LITERATURE REVIEW

The rhizosphere is the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root. Complex gradient of substrate availability, water potential, and redox state distinguish this habitat from bulk soil, and constrains the distribution and the activity of the tremendously diverse rhizosphere microbiota (Kumar et al. 2013). The region around the root is relatively rich in nutrients, due to the loss of as much as 40% of plant photosynthates from the roots as root exudates (Lynch and Whipps, 1991). Root exudates include amino acids, organic acids, carbohydrates, sugars, vitamins, mucilage and proteins. The exudates launch signals and attract the microbes towards root by stimulating biological and physical interactions between roots and soil microorganisms (Brevic, 2012). Consequently, the rhizosphere supports large and active microbial populations capable of exerting positive, neutral, or negative effects on plant growth (Reddy, 2013). According to Raynaud and Nunan (2014) the vast majority of soil organisms in the rhizosphere were bacteria, with densities as high as 10^8 cells per gram of bulk soil which depends on biotic conditions like soil pH, temperature and moisture.

Plant growth promoting rhizobacteria (PGPR) are natural microflora of soil that are able to colonize plant roots and stimulate plant growth when applied to roots and other propagules (Egamberdieva et al. 2010). The term PGPR was first introduced by Kloepper and Schroth (1978) to describe soil bacteria that colonize the roots of plants following inoculation on to seed and that improve the plant growth. Some authors also used the term plant health promoting rhizobacteria (PHPR) or nodule promoting rhizobacteria (NPR) for the PGPR and are associated with the rhizosphere, an important soil ecological environment for plant–microbe communications (Burr and Caesar, 1984). The relationship of PGPR with the host plants may be of two types and PGPR can be divided into two groups: symbiotic bacteria and free-living rhizobacteria (Khan, 2005). Several workers also designated PGPR two groups according to their residing sites: iPGPR (i.e., symbiotic bacteria), which live inside the host cells, produce nodules, and are localized inside the nodule; and ePGPR (i.e., free-living rhizobacteria), which do not produce nodules, but still can prompt plant

growth (Gray and Smith, 2005). The best-known iPGPR are Rhizobia, which produce nodules in leguminous plants and provide nitrogen to them (Hayat et al. 2010). The PGPR facilitate plant growth and development by both direct and indirect mechanisms (Glick, 1995). The direct mechanism of plant growth promotion include, production of phytohormones, providing plants with fixed nitrogen and soluble phosphate, while indirect stimulation of plant growth includes preventing phytopathogens by secretion of siderophore that sequester iron from the soil and thus preventing phytopathogens from pathogenesis and thus, promoting plant growth and development (Bashan and Glick, 1997). The review presented below has been compiled mainly to understand the present status knowledge regarding mechanisms of plant growth promotion and disease control by plant growth promoting bacteria (PGPR).

2.1. The mechanism of Plant growth promotion by PGPR

Plant growth promoting rhizobacteria (PGPR) are a common group of bacteria that can actively colonize plant roots and increase plant growth (Kloepper and Schroth, 1978). These PGPR can prevent the deleterious effects of phytopathogenic organisms on the environment. The mechanisms by which PGPR can influence plant growth may differ from species to species as well as from strain to strain. It may promote growth directly by producing phytohormones, increase the phosphorous uptake by solubilization of inorganic phosphates, by fixing the atmospheric N_2, increasing the availability to plants and ammonia production were reported as best known mechanisms of plant growth promotion (Podile and Kishore, 2006; Zhang et al. 2012; Singh et al. 2013; Kumar et al. 2013). Production of siderophores and secretion of different antifungal compounds to inhibit the phytopathogens are considered as indirect methods of plant growth promotion.

2.1.1. Synthesis of phytohormones

Plant hormones play important role in plant growth and development. Several stages such as cell elongation, cell division, tissue differentiation, and apical dominance are controlled by the plant hormones, especially auxins and cytokinins. Auxins and cytokinins can be synthesized by both the plants and the microorganisms.

Auxin, indole-3-acetic acid (IAA), is an important phytohormone produced by a number of PGPR, and treatment with auxin-producing rhizobacteria increased the

