop plants. The *Bacillus* species such as *B. brevis*, *B. cereus*, *B. circulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, *B. mesentericus*, *B. mycoides*, *B. polymyxa*, *B. pumillus*, *B. pulvifaciens* and *B. subtilis* were reported from the rhizosphere of legumes, cereals (rice and maize), arecanut palm, oat, jute and chilli (Sundara Rao and Sinha, 1963; Kole and Hajra, 1998).
Soil microorganisms can promote plant growth through the production of different hormones, such as, cytokinins, auxins and or ethylene, gibberellins and nitrogen fixing ability or by the suppression of plant diseases caused by deleterious microorganisms (Bloemberg and Lugtenberg, 2001; Loon, 2007). Some spore forming bacteria, in particular, gram-positive bacilli and *Streptomyces*, have attracted special attention due to their advantages over non-spore formers in product formulation and stable maintenance in soil (Emmert and Handelsmann, 1999). Among these PGPR, *Paenibacillus polymyxa* is known to have a broad host range.

Multiple *Bacillus* and *Paenibacillus* sp. can be cultured from both bulk and rhizosphere soils. Culturable counts of these bacteria generally range from log$_5$ to log$_6$ cells per gram fresh weight with soil counts typically exceeding those obtained from the maize rhizosphere (Halverson *et al.*, 1993; Mahaffee and Kloepfer, 1997; Seldin *et al.*, 1998; Ayala *et al.*, 2000).

Aerobic Endospore Forming Bacteria (AEFB), such as, *Paenibacillus* and *Bacillus* are essentially ubiquitous in agricultural systems. Common physiological traits important to their survival, include, production of a multilayered cell wall structure, formation of stress-resistant endospores, and secretion of peptide antibiotics, peptide signal molecules and extracellular, enzymes. Multiple species of *Paenibacillus* and *Bacillus* can be detected in the soils and rhizosphere (Seldin *et al.*, 1998). *Paenibacillus* is widely distributed throughout the environment, particularly in air, soil and
decomposing plant residue (Claus and Berkeley, 1986). A bacterium that
belongs to the genus, *Paenibacillus*, are promising inoculants because these
spore forming microbes can persist in soil for long periods of time and can
also be produced and stored for commercial purposes (Seldin *et al.*, 1998).

Karuppiah and Rajaram (2011) reported from the sixty three different
*Bacillus* sp. from twenty five soil samples were isolated from rice field.
Among the 63 isolates, the eight *Bacillus* sp. (BA1 to BA8) possesses
effective PGP activities. In eight different *Bacillus* isolates particularly
(BA1, BA3, BA4 and BA6) exhibited maximum plant growth promoting
and chromium reducing activities. In addition to these traits, plant growth
promoting bacterial isolates must be rhizosphereic competent, able to
survive and colonize in the rhizospheric soil.

Sandeep *et al.* (2011b) reported that the growth response of *Ayapana*
on inoculation with *Bacillus megaterium* isolated from different soil types of
various agroclimatic zones of Karnataka. The plants inoculated with *Bacillus
megaterium* isolates, the height, number of leaves, fresh and dry weight of
roots and shoots, nitrogen content, P content and chlorophyll content
remained higher than the uninoculated maize plants.

Shobha and Kumudini (2012) reported that the nineteen rhizosphere
soil samples from various plants *viz.*. ragi, brinjal, paddy, tomato, beans,
mango, chilly, *Okra anamika* and marigold were collected from different
regions in and around Bangalore and Krishnagiri. The seven isolates of
*B. megaterium* JUMB1, JUMB2, JUMB3, JUMB4, JUMB5, JUMB6 and
JUMB7 were screened in vitro for their plant growth promoting traits like production of indole acetic acid (IAA), ammonia, HCN, phosphate, siderophore and evaluated for the ability to suppress fusarial growth. *In vitro* screening for antagonism activity against *Fusarium oxysporum* revealed significant inhibitory effects of mycelia radial growth by all the seven isolates.

### 2.1.5. OTHER BENEFICIAL MICROBES

Several members belonging to *Escherichia freundii, E. intermedia, Serratia, Achromobacter, Brevibacterium, Corynebacterium, Erwinia, Micrococcus, Sarcina* and *Xanthomonas; Cyanobacterial* members such as *Anabaena* sp., *Calothrix brauni, Nostoc* sp., *Scytonema* sp. and *Tolypothrix ceylonica* were isolated as phosphate solubilizers (Gupta et al., 1998).

Arun et al. (2012) isolated the four bacterial strains *Isosericola variabilis* B10, *Agrobacterium tumifaciens* B12, *Bacillus safensis* NF and *Mesorhizobium* sp. NL form the rhizosphere of *Cassia occidentalis* medicinal plant. These isolates were used as inoculants of luxuriantly growing common weed, *C.occidentalis*, showed the potential to positively affect growth of *V. radiate*. This leads to the assumption that weed microflora is a rich source of PGPR, which can be assayed as non-rhizobial inoculants for leguminous crops in field trails.

Sang-Mo Kang et al. (2012) isolated and identified the gibberellins-producing *Burkholderia* sp. KCTC 11096 from agricultural field soil. The
culture filtrate of PGPR significantly increased the germination and growth of lettuce and Chinese cabbage seeds. The ethyl acetate extract of the culture showed significantly higher rate of lettuce seed germination and growth as compared to the distilled water treated control.

2.2. FUNCTIONS OF PLANT GROWTH PROMOTING RHIZOACTERIA

The PGPB are capable of fixing atmospheric nitrogen, solubilizing phosphorus, iron and producing plant hormones *viz.*, auxins, gibberellins and cytokinins and promote plant growth by directly affecting the metabolism of the plants by providing substances that are usually in short supply. Additionally, they improve plant tolerance to drought, high salinity and metal toxicity through the production of enzyme 1-amino cyclopropane carboxylate deaminase. PGPB prevents the deleterious effects of phytopathogenic microorganisms. They produce antibiotics that harm or inhibit other microbes, but not plants, by limiting the availability of iron to pathogens or by altering the metabolism of the host plant to increase its resistance to pathogen infection.

The plant responses to inoculation with PGPB include enhanced nitrogen fixing ability with minimal inoculation, direct increases of various growth parameters like plant dry weight, development and morphology of root system, grain yield, protein and mineral nutrient content, displacement of deleterious and pathogenic rhizosphere microorganisms, increased
phosphorus solubilization and enhanced VA-mycorrhizal colonization (Rennie et al., 1981).

The mechanisms attributed for plant growth stimulation, is mainly due to improvement of water and mineral uptake (Bashan and Levanony, 1991; Bertrand et al., 2000) and production of biologically active substances, such as vitamins, amino acids, phytohormones (Garcia de Salamone et al., 2001; Glick, 1995 and Persello-Cartieux et al., 2003) and antibiotics (Giacomodonato et al., 2001).

Ahmad et al. (2008) compiled the possible mechanisms PGPR employ as (1) the ability to produce or change the concentration of plant growth regulators like indole acetic acid (IAA), gibberellic acid, cytokinins and ethylene, (2) asymbiotic N₂ fixation, (3) antagonism against phytopathogenic microorganisms by production of siderophores, antibiotics and cyanide, (4) solubilization of mineral phosphates and other nutrients. Diazotrophs are believed to affect plant growth promotion both by (a) direct and indirect means, (b) employing all or a combination of some of the above mentioned mechanisms (Dobelaere et al., 2003).

2.2.1. PHYTOHORMONE PRODUCTION BY PGPR

Plant growth promoting effects exerted by some plant-beneficial bacteria are due to the bacterial production of plant hormones, such as, indole-3-acetic acid (IAA), cytokinins and gibberellins (Bloemberg and
Lugtenberg, 2001; Bottini et al., 2004). IAA was detected in 80 per cent of bacteria isolated from the rhizosphere (Loper and Schroth, 1986).

The production of plant growth promoting compounds by *Paenibacillus polymyxa*, similar in activity to indole-3-acetic acid, has been suggested for the stimulation of growth in crested wheatgrass (Holl et al., 1988). Apart from this compounds, the organism also produced iso-pentenyl adenine and one unknown cytokinin like compound during its stationary phase of growth which promotes seed germination, *de novo* bud formation, release of buds from apical dominance, stimulation of leaf expansion, and reproductive development and retardation of senescence (Mok, 1994) in wheat (Lindberg et al., 1985; Lindberg and Granhall, 1986).

Host plants may also be affected by hormones known to be secreted by various microbial species including *Bacillus subtilis* (Priest et al., 1981). Such compounds (i.e. auxins, gibberellins and cytokinins) mediate processes such as plant cell enlargement, division and extension in symbiotic as well as non symbiotic roots.

### 2.2.1.1. Indole acetic acid (IAA) production

*Azospirillum* uses L-tryptophan as precursor for IAA production (Reynders and Vlassak, 1979). Since root exudates contain tryptophan, IAA production by *Azospirillum* will be significant in the rhizosphere regions (Tien et al., 1979). Some microorganisms produce auxins in the presence of
a suitable precursor such as L tryptophan. The tryptophan increases the production of IAA in Bacillus amyloliquefaciens. Tien et al. (1979) showed that Azospirillum is able to produce auxins when exposed to tryptophan. Plants inoculated with the rhizobia together with Ag⁺ ion and L-tryptophan (Trp), give the highest root dry weight, and significantly increase the uptake of N, P and K compared to non-inoculated control plants. A. brasilense produced extremely high amounts of IAA compared to A. lipoferum (Horemans et al., 1986; Mascarina-Esparca et al., 1988; Martin et al., 1989).

Cultures of A. brasilense and A. lipoferum excreted high amounts of IAA in logarithmic growth phase (Zimmer and Bothe, 1988). On the other hand it was contradicted stating that the concentration of IAA was low during logarithmic growth phase and increased rapidly with the beginning of stationary phase (Baca et al., 1994). The mutant with increased phytohormone production significantly affected root morphology. In general, increased plant biomass and N₂-fixation were recorded in strains having increased production of indole compounds (Kundu et al., 1997).