plant growth (Vessey, 2003; Erturk et al. 2008). Diverse bacterial species possess the ability to produce the phytohormone IAA. At present, auxin synthesizing rhizobacteria are the most well-studied phytohormone producers (Tsavkelova et al. 2006; Spaepen et al. 2007). Different biosynthesis pathways have been identified and redundancy for IAA biosynthesis is widespread among plant-associated bacteria. It has been estimated that 80% of bacteria isolated from the rhizosphere can produce plant growth regulator IAA (Patten and Glick, 1996). IAA production by bacteria can vary among different species and strains, and it is also influenced by factors as culture condition, growth stage and substrate availability (Mutluru and Konada, 2007). The bacteria synthesize IAA generally through two pathways- *Rhizobium*, *Bradyrhizobium*, and *Azospirillum* synthesize IAA via the Indole-3-pyruvic acid (IPyA) pathway (Costacurta and Vanderleyden, 1995; Patten and Glick, 1996; Burdman et al. 2000). On the other hand, the indole-3-acetamide (IAM) pathway is used by some pathogenic bacteria such as *Pseudomonas syringae, Agrobacterium tumefaciens*, and *Erwinia herbicola* to synthesize IAA (Dobbelaere et al. 2003). Among PGPR species, *Azospirillum* is one of the best studied IAA producers (Dobbelaere et al. 1999). Other IAA producing bacteria belonging to *Aeromonas* (Halda-Alija, 2003), *Azotobacter* (Zahir et al. 2000), *Bacillus* (Swain et al. 2007), *Burkholderia* (Halda-Alija, 2003), *Enterobacter* (Shoebitz et al. 2009), *Pseudomonas* (Hariprasad and Niranjana, 2009) and *Rhizobium* (Ghosh et al. 2008) have been isolated from different rhizosphere soils. Culture filtrates of plant growth-promoting rhizobacteria (PGPR) *Bacillus amyloliquoefaciens* (FZB24, FZB42 and FZB45) and *Bacillus subtilis* FZB37 which are reported to produce IAA, have a strong growth-promoting activity. During the bioassays, seedling segment elongation and coleoptiles bending performed with diluted *Bacillus* culture filtrates demonstrated that length growth of maize seedlings was significantly enhanced. *Bacillus amyloliquefaciens* FZB42 exhibited the highest enhancement on plant growth comparable with concentrations of 10^{-6} to 10^{-7} mol/l IAA (Idris et al. 2007). In a study by Khalid et al. (2004) focused on the screening of effective PGPR strains on the basis of their potential for *in vitro* auxin production and plant growth promoting activity under gnotobiotic conditions. A large number of bacteria were isolated from the rhizosphere soil of wheat plants grown at different sites. Thirty isolates showing prolific growth on agar medium were selected and evaluated for their potential to produce auxins *in vitro*. Colorimetric analysis showed variable amount of auxins (ranging from 1.1 to

12.1 mg L^{-1}) produced by the rhizobacteria *in vitro* and amendment of the culture media with l-tryptophan (l-TRP), further stimulated auxin biosynthesis (ranging from 1.8 to 24.8 mg L^{-1}). HPLC analysis confirmed the presence of indole acetic acid (IAA) and indole acetamide (IAM) as the major auxins in the culture filtrates of these rhizobacteria. A series of laboratory experiments conducted on two cv. of wheat under gnotobiotic (axenic) conditions demonstrated increases in root elongation (up to 17.3%), root dry weight (up to 13.5%), shoot elongation (up to 37.7%) and shoot dry weight (up to 36.3%) of inoculated wheat seedlings. Linear positive correlation (r = 0.99) between *in vitro* auxin production and increase in growth parameters of inoculated seeds was found. Based upon auxin biosynthesis and growth-promoting activity, four isolates were selected and designated as plant growth-promoting rhizobacteria (PGPR). Auxin biosynthesis in sterilized vs nonsterilized soil inoculated with selected PGPR was also monitored that revealed superiority of the selected PGPR over indigenous microflora. Peat-based seed inoculation with selected PGPR isolates exhibited stimulatory effects on grain yields of tested wheat cv. in pot (up to 14.7% increase over control) and field experiments (up to 27.5% increase over control); however, the response varied with cv. and PGPR strains. It was concluded that the strain, which produced the highest amount of auxins in non-sterilized soil, also caused maximum increase in growth and yield of both the wheat cv. Their study suggested that potential for auxin biosynthesis by rhizobacteria could be used as a tool for the screening of effective PGPR strains. Ahmad et al. (2005) reported that 10 *Azotobacter* and 11 fluorescent *Pseudomonas* sp. showed IAA production ability in presence and absence of tryptophan. In absence of additional tryptophan, the *Azotobacter* strains showed low amount of IAA production (2.68-10.80 mg/ml).The *Azotobacter* isolates showed high level (7.3 to 32.8 mg/ml) production of IAA at 5 mg/ml of tryptophan while at 1 and 2 mg/ml the production was in the range of 1.47 to 11.88 and 5.99 to 24.8 mg/ml, respectively. Production of IAA in fluorescent *Pseudomonas* isolates increased with an increase in tryptophan concentration from 1 to 5 mg/ml in the bulk of isolates. In the presence of 5 mg/ ml of tryptophan, 5 isolates of *Pseudomonas* produced high levels (41.0 to 53.2 mg/ml) of IAA while 6 other isolates produced IAA in the range of 23.4 to 36.2 mg/ml. It has been further observed that *Pseudomonas* isolates (Ps1, Ps4 and Ps7) negatively affect the growth of root elongation of *Sesbania aculeata* and *Vigna radiata* at all concentrations of tryptophan compared to the control. Boiero et al. (2007) has reported significant shoot