The study by chemical method and HPLC revealed that A. brasilense Sp245 showed a high motive force for tryptophan synthesis from chorismic acid and for IAA synthesis from tryptophan and this makes it unlikely that anthranillic acid and indole act as the precursors to IAA in a tryptophan-independent pathway (Zakharova et al., 1999). Vitamins may also play a role in the regulation of IAA synthesis in A. brasilense. Very low levels of
the B vitamins, especially pyridoxine and nicotinic acid, increased production of IAA in *A. brasilense* (Zakharova *et al.*, 2000).

The cell-free supernatant of *A. brasilense* Cd applied to soybean plants induced the highest number of roots and increased root length (Molla *et al.*, 2001b). A mutant of *A. brasilense* with low production of phytohormones, but with high nitrogenase activity did not enhance root growth over uninoculated controls.

Confirmatory studies on IAA production by several strains of *Azospirillum* showed that production depended on the type of culture media and availability of tryptophan as a precursor. *A. brasilense* Cd produced the highest level of 380 μmol of IAA L⁻¹ among the strains tested (El-Khaswas and Adachi, 1999). The pH has a significant effect on the amount of IAA produced (Ona *et al.*, 2003).

*Bacillus, Pseudomonas, Azotobacter* and *Azospirillum* isolated from the rhizosphere of field grown *Trigonella* were found to produce Indole acetic acid (Tank and Saraf, 2003). Inoculation of *Azospirillum* isolates produced plant growth hormones like IAA and GA₃ in addition to increase NR activity of tea leaf (Baliah *et al.*, 2003). *Azospirillum* and *Pseudomonas* isolates of pearl millet were able to produce IAA under *in vitro* conditions and reduced acetylene to ethylene (Tiwari *et al.*, 2003).
The release of plant growth regulators like polyamines and ethylene by *Azospirillum* was reported by Thuler *et al.* (2003). *Azospirillum* strains produced IAA in the range 16.5 – 38 µg IAA mg⁻¹ protein in culture medium supplemented with tryptophan (Pedraza *et al.*., 2004). The isolates of *Azospirillum*, *Pseudomonas* from different medicinal plants *viz.*, *W. somnifera*, *C. forskholii*, *C. roseus* produced IAA to the tune of (72.6 µg of IAA 25 ml⁻¹ of culture medium) (Gopal, 2004). *P. fluorescens* Pfc-2 isolated from the rhizosphere of soybean produced 3-8 µg and 0.9 µg IAA ml⁻¹ of culture medium in the presence and absence of tryptophan respectively (Sridar, 1996). De Salamone *et al.* (2001) reported that *P. fluorescens* G20 produced significant amount of cytokinins *viz.*, isopentenyl adenosine (IPA), trans–zeatinribose (ZR) and dihydrozeatinribose (DHZR) during stationary phase.

Saikia and Bezabarauah (1995) reported that *Azotobacter* to produce plant growth promoting substances such as phytohormone, IAA and siderophore azotobactin. *A. chroococcum* able to produce IAA and other auxins such as gibberellins and cytokinins (Martinez *et al.* 1993; Neito and Frankenberger, 1989; Verma *et al.*, 2001).

Verma *et al.* (2004) conducted research on the comparative performance of phytohormone producing and non-producing strains of *A. chroococcum* on wheat. The strain which is capable of producing two phytohormones, when applied with a third phytohormone, exogenously had
synergistic effects on plant growth. When all the three phytohormones were supplied exogenously with the non-producer strain, the non-producer strain could not compete with the strain that produced all three phytohormones in augmenting plant growth. This indicates that plant growth promotion is caused by the cumulative effects of more than one factor.

IAA (indole-3-acetic acid) is the member of the group of phytohormones and is generally considered the most important native Auxin (Ashrafuzzaman et al., 2009). It functions as an important signal molecule in the regulation of plant development including organogenesis, tropic responses, cellular responses such as cell expansion, division, and differentiation, and gene regulation (Ryu and Patten, 2008). Diverse bacterial species possess the ability to produce the auxin phytohormone IAA. Different biosynthesis pathways have been identified and redundancy for IAA biosynthesis is widespread among plant-associated bacteria. Interactions between IAA-producing bacteria and plants lead to diverse outcomes on the plant side, varying from pathogenesis to phytostimulation.

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

Ramazan et al. (2007) reported that IAA producing PGPB, such as, Bacillus species OSU-142, P. polymyxa RC05, P. putida RC06, and R. capsulatus RC04 might be favourably affected the plant growth development, and improved nutrition uptake of the plant. They also reported that IAA producing PGPB could change root growth, morphology which eventually leads to it leads to increase in the plant growth, and also influence the density of micro-organisms in rhizosphere of barley.

Khakipour et al. (2008) evaluated the auxin productivity potential in studied Pseudomonas strains through chromatography, using HPLC devise; comparing the methods used and appointing IAA synthesize method by the studied strains in the applied cultivars. In fact, a variety of auxins like indole-3-acetic acid (IAA), indole-3-pyruvic acid, indole-3-butyric acid and indole lactic acid; cytokinins and gibberellins are detected, with auxin production being quantitatively most important. Azospirillum brasilense strain SM has the potential to be a competent rhizospheric bacterium as it triggers the IAA accumulation under nutrient stresses, likely environmental fluctuations and long-term batch cultures and beneficially influences the
growth of sorghum. Further, it also has the ability to promote the growth of a number of other plants like Mung bean, Maize, and Wheat.

A total of eleven selected isolates of *Azotobacter*, fluorescent *Pseudomonas* and *Bacillus* were tested for the quantitative estimation of IAA in the presence of different concentration of tryptophan. The production of IAA was highest in isolates of fluorescent *Pseudomonas*, followed by *Azobacter* and *Bacillus*, respectively (Ahmad et al., 2008).

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

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

Karnwal (2009) obtained thirty fluorescent *Pseudomonas* isolates from different plant rhizospheres and were characterized on the basis of biochemical tests and plant growth-promoting activities. *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* showed the best plant growth-promoting activity. These isolates were tested for their ability to produce indole acetic acid in pure culture in the absence and presence of L-tryptophan at 50, 100, 200 and 500 µg ml⁻¹. For both strains, indole production increased with increases in tryptophan concentration (0.5, 1.2, 4.3 and 9.3 µg ml⁻¹; and 0.2, 0.7, 3.8, and 8.3 µg ml⁻¹, respectively). *Pseudomonas aeruginosa* was less effective in production of indole acetic acid than *Pseudomonas fluorescens*. Inoculation of rice seeds with *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* showed a good level of indole acetic acid compared to uninoculated seeds.

Maleki *et al.* (2010) reported that isolated *Pseudomonas fluorescens* Cv6 strain to produce considerable amount of siderophore and indole-3-acetic acid (IAA). With the addition of tryptophan from 50 to 500 mg/ml the production of IAA was increased upto 15.3 µg ml⁻¹, respectively. Shokri and Emitiazi (2010) reported the maximum production of IAA by *Paenibacillus* under in vitro conditions.
Mandira Kochar et al. (2011) analyzed the biocontrol strain *Pseudomonas fluorescens* for indole-3-acetic acid (IAA) biosynthesis and studied the effect of its consequent manipulation on its plant-growth-promoting (PGP) potential. While the indole pyruvic acid (IPyA) pathway commonly associated with PGPB was lacking, the indole acetamide (IAM) pathway generally observed in phytopathogens was expressed in strain *Pseudomonas fluorescens*. Overexpression of IAM pathway genes *iaaM-iaaH*, from *Pseudomonas syringae* subsp. *savastanoi* drastically increased IAA levels and showed a detrimental effect on sorghum root development.

Acuna et al. (2011) was isolated bacilli strains from rhizosphere of pasture containing graminaceous family plants. The production *in vivo* of IAA by *Paenibacillus* sp. SPT-03 was significantly increased (7-fold) when incubated in tenfold diluted culture medium at low pH (pH<5), phytase of cell-associated proteins and IAA production of *Bacillus* sp. MQH-19 was decreased, where as they were increased in *Paenibacillus* sp. SPT-03. Moreover, these activities in both bacilli strains were significantly inhibited by 30-100% and 44-70% by concentrations of 10 mM and 350 μM Fe³⁺ and Al³⁺, respectively.

Vikram Patil (2011) reported that the six bacterial isolates were isolated from different rhizosphere soils. These isolates were able to produce IAA in the medium with 0, 1, 2, and 5 mg ml⁻¹ of tryptophan. A few amount of IAA production was recorded by *Azotobacter* strain without tryptophan addition. The production of IAA in *Azotobacter* increased with increase in
tryptophan concentration from 1 to 5 mg ml\(^{-1}\). In presence of 5 mg ml\(^{-1}\) of tryptophan, \textit{Azotobacter} produced high levels of IAA. Production of IAA was further confirmed by 3 isolates of \textit{Azotobacter} (Azb 3, Azb 5, Azb 7) and subsequent TLC analysis. \textit{Azotobacter} isolates (Azb 3, Azb 5, Azb 7) showed inhibitory effects on the growth of root elongation of ground nut at all the concentrations of tryptophan compared to control.

A total of 118 isolates of \textit{Bacillus} species were obtained from the rhizosphere of soybean, and examined for plant growth promoting activities. Among them 90 isolates were positively produced phytohormone \textit{viz.}, indole acetic acid (IAA). It was observed that all IAA producing isolates could promote root length, shoot length or number of lateral root of the seedling enhanced significantly increased of soybean under \textit{in vitro} condition (Aris et al., 2011).

Kapoor et al. (2012) reported that the total of thirty \textit{Pseudomonas} strains was isolated from the rhizosphere of apple and pear plants. Among the four strains \textit{viz.}, PN-4-SAN, PN-10-SAN, AN-2-NAG and AN-4-NAG were selected on the basis of their higher auxin production. The maximum production of IAA was observed at 72 h incubation period at pH 7.0 under shaken condition at 28\(^{\circ}\)C. The highest IAA was produced by strain AN-2-NAG (30\(\mu\)g ml\(^{-1}\)) and PN-4-SAN (30\(\mu\)g ml\(^{-1}\)) respectively.

A total eighteen rhizobacteria were isolated from various rhizosphere soils of paddy, tomato, chickpea and carrot in Mandalay region, Myanmar.
Among them, four isolates belonged to *Bacillus* spp. and five strains were recognized as *Serratia* spp. All the eighteen isolates were screened for indole-3-acetic acid (IAA) production and qualitative determination of IAA was done for all strains by UV-Vis spectrophotometer with the 2 days intervals during 10 days incubation. All isolates had different optimum IAA production periods and rhizobacteria strain R1 was the best IAA produces strain with 121.1 ppm. It was observed that *Bacillus* spp. produced IAA ranging from 53.1 ppm to 71.1 ppm optimally and *Serratia* spp. were regarded as poor producers (Lwin *et al*., 2012).

Lenin and Jayanthi (2012) reported that the PGPR strains *viz.*, *Azospirillum lipoferum, Azotobacter chroococcum, Pseudomonas fluorescens* and *Bacillus megaterium* were isolated from rhizosphere region of *Catharanthus roseus* in 20 different locations of Cuddalore districts. The twenty isolates were found to be producing IAA and GA3. *Azospirillum lipoferum* CRAS-2 strain (74.2 μg 25 ml⁻¹ of broth 7.10 μg 25 ml⁻¹ of broth) produce maximum amount in nitrogen free malate broth.

Sakthivel and Karthikeyan (2012) reported that the PGPR isolates *viz.*, *Azospirillum, Azotobacter, Bacillus* and *Pseudomonas* were isolated from the rhizosphere soils of *Coleus forskohlii*. The isolates *Pseudomonas fluorescens* (SPf-1) produced the highest amount of IAA (7.60 μg ml⁻¹) followed by *Azospirillum lipoferum* (SAzs-1) produced IAA of 6.80 μg ml⁻¹, and followed by *Azotobacter* and *Bacillus* isolates.
2.2.1.2. Gibberellins production

A beneficial effect of *Azospirillum* sp. on plants has been suggested to be partially caused by the production of gibberellins. Application of gibberellins had effects similar to *Azospirillum* inoculation in increasing root hair density (Bashan *et al.*, 2004). When *A. lipoferum* USA5b, a gibberellin-producing strain, was cultured in the presence of glucosyl ester or glucoside of gibberellin A$_{20}$, both conjugates were hydrolyzed. These *in vitro* results supported the hypothesis that growth promotion in plants induced by *Azospirillum* inoculation resulted from a combination of both gibberellin production and gibberellin-glucoside/glucosyl ester utilization by the bacterium (Piccoli *et al.*, 1997).

The effect of water potential or O$_2$ concentration on the growth and gibberellin A$_3$ production in *A. lipoferum* showed that gibberellin A$_3$ produced by it was reduced considerably at high water potentials or low O$_2$ concentrations (Piccoli *et al.*, 1999). The involvement of gibberellin A$_3$ produced by *Azospirillum* sp. in promoting maize growth was reported (Lucangeli and Bottini, 1997). *A. brasilense* Cd and *A. lipoferum* USA 5b promoted elongation of root sheaths in GA-deficient dwarf rice mutants, (Cassan *et al.*, 2001).

Cassana *et al.* (2009) tested the *Azospirillum brasilense* strain Az39 and *Bradyrhizobium japonicum* strain E109 were previously shown to produce indole-3-acetic acid, gibberellic acid and zeatin for early growth promotion in inoculated corn and soybean seedlings.
Ansary et al. (2012) reported that *Pseudomonas fluorescens* also produced gibberelaline content of water deficit levels of maize plant. The water deficit treatments the gibberellins production *viz.* T 45 % has produced 14.6 % and 27.5 % more gibberellins than T 60 % and T 75 %, respectively. Among the three water deficit, highest gibberellins content was observed in T 45 %.

Lenin and Jayanthi (2012) reported that the PGPR strains *viz.*, *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Pseudomonas fluorescens* and *Bacillus megaterium* were isolated from rhizosphere region of *Catharanthus roseus* in twenty different locations of Cuddalore districts. The twenty isolates were screened for gibberelic acid (*GA₃*) production. The CRAS-2 strain *Azospirillum* produced the gibberelic acid (7.10 μg 25 ml⁻¹ of broth) followed by other isolates.

### 2.2.2. Phosphate solubilization

Phosphorus (P) is a major essential macronutrient for biological growth and development. P in soil is immobilized or becomes less soluble either by absorption, chemical precipitation, or both. A survey of Indian soils revealed that 98 per cent of them needed phosphorus fertilization either in the form of chemical or biological fertilizer. Under such conditions, microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants.
Phosphate solubilizing microorganisms (PSM) include largely bacteria and fungi, which can grow in media containing tricalcium, iron and aluminium phosphate, hydroxyapatite, bonemeal, rock phosphate and similar insoluble phosphate compounds as the sole phosphate source. Such microbes not only assimilate P but a large portion of soluble phosphate is released in quantities in excess of their own requirement.

The most efficient PSM belong to genera *Bacillus* and *Pseudomonas* among bacteria and *Aspergillus* and *Penicillium* among fungi. Pikovskaya (1948) made a pioneer attempt in isolating “phosphorite” an organism capable of actively solubilizing tricalcium phosphate (TCP) and coined the name “Bacterium P”. Sperber (1957) and Katznelson and Bose (1959) designed enrichment technique and special media for the isolation of phosphate dissolving microorganisms from soils and from rhizosphere. Solubilization of insoluble phosphate by gram positive and gram-negative rods and cocci and several fungi including yeast was reported (Ahmad and Jha, 1968).

Anjani *et al.* (1996) isolated plant growth promoting fluorescent pseudomonad which had the characteristics of phosphate solubilization and siderophore production.

*Azotobacter* like other soil microorganisms plays a significant role in solubilization of P from the native soil P pool, as well as from added insoluble phosphates such as rock phosphates, for plants to use. The rates of solubilization vary with the inorganic P source and the *Azotobacter* strains
involved. The phosphate solubilizing *Azotobacter* strain may produce effective solubilizing agents, such as organic acids (or) chelating substances in micro environments in the vicinity of rock phosphate or in the rhizosphere (Behl *et al.*, 2006).

Narula *et al.* (1995) isolated six phosphate solubilizing isolates of *A. chroococcum*, from the rhizosphere soils of oil seed crops, which solubilized tricalcium phosphate (TCP) and rock phosphate (RP) at 37°C and 42°C respectively by “rock phosphate enrichment technique”. Twenty isolates of *Azospirillum* showing phosphorus solubilizing property were obtained by Tamilvendan (1995). However, these isolates were found inferior to *B. megaterium* var. *phosphaticum* in phosphorus solubilization.

The enzyme phosphatase found involved in the hydrolysis of organic phosphorus to inorganic form originated from varied sources in soil such as plant roots mycorrhizal fungi and rhizosphere soil (Taraftdar and Claassen, 1988).

Goldstein and Rogers (1999) demonstrated that an efficient mineral phosphate solubilizing phenotype in Gram-negative bacteria resulted from extracellular oxidation of glucose to gluconic acid via the quinoprotein glucose dehydrogenase. Kumar and Narula (1999) evaluated phosphate solubilizing strains of *A. chroococcum* isolated from the wheat rhizosphere for their ability to produce solubilize tricalcium phosphate (TCP) and Mussoorie rock phosphate (MRP).
Orthophosphate enrichment at 5 mg l\(^{-1}\) for *Bacillus* and *Staphylococcus* cultures isolated from fresh water pond system showed phosphatase (Sudha and Purushothaman, 2000).

Strain NBR12601 (Nautiyal *et al.*, 2001) isolated from the rhizosphere of chickpea and alkaline soils could solubilize phosphorus in the presence of 10% salt, pH 12 at 45°C suggesting that extensive diversity searches in appropriate habitats may lead to recovery of effective bacteria.

A phosphate solubilizing bacterium, *Enterobacter intermedicum* isolated from rhizosphere, exhibited a strong ability to solubilize insoluble phosphate which mediated by the production of organic acids especially 2-ketogluconic acid (2-KGA) in culture media (Kim *et al.*, 2002). The enzymes responsible for the formation of 2-KGA has also been produced by certain species of *Pseudomonas*, *Klebsiella*, *Serratia* and acetic acid bacteria.

In a screening of 4800 bacterial isolates from the root-free soil, rhizosphere and rhizoplane of *Prosophis juliflora* growing in alkaline soils, 857 morphotypes solubilized phosphate in agar. The incidence of PSB was highest in the rhizoplane, followed by rhizosphere and root-free soil. Phosphate solubilizing ability of strain NBR14 was higher than control in the presence of salts (NaCl, CaCl\(_2\) and KCl) at 30°C and it further increased at 37°C (Gaur *et al.*, 2004).
Five mutants solubilized P in the range of 1.5 – 1.7 μg ml⁻¹ of TCP and 0.19-0.22 μg ml⁻¹ of MRP than the wild strains. Proton release is correlated as one of the main mechanisms that increase phosphate availability (Illmer and Schinner, 1995; Villegas and Fortin, 2002). Other phosphate solubilizing mechanisms are to be explored (Bajpai and Sundara Rao, 1971; Banic and Dey, 1981; Staunton and Leprince, 1996; Whitelaw, 2000).

Ratti et al. (2001) reported that inoculation of Bacillus polymyxa and A. brasilense increased the uptake of tricalcium phosphate which is otherwise not used by the plants and their addition at 200 mg kg⁻¹ of soil gave higher productivity to palmarosa plants.

Dave and Patel (2003) tested various compounds of carbon and nitrogen for their effect on solubilization of tricalcium phosphate (TCP) and rock phosphatase (RP) by P. fluorescens. Glucose and galactose were found to be the best carbon sources while ammonium sulphate was the best nitrogen source. Optimum C:N ratio for the solubilization was found to be 40 and the pH drift was always towards acidic soils.