growths in maize and rice dwarf mutants, promoted by gibberellins-like substances excreted by *Azospirillum* spp. In another study Tsavkelova et al. (2007) reported an increase in the germination of seeds of *Dendrobium moschatum* inoculated with *Sphingomona* ssp. and IAA producing *Mycobacterium* sp. Biostimulant species of *Pseudomonas* and *Bacillus* can produce yet not well characterized phytohormones or growth regulators that cause crops to have greater amounts of fine roots which have the effect of increasing the absorptive surface of plant roots for uptake of water and nutrients. Rhizobia are the first group of bacteria, which are attributed to the ability of PGPR to release IAA that can help to promote the growth and pathogenesis in plants (Mandal et al. 2007). Acuña et al. (2011) reported IAA production in *Bacillus* and *Paenibacillus* sp. They have reported the effect of pH and metal ions on IAA production. The production *in vivo* of IAA by *Paenibacillus* sp. SPT−03 was increased 7−fold when incubated in tenfold diluted culture medium, compared to the full–strength medium. At low pH IAA production of *Bacillus* sp. MQH−19 was decreased, whereas they were increased in *Paenibacillus* sp. SPT−03. The ten *Pseudomonas fluorescens* and *Bacillus subtilis* isolates obtained from the rhizosphere of paddy showed IAA production. The IAA production by *Bacillus subtilis* was relatively low when compared to *Pseudomonas fluorescens*. The maximum IAA production by *Pseudomonas fluorescens* was recorded by the isolate PF-8 (28.80 µg/ml). The minimum production of IAA was found in PF–4 (7.36 µg/ml) isolates (Sivasakthi et al. 2013). Shim et al. (2015) reported IAA production by *Bacillus* sp. in presence of chromium. The strain JH 2-2, isolated from the rhizosphere of plants at a multi-metal contaminated mine site, has the potential to reduce Cr(VI) to Cr(III) and promote plant growth by reducing Cr toxicity and producing IAA.

Generally, rhizobacterial IAA interferes with the many plant developmental processes because the endogenous pool of plant IAA may be altered by the acquisition of IAA that has been secreted by soil bacteria (Glick, 2012; Ahemad and Kirbet, 2014). Evidently, IAA also acts as a reciprocal signalling molecule affecting gene expression in several microorganisms. Consequently, IAA plays a very important role in rhizobacteria-plant interactions (Spaepen and Vanderleyden, 2011). Moreover, down-regulation of IAA as signalling is associated with the plant defense mechanisms against a number of phyto-pathogenic bacteria as evidenced in enhanced susceptibility of plants to the bacterial pathogen by exogenous application of IAA or

IAA produced by the pathogen (Spaepen and Vanderleyden, 2011). IAA has been virtually associated with every aspect of plant growth and development, as well as defence responses. Generally, IAA affects plant cell division, extension, and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity and florescence; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions. IAA produced by rhizobacteria likely, interfere with the above physiological processes of plants by changing the plant auxin pool. Moreover, by increasing root surface area and length, bacterial IAA provides the plant greater access to soil nutrients. Also, rhizobacterial IAA loosens plant cell walls and as a result facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria (Glick, 2012; Ahemad and Kirbet, 2014). Reetha et al. (2014) reported the effect of IAA producing *Pseudomonas fluorescens* and *Bacillus subtilis* on the growth of Onion. Both the isolates are isolated from the rhizosphere of onion and analysed for *in vitro* indole acetic acid. In the quantitative measurements, the highest value of IAA production was obtained by *P. fluorescens* followed by *B. subtilis*, as they produced (15.38 ± 0.537) and (12.67 ± 0.325) respectively. Both bacteria demonstrated increase in root length, shoot length, root and shoot fresh and dry weight, on bacterial inoculated onion seeds over control.

Cytokinins, reported as adenine derivatives with an isoprenoid side chain, are other important phytohormones usually present in small amounts in biological samples and are often difficult to identify and quantify (Dobbelaere et al. 2003). The most noticeable effect of cytokinin on plants is enhanced cell division; however, root development and root hair formation have also been reported by few workers (Frankenberger and Arshad, 1995). Plants and plant associated microorganisms have been found to contain over 30 growth promoting compounds of the cytokinin group. Cytokinins are produced by bacteria such as *Azospirillum* and *Pseudomonas* spp. (Gaudin et al. 1994). Some PGPR strains were reported to produce cytokinins, such as *Arthrobacter* spp., *Rhizobium leguminosarum*, *Paenibacillus polymyxa*, and *Pseudomonas fluorescens* (Noel et al. 1996; Timmusk et al. 1999; Garcia de Salamone et al. 2001; Bent et al. 2001; Vessey, 2003). Hussain and Hasnain (2009) studied the effect of cytokinin producing soil isolates on cell division in cucumber