Tank and Saraf (2003) examined the phosphate solubilization by different bacterial cultures viz, Bacillus, Pseudomonas, Azotobacter, Rhizobium and Azospirillum isolated from the rhizosphere of field grown Trigonella. Pseudomonas isolate TP₂ and Rhizobium showed high tricalcium phosphate solubilizing ability in both solid and liquid medium.
Gopal (2004) examined the phosphate solubilization by different rhizobacterial isolates viz., *Bacillus*, *Azospirillum* and *Pseudomonas* from the rhizosphere of different medicinal plants. The strain APb-1 recorded the maximum solubilization of 18.11 mg of P from 100 mg of TCP on 15\textsuperscript{th} DAI and 29.10 mg of P from 100 mg TCP on 30\textsuperscript{th} DAI.

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

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

The use of phosphate solubilising bacteria as inoculants increases the P uptake by plants (Chen et al., 2006). Among the heterogeneous and naturally abundant microbes inhabiting the rhizosphere, the Phosphate Solubilising Microorganisms (PSM) including bacteria have provided an alternative biotechnological solution in sustainable agriculture to meet the P demands of plants. These organisms in addition to providing P to plants also facilitate plant growth by other mechanisms. Current developments in our understanding of the functional diversity, rhizosphere colonizing ability, mode of actions and judicious application are likely to facilitate their use as reliable components in the management of sustainable agricultural systems (Zaidi et al., 2009). PSM include largely bacteria and fungi. The most efficient PSM belong to genera Bacillus, Rhizobium and Pseudomonas among bacteria, and Aspergillus and Penicillium among fungi. Within rhizobia, two species nodulating chickpea, *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum*, are known as good phosphate solubilizers (Rivas et al., 2006). However, it is known that every aspect of the process of nodule formation is limited by the availability of Phosphorous.
The PSB strains exhibit inorganic P-solubilizing abilities ranging between 25-42 μg P ml⁻¹ and organic P mineralizing abilities between 8- 18 μg P ml⁻¹, respectively (Tao et al., 2008). *Pseudomonas putida, P. fluorescens* Chao and *P. fluorescens* Tabriz released 51, 29 and 62% P, respectively; with highest value of 0.74 mg P/ 50 mL from Fe₂O₃ (Ghaderi et al., 2008). *Pseudomonas striata* and *Bacillus polymxa* solubilized 156 and 116 mg l⁻¹, respectively (Rodriguez and Fraga, 1999). *Pseudomonas fluorescens* solubilized 100 mg P l⁻¹ containing Ca₃(PO₄)₂ or 92 and 51 mg P l⁻¹ containing AlPO₄ and FeSO₄, respectively (Henri et al., 2008).

Noori and Saud (2012) reported that the twenty *Pseudomonas* strains isolated from the rhizosphere soils of paddy areas in Malaysia. Among the 20 isolates, 18 isolate (90 %) phosphate solubilization on NBRIP medium. All the strains are identified as potential phosphate solubilizers based on their capacity to solubilize tricalcium phosphate [Ca₃(PO₄)₂] by the formation of clear halozone on NBRIP medium. According to the PSB index for each isolates, the maximum amount of soluble phosphates was released by TS3C8 (341) and the least by DL26 (129). A significant difference (P< 0.05) was observed between all the isolates.

**2.2.3. NITROGEN FIXATION**

N₂ fixation was the first mechanism suggested to promote the growth of plants by *Azospirillum*. The majority of evidence collected during the last 3 decades concerning this mechanism has generated controversy. Some
greenhouse and field experiments have shown repeatedly that the transfer of nitrogen, measured as transfer of $^{15}$N$_2$ fixed by *Azospirillum* sp. to the plant is minimal (Kennedy *et al.*, 1997; Kennedy and Chellapillai, 1998; Bashan and Holguin, 1997a and 1997b). Yet others added that bacteria cannot fulfill all of the nitrogen requirements of plants, but contribute significant amounts of nitrogen. Furthermore, inoculation commonly and significantly reduced the required doses of nitrogen fertilization in numerous greenhouse and field experiments of many plant species (Bashan and Levanony, 1990, Bashan and Holguin, 1997a).

Biological nitrogen fixation is a biochemical process in which atmospheric nitrogen is converted into substrates of nitrogen that plant can use. Worldwide 140-170x10$^6$ tonnes of nitrogen/year, valued at approximately $90$ billion, is annually added to the biosphere by nitrogen fixing microorganisms in both agricultural and natural ecosystems (Bezdicek and Kennedy, 1998).

Nitrogen fixation by *Azospirillum* has been confirmed beyond doubt by several workers, using not only conventional Microkjeldhal assay, but also the more definite method of isotopic enrichment involving $^{15}$N (Laxmikumari *et al.*, 1976; Okon *et al.*, 1976; Barber *et al.*, 1978 and Scott and Scott, 1978). Lima *et al.* (1987) demonstrated that up to 50 per cent of the nitrogen requirement of crops such as sugarcane, *Panicum maximum* and
*Paspalum notatum* could be supplied by associative nitrogen fixers mainly *Azospirillum*.

Mallik *et al.* (1987) showed using *A. brasilense* DSM 1891 and $^{15}$N technique that the extent of nitrogen in kollar grass derived from atmospheric nitrogen varied between 30 and 60 per cent. *A. lipoferum* and *A. brasilense* showed nitrogen fixation in the range of 7.54 to 24.53 mg of nitrogen g$^{-1}$ malic acid after seven days at 28°C under static conditions (Tamilvendan and Purushothaman, 1996). *A. lipoferum* contributed about 66 per cent of the total nitrogen in rice plants by Mallik *et al.* (1997). *Azospirillum* nitrogen fixation to the tune of 4.0 g N ha$^{-1}$ day$^{-1}$ in sorghum and 15 to 25 g ha$^{-1}$ day$^{-1}$ in corn was reported by Bashan and Dubrovsky (1996). Biological nitrogen fixation potential by diazotrophic bacteria and their effect on growth of many cereals and grasses were reviewed by Dobbelare *et al.* (2003).

*Azotobacter* is capable of converting nitrogen to ammonia (Newton *et al.*, 1953; Bishop *et al.*, 1982), which in turn is taken up by the plant. Mutant strains that have nitrogenase synthesis and are blocked in their ability to utilize ammonia, excrete ammonia ions into the medium. Nitrogen fixation activity (nitrogenase) is repressed by ammonium in *Azotobacter* spp. Therefore, the presence of high amounts of nitrogenous fertilizers in the field reduces their effectiveness as biofertilizers (Merrick, 1992).
It is important to isolate depressed mutants for agronomic use and develop them in order to offer a new approach in the field of biofertilizers. There are reports of isolated mutants that can fix nitrogen in the presence of ammonium and excrete ammonia (Gordon and Brill, 1972; Gordon and Jackson, 1983; Bela et al., 1986). Constitutive mutants that overproduce nitrogenase and excrete ammonia in nitrogen free un-supplement medium, suggest that they could be better bio-fertilizers. Bela et al. (1986) isolated mutants resistant to analogues of ammonia viz., methyl alanine (mal), methionine sulfoximine (MSX) and methyl ammonium chloride (Mac). Derepressed nitrogenase activity and ammonia excretion were studied in all the mutants however Mac mutants showed higher nitrogenase. Lakshminaryana et al. (2000) isolated and tested mutants of *Azotobacter* strain A 103, resistant to the three metabolic analogues (Msx, Mal and Mac), for ammonium.

Several bacteria help to derive maximum benefit from root exudates by their ability to attach to the root surfaces. Since associative interactions of plants and microorganisms must have come into existence as a result of co-evolution, the use of latter group as bio inoculants must be pre-adapted, so that it fits into a long term sustainable agricultural system. PGPR are commonly used as inoculants for improving the growth and yield of agricultural crops and offers an attractive way to replace chemical fertilizers, pesticides, and supplements (Ashrafuzzaman et al., 2009). The use of
biofertilizer and bioenhancer such as N₂ fixing bacteria and beneficial microorganism can reduce chemical fertilizer applications and consequently lower production cost. Utilization of PGPR in order to increase the productivity may be a viable alternative to organic fertilizers which also helps in reducing the pollution and preserving the environment in the spirit of an ecological agriculture (Stefan et al., 2008). Thus, rhizospheric bacteria can be a promising source for plant growth promoting agent in agriculture (Chaiharn et al., 2005) and are commonly used as inoculants for improving the growth and yield of agricultural crops.

The use of PGPR isolates as inoculants biofertilizers is beneficial for rice cultivation as they enhance growth of rice and by inducing other plant growth promoting traits. Applying the combined inoculation of PGPR as biofertilizer affects beneficially the yield and growth of chickpea in field conditions (Rokhzadi et al., 2008). Biological nitrogen fixation contributes 180 × 10⁶ metric tons year⁻¹ globally, out of which symbiotic associations' produces 80% and the rest comes from free-living or associative systems. The ability to reduce and derive such appreciable amounts of nitrogen from the atmospheric reservoir and enrich the soil is confined to bacteria and Archaea. These include symbiotic nitrogen fixing forms, viz., Rhizobium, the obligate symbionts in leguminous plants and Frankia in non-leguminous trees, and non-symbiotic (free-living, associative or endophytic) N₂-fixing forms such as cyanobacteria, Azospirillum, Azotobacter, Acetobacter diazotrophicus, Azoarcus etc.
Non-symbiotic nitrogen fixation has a great agronomic significance. One main limitation that it faces is the availability of carbon and energy source for the energy intensive nitrogen fixation process. However, this limitation can be compensated by moving closer to or inside the plants, *viz.*, in diazotrophs present in rhizosphere, rhizoplane or those growing endophytically. Some important non-symbiotic nitrogen-fixing bacteria include *Azoarcus* sp., *Gluconacetobacter diazotrophicus*, *Herbaspirillum* sp., *Azotobacter* sp. (Vessey, 2003; Barriuso et al., 2008), *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enerobacter*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum*, *Rhodopseudomonas* and *Xanthobacter* (Saxena and Tilak, 1998).

Mirzakhani *et al.* (2009) evaluate the effect of *A. chroococcum* inoculation on seed yield and yield components of safflower, which significantly affected, because the biofertilizers can fix atmospheric nitrogen and increase phosphorus availability in soil and enhanced elements absorbance by safflower.

The effect of PGPR inoculation on growth and N\textsubscript{2}-fixation of tissue cultured banana evaluated by Baset *et al.* (2010) who recorded that due to the PGPR inoculation, beside higher shoot growth and N yield, the inoculated banana plants showed higher growth attributes, leaf area, chlorophyll content and the total biomass was higher in compare to uninoculated control.
Rajeawari (2011) revealed that *Azotobacter*, even at low densities, can activity fix nitrogen, the pot experiment of his study showed a significant increase in shoot, root length of the wheat plants, he recommend that *Azotobacter* can survive in soil and fix atmospheric nitrogen, and can be used as a suitable biofertilizer in order to reduce the usage of chemical fertilizer which is potent harmful substances mainly protochemicals.

2.2.4. SIDEROPHORE PRODUCTION

Siderophores are iron chelating compounds secreted by PGPR, which sequester iron in the root zone making it unavailable to certain rhizosphere microorganism. They act differently depending on the nature of organism.

Siderophores are low molecular weight compounds that are produced under iron limiting conditions, found to chelate the ferric iron (Fe) with a high specific activity and serve as a vehicle for the transport of Fe (III) into microbial cells.

Siderophores are also known to bind other metals, such as Mn, Cr, Ga and Pb (Birch and Bachofen, 1990). Siderophores are also known to act as growth factors and as phytopathogenic suppressive agents (Calvente et al., 2001).

Iron salts have been reported to be essential for nitrogen fixation (Camahan and Castle, 1958). Several *Azospirilla* are endowed with a very efficient Fe-acquisition system. Saxena et al. (1986) reported that under iron limiting conditions, *A. lipoferum* D₂ excreted the phenolate siderophores 2, 3
and 2, 5 dihydroxy benzoic acid (DHBA). They conjugated with lysine and leucine as well as salicylic acid. *Azospirillum* sp produced salicylate type of siderophores which ranged from 1.28 to 3.20 mg\(^{-1}\) and *A. lipoferum* produced higher quantity of siderophores than other species (Sridar and Balasubramanian, 1996).

Soil pseudomonads generally produce fluorescent, yellow green, water soluble siderophores with hydroxamate and phenolate group. Siderophores synthesized by fluorescent *Pseudomonas* is called pyoverdine (Meyer and Abdullah, 1978). Kloeper et al. (1980) reported that PGPR produced extra cellular siderophores, Pseudobactin which efficiently complexes environmental iron, making it less available to certain native microflora.

The fluorescent *Pseudomonas* isolated from tomato rhizoplane, produced siderophore in succinate medium deficient in iron (Kumar and Dube, 1993). The presence of hydroxamate siderophores in the crude extract of *Pseudomonas* strain was isolated from the rhizosphere of tea (Kumar and Bezbaruah, 1996), and hydroxamate siderophores in the rice soil *Pseudomonas* strain (Karthikeyan, 1999) was reported.

Plant assay technique showed that *Pseudomonas aeruginosa* Sc1 was more effective in suppressing the growth of plant pathogens *viz.*, *Fusarium oxysporum* and *Aspergillus flavus*. The amount of siderophores produced by Sc1 and Sc4 was studied on different media and per cent siderophore units were calculated to be 57 and 50, respectively (Manwar et al., 2000). The
crown gall biocontrol agent *Agrobacterium rhizogenes* strain K-84 produced a hydroxamate iron chelator in large amounts (Penyalver *et al.*, 2001).

Sharma and Johri (2003) compared siderophore production in fluorescent *Pseudomonas* sp. GRP3A, PRS9 and *Pseudomonas chlororaphis* ATCC 9446 in standard succinate medium for siderophore production, and found that iron limitation increased siderophore production in all the strains. Maximum siderophore level of 216.23 µg ml\(^{-1}\) was produced by strain PRS9.

Temirov *et al.* (2003) studied the siderophore of thermophilic and thermoresistant strains of *Bacillus* on a medium containing Chrome Azurol Sulphonate. It was found that the *Bacillus licheniformis* VK21 strain dramatically increased the secretion of siderophore in response to addition of manganese (II) salts.

The production of siderophore pyochelin by *Pseudomonas aeruginosa* in the culture medium was reported by Schlegel *et al.* (2004). Bano and Musarrat (2004) isolated *Pseudomonas* sp NJ-101 from agricultural soil and found its ability to produce hydrogen cyanide and siderophores.

Siderophore production in the rhizosphere of different medicinal plants such as *W. somnifera, C. forskohlii* and *C. roseus* were reported by Gopal (2004). Among three groups of rhizobacterial isolates, *P. fluorescens* APs-1 produced the maximum amount of 7.31 and 8.92 µg ml\(^{-1}\) of catechol and salicylate types respectively. *Bacillus-APb*-1 produced 5.65 and 7.65 µg ml\(^{-1}\)
catechol and salicylate of respectively type. *Azospirillum* isolates AAs-11 produced 3.23 and 5.10 μg ml⁻¹ of catechol and salicylate types siderophore.

*A. chroococcum* is known to produce siderophores (Knosp *et al.*, 1984; Suneja and Lakshminarayana 1996). Antagonistic action of *A. chroococcum* on phytopathogens has been studied by various researchers (Schroth and Hancock, 1982; Meshram and Jager, 1983; Weller, 1988; Verma *et al.*, 2001), but whether this inhibition is due to siderophores or antifungal properties was not clear.

Beniwal *et al.* (1996) conducted extensive field experiments to evaluate the effect of *A. chroococcum* strain / mutants on the incidence of flag smut. Flag smut incidence in wheat was significant by less under bioinoculation compared to the control. The maximum disease reduction of 37.96 per cent was observed with the strain Mac 21. An increased incidence of flag smut in some strains namely, Mac 54, Mac 68 and in parent isolate 103, was also observed.

Out of four rhizospheric diazotrophs tested, *A. chroococcum* (HT 54) showed maximum reduction in nematode infection (48%) followed by *Pseudomonas* (11%) and *Azospirillum* (4%) in wheat (Bansal *et al.*, 1999).

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

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

Although various bacterial siderophores differ in their abilities to sequester iron, in general, they deprive pathogenic fungi of this essential
element since the fungal siderophores have lower affinity. Some PGPR strains go one-step further and draw iron from heterologous siderophores produced by cohabiting microorganisms. *Pseudomonas* sp. have the capacity to utilize siderophores produced by diverse species of bacteria and fungi, and *Pseudomonas putida* can utilize the heterologous siderophores produced by rhizosphere microorganisms to enhance the level of iron available to it in the natural habitat (Loper and Henkels, 1999). The two strains of *Pseudomonas fluorescens* along with *Pseudomonas putida* produce maximum yield of hydroxamate type of siderophore in the modified succinic acid medium (SM).

Soil bacteria isolates including *Azotobacter vinelandii* and *Bacillus cereus* produces siderophores and they can be used as efficient PGPR to increase the yield of the crop (Husen, 2003). *Bacillus megaterium* from rhizosphere is able produce siderophore and thus it helps in the plant growth promotion and reduction of disease intensity. Specific strains of the *Pseudomonas fluorescens* group have recently been used as seed inoculants on crop plants to promote growth and increase yields of various crops. These results prompted Kloeper et al. (1980) to investigate the mechanism by which plant growth was enhanced. A previous study indicated that PGPR increase plant growth by antagonism to potentially deleterious rhizoplane fungi and bacteria, but the nature of this antagonism was not determined. They presented evidence that PGPR exert their plant growth-promoting activity by depriving native microflora of iron. PGPR produces extracellular
siderophores which efficiently complex environmental iron, making it less available to certain native microflora. The siderophores production by *Bacillus* and *Pseudomonas* when assessed both in the presence and in absence of technical grade of herbicides show that the metabolic activities of plant growth promoting rhizobacteria decline following herbicides application (Munees and Mohammad, 2009).

Waseem and Qirong (2010) reported the ability of *Paenibacillus polymyxa* SQR-21 to produce siderophore under differential iron availability conditions such as 0, 2, 20 µM. They suggested that *Paenibacillus polymyxa* to have more than one type of iron acquisition mechanism including gradual release of organic acids, cell surface ferric reductases, extracellular reductants, and secretion of low molecular weight hydroxamates chelators.

Yu *et al.* (2011) reported the ability of *Bacillus subtilis* CAS15, obtained from rhizosphere of pepper, were positive for the siderophore production on Chrome Azurol Sulphonate (CAS) agar plate assay, which mediated the biocontrol activity and induced the systemic resistance against *Fusarium* wilt in pepper.

Lenin and Jayanthi (2012) reported that the *Pseudomonas fluorescens* CRPS-2 secreted highest amount of both catechol and salicylate type (9.42 µg ml⁻¹ and 9.84 µg ml⁻¹) of siderophores followed by *B. megaterium* CRBA-4 secreted the catechol and salicylate type (7.12 µg ml⁻¹ and 8.96 µg ml⁻¹) of siderophores respectively.
Sakthivel and Karthikeyan (2012) reported the *Pseudomonas* spp., was produced highest amount of siderophore (18.22 %) followed by *Azospirillum* (16.22 %), *Bacillus* spp., (10.00 %) respectively.