cotyledons. They have screened 33 rhizospheric isolates and selected three most promising isolates. *Bacillus licheniformis* Am2 strain isolated from crop plant *Brassica campestris* was the most efficient cytokinin secreting bacteria among the strains studied. Two species of cytokinins were detected in the culture media of *Bacillus licheniformi*s Am2 strain. Bacterial cytokinin was significantly correlated to cell division as well as cotyledon expansion in the dark. However in light grown cotyledons bacterial cytokinin was only significantly correlated to cell division but not to the cotyledon expansion. Liu et al. (2013) studied the effects of *Bacillus subtilis* on hormone concentration, drought resistance, and plant growth under water-stressed conditions. Under well-watered conditions, leaves of inoculated *Platycladus orientalis* seedlings under drought stress had higher relative water content and leaf water potential compared with those of un-inoculated ones. Regardless of water supply levels, the root exudates, namely sugars, amino acids and organic acids, significantly increased because of *B. subtilis* inoculation. Water stress reduced shoot cytokinins by 39.14%. However, inoculation decreased this deficit to only 10.22%. *B. subtilis* inoculation increased the shoot dry weight of well-watered and drought seedlings by 34.85 and 19.23%, as well as the root by 15.445 and 13.99%.

A number of authors have reported that PGPR also produced gibberellins (GAs). Dobbelaere et al. (2003) reported that over 89 GAs are known to date and are numbered GA1 through GA89 in approximate order of their discovery (Frankenberger and Arshad, 1995; Arshad and Frankenberger, 1998). The most widely recognized gibberellin is GA3 (gibberellic acid); the most active GA in plants is GA1, which is primarily responsible for stem elongation (Davies, 1995). PGPR such as *Rhizobium phaseoli*, *Azospirillum lipoferum*, *Azotospirillum brasilense*, *Acetobacter diazotropicus*, *Herbospirillum seropedicae*, *Bacillus licheniformis*, *B. pumilus*, *Bacillus cereus* MJ-1, *Bacillus macroides* CJ-29 were reported to produce GAs (Janzen et al. 1992; Bastian et al. 1998; Gutierrez-Manero et al. 2001; Joo et al. 2004). Joo et al. (2004) reported that the growth of red pepper plug seedlings was increased by *Bacillus cereus* MJ-1, *B. macroides* CJ-29, and *B. pumilus* CJ-69, though the number of leaves and stem diameter were not significantly changed. The greatest increase is in the height and the root fresh weight of the seedlings was by *B. pumilus*, which could increase the height by 12% and the root fresh weight by 20%. Pandya and Desai (2014) reported the GA production from rhizospheric *Pseudomonas*

monteilii NPB20. The isolate was bioassayed on wheat and chana for its growth promoting capacity. The microbial broth suspension of NPB20 significantly promoted growth of wheat and chana seedlings. In both crops, seed germination, the root length, and shoot length parameters significantly promoted compared to positive control.

Another mechanism of plant growth promotion involving ethylene has been proposed by Burdman et al. (2000). Ethylene is a gaseous plant growth hormone produced endogenously by almost all plants. It is also produced in soil through a variety of biotic and abiotic mechanisms, and plays a key role in inducing diverse physiological changes in plants at cellular level. Apart from being a plant growth regulator, ethylene has also been established as a stress hormone. Under stress conditions like those generated by salinity, drought, water logging, heavy metals and pathogenicity, the endogenous production of ethylene is accelerated significantly which adversely affects the plant physiology (Saleem et al. 2007). According to Glick et al. (1998) some bacteria contain 1-aminocyclopropane-1-carboxylate (ACC) deaminase that could cleave ACC, the immediate precursor to ethylene in the biosynthetic pathway for ethylene in plants. It has been postulated that ACC deaminase activity would decrease ethylene production in the roots of host plants and results in root lengthening (Kaymak, 2010). Several reports have indicated that under laboratory conditions, inoculation with rhizobacteria containing ACC-deaminase increased growth of the inoculated plants primarily through regulation of ethylene synthesis in the inoculated roots (Jacobson et al. 1994; Glick et al. 1995; Li et al. 2000; Penrose et al. 2001; Ghosh et al. 2003; Shaharoona et al. 2006).

Some workers have reported that the growth promotion effects of ACC deaminase producing PGPR is the best expressed in stress conditions including drought (Zahir et al. 2008) and salt (Nadeem et al. 2007; Zahir et al. 2009) stress. ACC deaminase has been widely reported in numerous microbial species of gram negative bacteria (Wang et al. 2000; Babalola et al.2003), gram positive bacteria (Belimov et al. 2001; Ghosh et al. 2003), rhizobia (Ma et al. 2003; Uchiumi et al. 2004) and endophytes (Sessitsch et al. 2005). It is extensively studied in numerous species of plant growth promoting bacteria like *Agrobacterium genomovars* and *Azospirillum lipoferum* (Blaha et al. 2006), *Alcaligenes* and *Bacillus* (Belimov et al. 2001), *Burkholderia* (Pandey et al. 2005; Sessitsch et al. 2005), *Enterobacter* (Penrose and Glick, 2001), *Methylobacterium fujisawaense* (Madhaiyan et al. 2006),