2.3. PRODUCTION OF MICROBIAL INOCULANT AND FORMULATION

Efficient strains of nitrogen fixing and phosphate solubilizing microorganisms are mass multiplied under laboratory condition and mixed with a carrier. These carrier based inoculants are supplied to farmers for crop inoculation. Carrier is a medium or matrix on which inoculant microorganisms grow to a reasonably higher population for an initial period and thereafter decline. The nature of the carrier often determines the subsequent performance of the inoculant. The criteria for a good carrier material are no toxicity to the introduced microorganisms, good absorption capacity, suitable pH, and fine particle size for better adherence to seed, good water holding capacity and availability of materials at cheaper cost. Different carriers have been tested and used for inoculation throughout the world. Some of the carriers used by different manufactures in the country and abroad are peat, lignite, vermiculite, charcoal, pressmud, coal, polyacrylamide and alginates. The carrier based inoculant improves their shelf life and efficiency of biofertilizers (Palaniappan and Arangarasan, 1996).
2.3.1. Carrier based inoculant

Jauhri et al. (1979) reported higher survival of *Rhizobium* and *Azotobacter* in modified charcoal carrier. Jauhri and Philip (1982) reported that pressmud with charcoal in a proportion of 13:1 was a superior inoculant base. Geels and Schippers (1983) formulated the suspension of *Pseudomonas* cells by mixing cell suspension with 0.2 per cent protease peptone and 2 per cent carboxyl methyl cellulose in distilled water for potato tuber treatment.

Lignite based inoculants are widely accepted and used for seed treatment of various crops (Rasal et al., 1994). Thangaraju (1996) recommended the use of decomposed coirpith with lignite or peat (1:1) for better survival of *Rhizobium*. Govindarajan (1996) studied the growth and survival of *A. lipoferum* in peat, coirpith and mixture of peat and coir pith. The peat supported higher proliferation of the inoculated organisms than other carriers.

Lignite is the preferred and widely used carrier in most of the biofertilizer manufacturing plants all over India (Khungar, 1998). Among the four different bioinoculant carriers (Paddy husk, groundnut shell, Lignite and sawdust). The population was maximum in lignite at all temperatures studied (Saha et al., 2001). Addition of various polymers, amendments and chemicals in both sterile and unsterile carriers resulted in increased shelf life of *A. lipoferum* (Sureshbabu et al., 2002).
Gopal (2004) showed the better shelf life and effectiveness of lignite based rhizobacterial inoculant than other carrier based inoculants on Ashwagandha a commercially grown medicinal plant. Narendranath et al. (1996) reported that higher survival of groundnut Rhizobium in peat followed by pressmud and lignite and suggested pressmud amended with soymeal as an alternative carrier to peat in inoculant preparation. Tilak and Subba Rao (1978) found that soil + FYM in 1:1 proportion had higher Azospirillum count followed by soil + FYM + Vermiculite in 5:3:2 proportion.

Vermicompost as an alternative carrier to lignite was suggested in the inoculant preparation of phosphobacteria, Azospirillum and Acetobacter diazotrophicus (Muthukumarasamy et al., 1996).

Pressmud supported higher population of phosphobacteria, Rhizobium, Azospirillum and Azotobacter than peat, lignite and composted coirpith (Rajannan et al., 1996). Lignite supported higher survival of P. fluorescens and Bradyrhizobium japonicum than peat and suggested it may be used as an alternative to peat (Kalaivani, 1998).

Gaind and Gaur (2004) studied the usage of fly ash carrier material and found to support the maximum viability of A. chroococcum, A. brasilense and Bacillus circulans. While Pseudomonas striata proliferated well in soil: flyash of 1:1 combination.
The addition of polymers and amendments improved the survival of Azospirillum. The hydrophilic polymers, Jalsakthi and terra cotton both at 2% and 4% levels and amendments, polyvinyl pyrrolidone (PVP) and skim milk at 1% and 2% levels were found suitable for retaining moisture and favouring the survival of Azospirillum cells in both sterilized and un-sterilized carrier based inoculants (Santhanakrishnan and Thangaraju, 2002).

Sarma et al. (2009) studied the vermiculite was used to develop inorganic carrier-based formulations of fluorescent pseudomonad strains R62 and R81. The effect of bio-inoculation of fluorescent pseudomonad strains R62 and R81 on growth responses of Vigna-mungo under field condition was enumerated. The combined bio-inoculation of these two organisms in a formulation increased the pods yield by 300 % in comparison to the control crop, these was also significantly increment in the other plant growth responses such as dry root weight, dry shoot weight, shoot length and number of branches per plant.

Sobhan Aridakani et al. (2010) studied the formulation included a talc powder and bentonite-based powder as inorganic carriers for peat and rice bran as organic carriers for increasing stability in interaction between associated Pseudomonas fluorescens in different treatments for significantly promoting seedling height, root length, seedling dry weight and root dry weight of cotton seedlings.
Crop response to *Azotobacter chroococcum* strain SDSA-I 12/2 with vermicompost as inoculants carrier was more pronounced and formed to be higher as compared to charcoal, lignite and cured compost as carriers. Among the carrier materials vermicompost was most effective in improving the growth, yield parameters of rice, plant height, ‘N’ uptake, dry matter production, grain yield, protein and oil content were improved by 48 %, 45 %, 44 %, 43 %, 33 % and 33 % respectively over control of summer (ahu) rice cv. IR-36 (Deb Roy, 2010).

### 2.3.2. Beaded inoculant

Polymers were demonstrated as potential bacterial carrier (Jung *et al.*, 1982) offering substantial practical advantages over peat (Amiet Charpentier *et al.*, 1998; Amiet charpentier, 1999). These formulations encapsulate the living cells, protect the microorganism against many environmental stresses and release them to the soil gradually when soil microorganisms degrade the polymers. They can be stored dry at ambient temperatures for prolonged periods, offer consistent batch quality and a better-defined environment for the bacteria. Beaded inoculant can be amended with nutrient to improve the short term survival of the bacteria as well as with associative PGPB to improve their efficiency (Bashan, 1986; Bashan, 1998).

Alginate is the most common polymer material for the encapsulation of microorganisms for various industrial microbiological purposes (Chen
and Huang, 1988; Fenice et al., 2000). Several alginate based preparations were evaluated for agricultural purpose including the encapsulation of biocontrol agents against soil-borne pathogens (De Lucca et al., 1990; Fravel et al., 1985; Lewis and Papavizas, 1985; Russo et al., 1996) and phosphate-solubilizing bacteria (Vassilev et al., 1997). This technology was also employed to encapsulate the PGPB like A. brasilense and P. fluorescens (Bashan, 1986) that were successfully used to inoculate wheat plants under field condition (Bashan et al., 1987). Encapsulated genetically engineered P. fluorescens released later into soil micro ecosystem showed significantly increased survival rates over non-encapsulated cell after three months (Elass et al., 1992).

Pankaj Trivedi et al. (2005) evaluated for the growth promotion and rhizosphere colonization of five different formulation using two plant growth promoting rhizobacteria (PGPR) viz., Bacillus subtilis and Pseudomonas corrugata. The best results were obtained in alginate-based formulation.

Ricardo Yabur et al. (2007) reported that Alginate extracted from the macro algae Sargassum sinicola was used as the raw material for co-immobilization of the micro algae Choleralla sorokiniana and growth promoting bacterium Azospirillum brasilense for waste water treatment and as an inoculant carrier of A. brasilense for plant growth promotion.

Prabakaran and Hoti (2008) developed the immobilization technique using Sodium alginate as the matrix to preserve the Bacillus thuringiensis
var. *israelensis* isolate for long time storage which enhanced the stability of both spores and toxin against several physico-chemical conditions and conferred reduced chance of contamination.

Minaxi and Jyoti Saxena (2011) introduced bioinoculants in soil by encapsulating the cells in biodegradable gel matrices which gradually released the microorganisms and also helped to increase the survival rate by protecting them against environment stress.

### 2.3.2a. Factors influencing the shelf life of microbial inoculants

The quality assured biofertilizer product ensures presence of viable prescribed microbial load between date of manufacture and expiry so to affect its biological activity. Hence it is desirable to use appropriate technology for longer shelf life during manufacturing processes. The following various factors such as moisture content, temperature of incubation, Aeration, carrier sterility, packaging materials determines the shelf life of biofertilizers.

#### 2.3.2.1. Temperature of incubation

Roughley (1968) studied the effect of storage temperature on the growth and survival of inoculant in sterilized and unsterilized carrier to purity the culture and the loss moisture during storage.

The moisture content of culture in cotton wool stoppered bottles wrapped in cellophane may be kept at a constant 50 % by storage at 2°C, but
storage at such temperature immediately after inoculant restricts initial multiplication and maximum numbers are not reached until 26 weeks. This period can be reduced to two weeks if cultures are incubated for one week at 26°C prior to storage at 4°C (Van Scherven, 1958).

Saha et al. (2001) studied the survival of *Rhizobium* in the paddy husk as carrier at different temperature using green fluorescent protein marker and reported that survival rate of *Rhizobium* transformant is less at higher temperature above 28°C.

Sangeetha and Stella (2012) reported the survival of PGPB *viz.*, *Azospirillum lipoferum* VAZS-18, *Azotobacter chroococcum* VAZB-6, *Bacillus megaterium* VBA-2 and *Pseudomonas fluorescens* VPS-19 inoculants on different carrier materials *viz.*, lignite, vermiculite, pressmud and alginate bead based on the consortium treatments to survival of population at 25°C and 30°C upto six month storage period, respectively.

2.3.2.2. Moisture content

Clover and cowpea type rhizobia are adversely affected with moisture content of 30 per cent, although other organisms occurring naturally in peat were less affected (Roughley, 1968). Levels in the range of 40 to 50 per cent were optimal for survival of *Rhizobium* (Date and Roughley, 1977).

In a sterilized peat, all strains are more tolerant to higher levels of moisture and growth is optimal in the range of 40 to 60 per cent moisture (Kannaiyan, 2000).
2.3.2.3. Aeration

Canadian and European workers observed rapid death of rhizobia in sealed containers, but with access to air their numbers remained high until the carrier became desiccated (Hedlin and Newton, 1948).

Roughley (1968) examined the growth and survival of clover and cowpea-type rhizobia in sterilized peat using cotton wool stoppered tubes, sealed cans and plastic film packets and with various gas exchange properties.

The practice of putting small holes in bags is unnecessary and harmful as in that moisture loss is increased. Also, they allow entry of contaminants in sterilized peat cultures (Smith, 1987).