Pseudomonas (Mayak et al. 1999; Blaha et al. 2006; Zahir et al. 2009), *Ralstonia solanacearum* (Blaha et al. 2006), *Rhizobium* (Ma et al. 2003, Uchiumi et al. 2004), *Rhodococcus* (Stiens et al. 2006), *Sinorhizobium meliloti* (Belimov et al. 2005) and *Variovorax paradoxus* (Belimov et al. 2001). Recent studies with ACC deaminase containing PGPR have given indication of their ability to boost plant growth under stressed conditions by regulating the production of ethylene. Under salinity stress, 1-aminocyclopropane-1-carboxylic acid-deaminase activity of *P. putida* (N21), *P. aeruginosa* (N39) and *Serratia proteamaculans* (M35) might have caused reduction in the synthesis of stress (salt)-induced inhibitory levels of ethylene (Zahir et al. 2009). Shahzad et al. (2013) assed the growth promoting activity of two ACC deaminase producing strains *Pseudomonas thivervalensis* (STF3) and *Serratia marcesens* (STJ5) in maize under axenic conditions. The rhizobacterial strains were investigated for their growth and yield promoting potential under field conditions with 50, 75 and 100% recommended chemical fertilizers (CF). Results of the study revealed that the rhizobacterial isolates, with 75 and 100% CF, significantly improved the growth and yield of maize compared to the uninoculated control. The growth and yield promoting effect of rhizobacterial strain *P. thivervalensis* (STF3) with 75% CF were similar to that of CF alone. However, with 100% CF, same rhizobacterial strain significantly increased plant height, total biomass, grain yield, 1000-grain weight and chlorophyll content compared to the uninoculated control. Varied ACC deaminase activity might be responsible for differential behaviour of *P. thivervalensis* (STF3) and *S. marcesens* (STJ5) under axenic and filed conditions.

2.1.2. Solubilization of Organic and Inorganic Phosphates

Phosphorus (P) is one of the major essential macronutrients for plant growth and development and is present at levels of 400–1,200 mg kg^{-1} of soil (Hayat et al. 2010). Most of the P is insoluble making it unavailable to plants. Even in P rich soil very little amount is readily available to plants. The plants are able to absorb their own mono and dibasic phosphate, but organic or insoluble forms of P need to be mineralized or solubilized (Ramaekers et al. 2010). Most of the insoluble P forms are present as aluminum and ironphosphates in acid soils (Mullen, 2005), and calcium phosphates in alkaline soils (Goldstein and Krishnaraj, 2007). The concentration of soluble P in soil is usually very low, normally at levels of 1 ppm or less (Goldstein, 1994).

Table 1: List of plant growth regulators/ Hormones produced by PGPR.

Plant growth regulators	**PGPR**	**References**
IAA	*Rhizobium leguminosarum* *Azotobacter sp.* *Enterobacter sp.* *Pseudomonas fluorescens* *Mesorhizobium loti* MP6 *Bacillus sp.* *Paenibacillus sp.* *Bacillus* RC23 *Pseudomonas putida*	Biswas et al.(2000) Zahir et al. (2000) Mirza et a. (2001) Dey et al. (2004) Chandra et al. (2007) Beneduzi et al. (2008) Erturk et al. (2008) Ahemad and Khan (2012)
Gibberellin	*Acetobacter diazotropicus* *Bacillus pumilus* *Azotobacter sp.*	Bastian et al. (1998) Guiterrez–Manero et al.(2001) Narula et al.2006
Cytokinin	*Paenibacillus polymyxa* *Pseudomonas fluorescens* *Bacillus licheniformis* *Bacillus subtilis BC1*	Timmusk et al. (1999) Garcia de Salmone et al. (2001) Hussain and Hasnain (2009)
ACC deaminase	*Pseudomonas putida* *Pseudomonas cepacia* *Pseudomonas putida* biovar B *Bacillus* sp. ACC3 *Enterobacter cloacae*	Mayak et al. (1999) Cattelan et al. (1999) Rodriguez et al. (2008) Bal et al. (2012) Singh and Jha (2015)

The plant takes up several P forms but major part is absorbed in the forms of $HPO4^{-2}$ or H_2PO4^{-1}. The phenomenon of P fixation and precipitation in soil is generally highly dependent on pH and soil type. Phosphate solubilizing bacteria (PSB), which are very common in soil, can be used to overcome the situation. Microorganisms offer a biological rescue system capable of transforming insoluble P to soluble monobasic and dibasic ions and may also solubilize inorganic phosphate (Kumar and Narula, 1999). The mechanism by which PSB solubilize P, involves process of acidification, chelation, exchange reactions, and production of gluconic acid (Rodriguez et al. 2004; Chung et al. 2005; Hameeda et al. 2008). However, acidification does not seem to be the only mechanism of P solubilization, as the ability to reduce the pH in some cases does not correlate with the ability to solubilize mineral phosphates (Subba Rao, 1982).

Kim et al. 1997 reported that DNA transfer from *Enterobacter agglomerans* showed mineral phosphate solubilization activity in *E. coli* JM109, although the pH of the medium was not altered. Members belonging to the genera *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aerobacter, Flavobacterium* and *Erwinia* have the ability to solubilize insoluble inorganic phosphate (mineral phosphate) compounds such as tricalcium phosphate, dicalcium phosphate, hydroxyl apatite and rock phosphate (Goldstein, 1986; Rodríguez and Fraga, 1999; Rodríguez et al. 2006). Organic P can constitute between 30 and 50% of the total P of the soil, a high proportion of it corresponding to phytate (Borie et al. 1989; Turner et al. 2003). In some soil the availability of organic phosphate compounds for plant nutrition could be a limitation due to precipitation with the soil particle ions. Three groups of enzymes viz. Nonspecific phosphatases, Phytases, Phosphonatases/Carbon-phosphate Lyases, can release phosphate from insoluble organic compounds in the soil. The activities of nonspecific acid phosphatases lead to the dephosphorylation of phospho-ester or phosphoanhydride bonds in the organic matter. Most of the PGPR strains possessed these enzymes which were formed by three molecular families, designated as molecular class A, B and C (Thaller et al. 1995). Phytases, which are responsible for specific release of phosphate from phytic acid. Most of the phytases (myo-inositol hexakisphosphate phosphorhydrolases) belong to high molecular weight acid phosphatases. According to Richardson (2001) phytate is the major component of organic phosphate in the soil.

In its basic form, phytate is the primary source of inositol and the major stored form of phosphate in the plant seeds and pollen. Phosphonatases and C-P Lyases perform C-P cleavage in organophosphonates. The main activity apparently corresponds to the work of acid phosphatases and phytases because of the predominant presence of their substrates in soil (Tarafdar and Jung, 1987; Tarafdar and Claassen, 1988). These processes have the potential to decrease the use of phosphate fertilizer by mobilizing the fertilizer constituents present in the soil and at the same time reducing costs and improving crop yields (Chaiharn and Lumyong, 2009). Microbial P release from organic P sources has been reported by several workers (Ohtake et al. 1996; McGrath et al. 1998; Rodríguez and Fraga 1999). Assimilation, storage and metabolism of phosphorus are of major importance to plant growth and development (Duff et al. 1994). De Freitas et al. (1997) isolated 111 strains from plant rhizospheric soil, and a collection of nine bacteria (PGPR) were screened for P-solubilization *in vitro*. The P-solubilizing isolates were identified as two *Bacillus brevis* strains, *Bacillus megaterium, B. polymyxa, B. sphaericus, B. thuringiensis* and *Xanthomonas maltophilia* (PGPR strains R85). Root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition. Davison (1998) reported that fluorescent pseudomonads enhanced plant growth by improving soil nutrient status, secreting plant growth regulators, and suppressing soil-borne pathogens. In addition to the phosphate-solubilizing capability of many *Pseudomonas* strains, they could promote plant growth by mechanisms such as the production of plant growth regulators and vitamins, enhancement of plant nutrient uptake and suppression of pathogenic or deleterious organisms Moreover, the tripartite association composed of legume plant, rhizobia and *Pseudomonas spp*. was reported to increase root and shoot weight, plant vigour, nitrogen (N) fixation and grain yield in various legumes (Dashti et al. 1998; Sindhu et al. 1999).

The phosphate solubilising bacteria (PSB) dissolved the soil P through production of low molecular weight organic acids mainly gluconic and keto gluconic acids (Deubel et al. 2000), in addition to lowering the pH of rhizosphere. The pH of rhizosphere was lowered through biotical production of proton/bicarbonate release (anion/cation balance) and gaseous (O_2/CO_2) exchanges. *Pseudomonas stutzeri* (Vazquez et al. 2000) and *P. putida* (Kumar and Singh, 2001) were also reported as

phosphate solubilizers. Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi (Whitelaw, 2000) and among the soil bacterial communities, ectorhizospheric strains from *Pseudomonas* and *Bacillus*, and endosymbiotic rhizobia were categorised as effective phosphate solubilizers. Vazquez et al. (2000) isolated 13 phosphate solubilizing bacteria along with a fungus from the rhizosphere of mangroves and reported that among the bacterium *Vibrio proteolyticus* was the most active P solubilizing species. Gupta et al. (2002) described *Pseudomonas* species as a potent phosphate solubilizer. Phosphorus solubilization ability of PSB had direct correlation with pH of the medium. Ectorhizospheric strains from pseudomonads and bacilli, and endosymbiotic rhizobia were also described as effective phosphate solubilizers (Igual et al. 2001). Growth and phosphorus content in two alpine *Carex* species were increased by inoculation with *Pseudomonas fortinii* (Bartholdy et al. 2001). Bacteria were also reported as more effective in phosphorus solubilization than fungi (Alam et al. 2002). Inoculation with PSB increased sugarcane yield by 12.6 percent (Sundara et al. 2002). Large amount of P applied as fertilizer enters in to the immobile pools through precipitation reaction with highly reactive Al^{3+} and Fe^{3+} in acidic, and Ca^{2+} in calcareous or normal soils (Gyaneshwar et al. 2002; Hao et al. 2002). Neelam and Meenu (2003) reported high tricalcium phosphate solubilizing ability of *Pseudomonas* sp. (TP2) isolated from rhizosphere of field grown *Trigonella*. Jeon et al. (2003) also reported phosphorous solubilization by three strains of *Pseudomonas fluorescens*.