2.3.2.4. Carrier sterility

The inoculant prepared with non-sterilized peat might contain 100-fold more rhizobia than sterilized peat because the death rate is higher in sterilized peat and is progressive with increase in storage period (Burton et al., 1967).

Sterilization has got great influence on the growth and survival of rhizobia and important to achieve consistently high cell densities in excess of $10^9$ g$^{-1}$ (Roughley and Vincent, 1967; Strijdom and Van Rensburg, 1981; Somasegaran and Halliday, 1982).
2.3.2.5. Packaging material

The choice of a method of inoculating sterilized peat without introducing contaminants depends on the type of packaging. *Rhizobium* needed a define gas exchange through packing materials (Roughly, 1968). Other hand inoculant in better survival of sealed bags than in open aerated bags was observed by Iswaran (1972). Higher survival (3.5 x 10^8 g⁻¹ to 62 x 10^8 g⁻¹) in inoculants packed of high density polythene bags of 0.31-0.32 mm gauge was reported by Strijdom and Deschodt (1976) and Narendranath *et al.* (1996). Double polythene bag having 300 gauge thickness supported higher survival of *Azospirillum* compared to single polythene bag (600 guage) at room temperature. Packing the peat based inoculant of *Azospirillum* in two layered polythene bag increased the shelf life when compared to packing in single polythene bag or polythene container (Govindarajan, 1996).

Beaded inoculant of *A. brasilense* enhanced the development of wheat and tomato seedlings growing in unfertile soil. Beads get degraded within 15 days in moist soil, by releasing gradually the immobilized inoculant organism (Bashan *et al.*, 2002).

2.4. CROP RESPONSE TO INOCULATION WITH PLANT GROWTH PROMOTING RHIZOBACTERIA

A large number of rhizosphere bacteria have potential for use as inoculants in crops. The plants inoculated with these bacteria have shown increased grain yield and plant biomass accumulation (Bashan and Levanony, 1990; Glick, 1995).
# Response of field crops to inoculation with Plant Growth Promoting Rhizobacteria

<table>
<thead>
<tr>
<th>S.No</th>
<th>Crop</th>
<th>Name of PGPR</th>
<th>Location</th>
<th>Types of Experiment</th>
<th>Parameter studied</th>
<th>Percentage increase over control</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Maize</td>
<td><em>Azospirillum</em></td>
<td>Isral</td>
<td>Field</td>
<td>Shoot fresh weight total N</td>
<td>18 to 200</td>
<td>Kapulnik <em>et al.</em> (1985)</td>
</tr>
<tr>
<td>2.</td>
<td>Sorghum</td>
<td><em>Azospirillum</em></td>
<td>USA</td>
<td>Field</td>
<td>Dry matter</td>
<td>Significant increase</td>
<td>Pacovsky <em>et al.</em> (1985)</td>
</tr>
<tr>
<td>3.</td>
<td><em>Cichorium intybus</em> (L.)</td>
<td><em>Azospirillum</em></td>
<td>India</td>
<td>Pot culture</td>
<td>Yield</td>
<td>Significant increase</td>
<td>Gunasekarn and Vissak (1986)</td>
</tr>
<tr>
<td>4.</td>
<td>Wheat</td>
<td><em>Azospirillum</em></td>
<td>India</td>
<td>Field</td>
<td>Grain yield, dry matter</td>
<td>9 to 35 to 12</td>
<td>Rasal and Patil (1990)</td>
</tr>
<tr>
<td>5.</td>
<td>Rice</td>
<td><em>Azospirillum</em></td>
<td>India</td>
<td>Field</td>
<td>Yield</td>
<td>19.23</td>
<td>Subramanian and Rangarajan (1990)</td>
</tr>
<tr>
<td>7.</td>
<td>Chilli</td>
<td><em>Azospirillum</em></td>
<td>India</td>
<td>Pot</td>
<td>Yield</td>
<td>Significant increase</td>
<td>Paramaguru and Natarajan (1993)</td>
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<tr>
<td>8.</td>
<td>Tomato</td>
<td><em>Burkholderia cepacia</em></td>
<td>Field</td>
<td>Yield</td>
<td>Significant increase</td>
<td>Martinez <em>et al.</em> (1993)</td>
<td></td>
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<tr>
<td>11.</td>
<td>Black gram</td>
<td><em>Bacillus</em></td>
<td>India</td>
<td>Field</td>
<td>Yield</td>
<td>37.2</td>
<td>Prabakaran <em>et al.</em> (1996)</td>
</tr>
<tr>
<td>S.No</td>
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<td>Location</td>
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<td>Percentage increase over control</td>
<td>Reference</td>
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<td>12.</td>
<td>Coffee</td>
<td><em>Azospirillum</em></td>
<td>India</td>
<td>Pot culture</td>
<td>Growth, flowering and yield</td>
<td>Significant increase</td>
<td>Hemavathi (1997)</td>
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<tr>
<td>13.</td>
<td>Wheat</td>
<td><em>Azotobacter</em></td>
<td>India</td>
<td>Field</td>
<td>Yield</td>
<td>Significant increase</td>
<td>Kumar and Narula (1999)</td>
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<tr>
<td>15.</td>
<td>Tea</td>
<td><em>Azospirillum</em></td>
<td>India</td>
<td>Field</td>
<td>Yield</td>
<td>Significant increase</td>
<td>Chakraborthy et al. (2003)</td>
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<tr>
<td>17.</td>
<td>Bamboo &amp; Maize</td>
<td><em>Azotobacter</em></td>
<td>India</td>
<td>Pot</td>
<td>Shoot fresh, root dry weight</td>
<td>Significant increase</td>
<td>Dhamangaonkar Sachin (2009)</td>
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<tr>
<td>18.</td>
<td><em>Vigna mungo</em></td>
<td><em>Pseudomonas</em></td>
<td>India</td>
<td>Field</td>
<td>dry root weight, dry shoot weight</td>
<td>Significant increase</td>
<td>Sarma et al. (2009)</td>
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<td>20.</td>
<td>Ginger</td>
<td><em>Azospirillum</em></td>
<td>India</td>
<td>Pot</td>
<td>Root length and yield</td>
<td>Significant increase</td>
<td>Govindan et al. (2009)</td>
</tr>
<tr>
<td>S.No</td>
<td>Crop</td>
<td>Name of PGPR</td>
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<td>22.</td>
<td>Soybean</td>
<td><em>P. aeruginosa</em></td>
<td>Indonesia</td>
<td>Pot</td>
<td>Fresh and dry weight of shoot, fresh and dry weight of root and yield</td>
<td>Significant increase</td>
<td>Khalima and Supraptta (2011)</td>
</tr>
<tr>
<td>23.</td>
<td>Wheat</td>
<td><em>Azotobacter</em></td>
<td>India</td>
<td>Pot</td>
<td>Shoot and root length</td>
<td>Significant increase</td>
<td>Rajewari (2011)</td>
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<td>24.</td>
<td>Vigna mungo (Black gram)</td>
<td>Phosphobacteria</td>
<td>India</td>
<td>Field</td>
<td>Yield</td>
<td>Significant increase</td>
<td>Selvakumar et al. (2012)</td>
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<tr>
<td>25.</td>
<td>Tomato</td>
<td><em>Bacillus, azotobacter</em></td>
<td>Egypt</td>
<td>Field</td>
<td>Plant height and fruit yield</td>
<td>Significant increase</td>
<td>Abdel- Monaim et al. (2012)</td>
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<tr>
<td>26.</td>
<td>Tomato</td>
<td><em>P.fluorescens, B. subtils</em></td>
<td>Saudi Arabia</td>
<td>Field</td>
<td>Plant height, fruit weight and fruit yield</td>
<td>Significant increase</td>
<td>Omar. A. Almaghrabi et al. (2013)</td>
</tr>
<tr>
<td>27.</td>
<td>Maize</td>
<td><em>P.fluorescens</em></td>
<td>Pakistan</td>
<td>Field</td>
<td>Plant height, total biomass and grain yield</td>
<td>Significant increase</td>
<td>Shahzad et al. (2013)</td>
</tr>
</tbody>
</table>
2.5. CROP RESPONSE TO CO-INOCULATION OF *Azospirillum*, *Azotobacter*, *Pseudomonas* AND PHOSPHATE SOLUBILIZERS

In addition to exploiting their individual plant growth promoting capacity, the potential of selected diazotrophs can be improved further through combined inoculation, with other microorganisms for additive and or synergistic effects. Combined inoculation of *Rhizobium* with *Azospirillum* or with *Azotobacter* increased the dry matter production, grain yield and nitrogen content of several legumes when compared with inoculation of *Rhizobium* alone (Burns et al., 1981; Rodelas et al., 1996).

Alagawadi and Gaur (1988 and 1992) observed that the combined inoculation of *A. brasilense* and *B. polymyxa* or *P. striata* had significant increase in the grain yield, dry matter yield, N and P uptake of sorghum over single inoculation. The rhizosphere population of *Azospirillum* and phosphate solubilizer was also higher in the respective inoculated treatment. Prabhakara and Ravi (1991) recommended the combined application of *A. brasilense* Sp7 and *Pseudomonas* sp. for getting higher dry matter, nitrogen and phosphorus content in maize.

Dual inoculation of *Azospirillum* and phosphobacteria resulted in higher root biomass and more bolls and kapas yield of cotton (Radhakrishnan, 1996). Dual inoculation of phosphobacterium and *Azospirillum* with 75 per cent recommended dose of NPK was superior to uninoculated control in increasing the yield of cumbu variety UCC 5 (Nirmala and Sundaram, 1996).
The combination of 2 kg *Azospirillum* with 150 kg N ha\(^{-1}\) recorded a higher dry matter production, flower head yield (1.340 t ha\(^{-1}\)) herbage and oil yield (0.167\%) and the highest uptake of P\(_2\)O\(_5\) and K\(_2\)O in Davana (Gandhi Kumar; 1996).