Some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus, respectively (Khiari and Parent, 2005). Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, Ca) and decrease the pH in basic soils (Stevenson, 2005). El-Komy (2005) studied the efficacy of strains of *Pseudomonas fluorescens*, *Bacillus megaterium* and *Azospirillum spp*. to solubilise Ca_3PO_4 under *in vitro* condition. *Pseudomonas fluorescens* and *Bacillus megaterium* strains were the most powerful phosphate solubilizers on Pikovskaya (PVK) plates and liquid medium. *Azospirillum lipoferum* strains showed weak zones of solubilisation on the PVK plates. Maximum pH reduction was 2.8, 1.2 and 0.5 units for *Pseudomonas fluorescens*, *Bacillus megaterium* and *Azospirillum lipoferum* strain 137, respectively. Alginate and agar immobilization of the tested bacteria or

combination of *A. lipoferum* 137 and *B. megaterium* significantly enhanced phosphorus solubilisation for four consecutive 4-day cycles. In a pot experiment, phosphorus mobilization in wheat (*Triticum aestivum* L. cv. BeniSwif 1) inoculated with *B. megaterium* or *A. lipoferum* 137 as single or mixed inocula (as free or alginate immobilized beads) was studied in presence of Ca_3PO_4. In a study, Chen et al. (2006) isolated, screened and characterized 36 strains of phosphate solubilizing bacteria (PSB) from Central Taiwan. Mineral phosphate solubilizing (MPS) activities of all isolates were tested on tricalcium phosphate medium by analyzing the soluble-P content after 72 h of incubation at 30°C. Identification and phylogenetic analysis of 36 isolates were carried out by 16S rDNA sequencing. Ten isolates belonged to genus *Bacillus*, nine to genus *Rhodococcus*, seven to genus *Arthrobacter*, six to genus *Serratia* and one each to genera *Chryseobacterium*, *Delftia*, *Gordonia* and *Phyllobacterium*. In addition, four strains namely, *Arthrobacter ureafaciens*, *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis* and *Delftia* sp. were reported for the first time as phosphate solubilizing bacteria (PSB) after confirming their capacity to solubilize considerable amount of tricalcium phosphate in the medium by secreting organic acids. P-solubilizing activity of these strains was associated with the release of organic acids and a drop in the pH of the medium. HPLC analysis detected eight different kinds of organic acids, namely: citric acid, gluconic acid, lactic acid, succinic acid, propionic acid and three unknown organic acids from the cultures of these isolates. An inverse relationship between pH and P solubilized was apparent from this study. Identification and characterization of soil PSB for the effective plant growth-promotion broadens the spectrum of phosphate solubilizers available for field application. Chakraborty et al. (2006) reported phosphate solubilization by *Bacillus megaterium* and thereby promoting the growth of tea plants. Phosphate solubilization by the tested organisms was accompanied with pH reduction of the culture medium. In another study, Orhan et al. (2006) reported that plant growth promoting effects of two *Bacillus* strains OSU-142 (N-fixing) and M3 (N-fixing and phosphate solubilizing) were tested alone or in combinations of organically grown primocane fruiting raspberry (cv. Heritage) plants and a significant increase in yield (33.9 and 74.9%), cane length (13.6 and 15.0%), number of cluster per cane (25.4 and 28.7%), and number of berries per cane (25.1 and 36.0%) were observed when compared with that of the control. Hameeda et al. (2008) reported that plant biomass increased with *Serratia marcescens* EB 67 and *Pseudomonas sp* CDB