The combined inoculation effects of diazotrophs such as *A. lipoferum*, *Herbaspirillum seropedicae* and phosphorus solubilizing bacterium *B. megaterium* var. *phosphaticum* to rice increased shoot and root length and grain yield. Among them, *Herbaspirillum* and phosphobacterial inoculation recorded 14 per cent higher grain yield, whereas, *Azospirillum* and phosphobacterial inoculation recorded only 9 per cent yield increase over uninoculated control (Arangarasan *et al.*, 1998).

Inoculation of maize with different strains of *A. chroococcum* and *A. brasilense* stimulated the populations of certain other beneficial groups of microbial communities, including actinomycetes and a group of bacteria that are capable to grow on N free medium (Pandey *et al.*, 1989).

Several studies showed that growth promotion effects are seen early in plant development, and these subsequently translated into higher yields (Hoffmann-Hergarten *et al.*, 1998; Polyanskaya *et al.*, 2000). Actinomycetes promote the plant growth possibly due to antibiotic production or by providing cross protection (Wave *et al.*, 2001). The observed effects of seed inoculation or plant may in part be due to the stimulation of already existing PGPR in and around roots.
Mangrove seedlings treated with a mixture of a slow growing, 
N₂ fixing bacterium *Phyllobacterium* sp. and a fast growing phosphate 
solubilizing bacterium *B. licheniformis* resulted in increased nitrogen 
fixation and phosphate solubilization (Rojas *et al.*, 2001).

Application of *P. fluorescens* strains to black pepper rhizosphere and 
enhanced uptake of the same which reflected in increased plant biomass 
(Paul *et al.*, 2003). *P. fluorescens* inoculation significantly increased plant 
growth dry matter production and yield of tomato crop (Yan *et al.* 2003). The 
increase in growth parameters, highest biomass yield and the contents, 
lignins, phyllanthin and hypophyllanthin of *P. amarus* improved with 
combined inoculation of *Azospirillum* and phosphobacteria (Chezhian *et al.*, 2003). Combined inoculation of biofertilizers viz., *Azospirillum* + 
Phosphobacteria + VAM improved the biometric as well as biochemical 
attributes of silk cotton (Vijayakumari and Janardhanan, 2003).

Tomato crop inoculated with *P. fluorescens*, *A. chroococcum*, 
*A. brasilense* either alone or in combination recorded higher plant growth as 
well as control of the nematode, *Meloidogyne incognita* (Siddiqui, 2003).

Zhinong Yan *et al.* (2003) studied in tomato the application of PGPR 
organism, *Pseudomonas fluorescens* and *Bacillus subtilis* as seen treatment 
and soilless medium in which the transplants are grown. Both methods of 
applications of these organisms enhanced the plant growth but the level of 
growth promotion was significantly greater with the application of in the soil 
less medium.
Selvaraj et al. (2003) evaluated the organic farming system using various components FYM 25 t ha\(^{-1}\), bio dynamic compost 5 t ha\(^{-1}\), neem cake 5 t ha\(^{-1}\), *Azospirillum* and Phosphobacteria (2 kg ha\(^{-1}\)), and foliar spray of 3 per cent panchakavlya, on the growth and yield of rosemary and reported significant increase in plant height, number of branches, number of leaves per bunch, leaf length, leaf width and oil content over control plot.

The application of Phosphobacteria and *Azospirillum* at 2 kg ha\(^{-1}\) along with FYM 10 t ha\(^{-1}\) and neem cake 2 t ha\(^{-1}\) in *Gloriosa superba* recorded the maximum vegetative growth, fruit set percentage and yield attributes than control (Sujai Kumar, 2001). Fertilization with 50 per cent N, P +100 per cent K + *Azotobacter* + Phosphobacteria + VAM recorded the highest plant height, number of leaves, number of branches, plant spread, leaf area, fresh herb yield and essential oil yield in patchouli (Manjunatha et al., 2002). The application of phosphobacteria and *Azospirillum* along with poultry manure at closer spacing in Bhumymalaki recorded the highest number of branches per plant, number of leaves, plant spread, and yield of lignins, phyllanthins and hypophyllanthins (Chezhiyan et al., 2003). The inoculation of PSB + *Azotobacter* increased seed yield and root yield in Ashwagandha (Thosar et al., 2005).

Kumutha et al. (2006) conducted pot culture experiment to text the influence of AM fungi and PGPR inoculants on growth of tomato. The result indicated that the culturing of PGPR organisms and AM fungi in tomato significantly enhanced the growth and yield.
An investigation with marjoram (*Majorana hortensis* L.) indicated that the use of combined treatments of biofertilizers gave better results for all studied traits. The oil percentage and yield per plant for three cuttings was almost twofold higher on fresh weight basis as a result of aqueous extracts of compost at low level + biofertilizers compare with control. The chemical composition of marjoram essential oil did not change due to the fertilization type or level (Gharib et al., 2008).

Constantino et al. (2008) evaluated the effect of two rhizobacteria (*Azotobacter chroococcum* and *Azospirillum brasilense*) and a commercial product containing multiple strains of arbuscular mycorrhizal fungi (AMF) and an NPK fertilizer on the growth and yield of habanero chilli (*Capsicum chinense* Jacquin). The highest yields were recorded for the treatments involving a single inoculation of *A. chroococcum* and for those with the multi strain of AMF, with average values of 2.5 and 2.3 kg plant$^{-1}$ respectively, compared with 1.0 kg plant$^{-1}$ obtained with the treatment in which NPK fertilizer was applied.

Minorsky (2008) studied the effect of PGPR isolated from the roots graminaceous plants has been shown to colonize the roots of various plants and to increase the height, flower number, fruit number and total fruit weight of tomato plants. PGPR organisms applied to the rice crop can able to increase the yield of rice through growth hormone production, phosphorous solution. (Asbra Fazzaman et al., 2009).
Mahato et al. (2009) studied the effect of *Azotobacter* and nitrogen on seed germination and seedling growth in Tomato. Results showed that application of biofertilizer resulted increase of shoot length and more number of leaves per plant.

Mishra et al. (2010) reported the isolates *Bacillus subtilis* and *Pseudomonas fluorescens* gave excellent result on the productivity of *Pelargonium graveolens*, increased herb yield over control by 9 and 27.6% respectively.

Gehan Mostafa and Abo-Baker (2010) studied the effect of biofertilizer and chemical fertilizers, separately and in different combinations, on the growth of sunflower (*Helianthus annuus* L.) to reduce the chemical fertilizers used, maximizing their use efficiency to obtain highest growth and productive parameters. The biofertilizers used as inoculums for seeds treatment of sunflower were *Azospirillum* and *Bacillus polymyxa* and their mixture. Both bacterial inoculants and their mixture show an increase in growth parameters, nutrient content and yield when compared to the control. The result reveals that biofertilization treatments of *Azospirillum + Bacillus* plus 100% chemical fertilizers produced the highest values in all growth and yield parameters compared with the control. The results also indicated that biofertilization, beside its ability to improve the nutrient supply in the soil, also increases the efficiency of added chemical fertilization.
Mehran et al. (2011) was investigation revealed that inoculation of sunflower with PGPR and animal manure improved growth, yield and phytohormonal changes. The treatments were animal manure (M), *Pseudomonas putida* (P), *Azotobacter chroococcum* (A) and *Azospirillum lipoferum* (Z), the results indicated that manure significantly affected grain yield (P<0.01), and the highest grain yield was achieved in the interaction of manure x *Azotobacter* x *Pseudomonas* (4.556 ton/ha). These four factors of the experiment significantly affected auxin, gibberellins and cytokinin content of sunflower.

Ordookhani et al. (2011) was investigation revealed that the inoculation of *Ocimum basilicum* roots with PGPR improved growth and accumulation of essential oils. The treatments were *Pseudomonas putida* strain 41, *Azotobacter chroococcum* strain 5 and *Azospirillum lipoferum* strain OF. In comparision to the control treatment, all factors were increased by PGPR treatments. The maximum root fresh weight (3.96 g/plant), N content (4.72%) and essential oil yield (0.82%) were observed in the *Pseudomonas + Azotobacter + Azospirillum* treatment. All factors were higher in the *Pseudomonas + Azotobacter + Azospirillum* and *Azotobacter + Azospirillum* treatments respectively.

Eleiwa et al. (2012) evaluated the inoculation of *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus polymyxa*, in combination with foliar application with micronutrients (Mn+Fe+Zn) can lead to higher wheat yield.
Ahmad Gholami et al. (2012) reported that the bacterial strains *Azospirillum lipoferum* S-21, *Azospirillum brasilense* DSM1690, *Azospirillum lipoferum* DSM1619, *Azotobacter chroococcum* S-5 and *Azotobacter chroococcum* DSM2286 inoculated for maize crop under field conditions. The growth parameters were increased dry weight of leaf, stem and grain and hence total biomass sampled at 90, 105 and 120 (harvest time). The greatest grain weight was produced by *Azospirillum* S-21 inoculation. Dual inoculation with *Azotobacter* S-5+ *Azospirillum* S-21 significantly increased total dry weight up to 115% respectively.

Moslemi et al. (2012), application of super absorbent polymer (powder or gel) and inoculation of grain with biofertilizer (*A. lipoferum*+ *P. putida*) increased grain and biological yield of maize in both stress and normal condition.

Abdel-Monaim et al. (2012) evaluated *Azotobacter* sp, *Bacillus cereus*, *B. megaterium* individually or combined with humic acid seedling treatment of tomato significantly increased the plant height, fruit weight, fruit yield under green house condition.

Moslemi et al. (2012), application of super absorbent polymer (powder or gel) and inoculation of grain with biofertilizer (*A. lipoferum*+ *P. putida*) increased grain and biological yield of maize in both stress and normal condition.
Omar A. Almaghrabi et al. (2013) reported that the effect of PGPR *Pseudomonas putida, Pseudomonas fluorescens, Serratia marcescens, Bacillus amyloliquefaciens, Bacillus subtilis* and *Bacillus cereus*, were studied on tomato plant growth and root knot nematode reproduction after 45 days from nematode infection and these treatment recorded the highest number of shoot dry weight, highest number of plant height, fruit weight and fruit yield under green house conditions.