35 under both glasshouse and field conditions. And also, seed treatment with EB 67 and CDB 35 increased the grain yield of field-grown maize by 85 and 64% compared with the uninoculated control. In an experiment Elkoca et al. (2008) showed that the controlled environment and in the field trials, single and dual N-fixing *B. subtilis* (OSU-142) and P-solubilizing *B. megaterium* (M-3) inoculations significantly increased height, shoot, root and nodule dry weight, N%, chlorophyll content, pod number, seed yield, total biomass yield, and seed protein content in chickpea compared with the control treatment, equal to or higher than N, P, and NP treatments. Jorquera et al. (2008) isolated P solubilizing bacteria from the rhizospheres of five cultivated plants (*Lolium perenne*, *Trifolium repens*, *Triticum aestivum*, *Avena sativa*, *Lupinus luteus*), which presented more than one mechanism for utilizing insoluble forms of phosphorus. Ahemad and Khan (2010) studied the effect of four fungicides, tebuconazole, hexaconazole, metalaxyl and kitazin on the plant growth promoting activities of the fungicide tolerant *Enterobacter asburiae* strain PS2 under *in vitro* conditions. *Enterobacter asburiae* strain PS2 was isolated from the mustard rhizosphere and was assessed for the fungicide-tolerance and production of plant growth promoting traits both in the presence and absence of fungicides. *Enterobacter asburiae* strain PS2 showed plant growth promoting activities even in the presence of fungicides which however, decreased progressively with the increase in fungicide concentration. Fungicides at recommended dose had little effect while the dose higher than the recommended one adversely affected the physiological traits, like, phosphate solubilization, siderophore, and indole acetic acid synthesis. The P-solubilizing potentials of *E. asburiae* strain PS2 in the presence of varying concentrations of fungicides was assayed both qualitatively and quantitatively using a solid and liquid Pikovskaya medium. In general, when the concentration of each fungicide was increased from 1X to 3X, the size of the halo decreased considerably. The effect of three times the recommended rate (3X) of each fungicide was most adverse on the halo formation compared to the recommended (1X) and two times the recommended rate (2X). The order of toxicity of the fungicides at 3X on the halo size (solubilization index) was: tebuconazole>hexaconazole>metalaxyl>kitazin. P-solubilized in the liquid medium decreased with the increasing concentrations of fungicides from the recommended to 3X. Maximum reduction of the P-solubilizing activity of the *E. asburiae* strain PS2 in the broth was found to be 67, 89 and 93% over the control when tebuconazole at 100, 200, 300 μg/L, respectively was added to the medium.

In a study, Castagno et al. (2011) isolated fifty isolates from *Lotus tenuis* rhizosphere in the Salado River Basin (Argentina) and through BOX-PCR analysis, 17 non-redundant strains were identified. Subsequently, they were found to be related to *Pantoea*, *Erwinia*, *Pseudomonas*, *Rhizobium* and *Enterobacter* genera, via 16S rRNA gene sequence analysis. All isolates were tested for their phosphate-solubilizing activity and selected strains were inoculated onto *L. tenuis* plants. The most efficient isolate, was identified as *Pantoea eucalypti*, a novel species in terms of plant growth-promoting rhizobacteria. The isolate showed highest phosphate solubilization activity in *in vitro* assay. Under soluble phosphate starvation, isolate M91 (*Pantoea*) was found to produce gluconic acid being this production responsible for medium acidification. Gluconic acid secretion reaches a threshold at 24 h p.i. and decreases at 72 h p.i. highly significant correlation was found between the amount of solubilized phosphate and the pH of the culture media ($r = -0.753$), at 72 h. the correlation value was even higher ($r = -0.956$). In green house study isolates from these genera caused a significant increase in plant height and dry weight of *L. tenuis* cv Pampa INTA plants. The growth promotion resulting from the inoculation with *Pa. eucalypti* strain M91, was dependent on the pH value and the N/P ratio on the media and apparently had no dependency on the indole acetic acid and zeatine production.Virue et al. (2014) demonstrated the effect of inoculation of PSB on growth and yield of maize. In their study, a pot culture experiment was conducted to investigate the effects of seven previously isolated PSB on early development of plants. Seeds were treated with each bacterial strain, and seedlings were harvested 30 days after inoculation. All strains showed a positive effect on plant growth. A significant increment in plant height (45%), shoot dry weight (40%) was determined in plants treated with *Pseudomonas tolaasii* IEXb, while *Pseudomonas koreensis* SP28 has remarkably increased P content compared to the uninoculated control. IEXb strain was selected and evaluated under field conditions in combination with triple superphosphate (TSP) as P fertilizer. The presence of IEXb strain stimulated seedling emergence (8%), shoot length (19%), grain yield (44%), 1000-grain weight (18%), total dry biomass (32%) and P content (56%) of maize plants.

Oteino et al. (2015) isolated endophytic strains of *Pseudomonas*, which can solubilize insoluble phosphate, and stimulate the growth of *Pisum sativum* L. plants. The efficacy of the isolates to solubilize phosphate estimated in the NBRIP

supernatant varied from 85 mg to 1312 mg L^{-1} with the highest solubilization recorded in L228 and L132. The lowest level of solubilized phosphate was recorded in S20 and L111. The culture supernatants were analyzed by HPLC in order to determine if organic acids were produced by the strains. All strains showed production of gluconic acid (GA) with a concentration ranging from 2840 to 33240 ± 230 mg L^{-1} (14–169 mM). Although other minor peaks did appear in the HPLC chromatographs, none of the retention times of these peaks corresponded to those of the other organic acids tested and in all cases represented. Ghosh et al. (2012) isolated six phosphate-solubilizing bacterial strains from the rhizosphere soils of two sea grasses *Halophila ovalis* (R.Br.) Hook and *Halodule pinifolia* (Miki) Hartog in the Vellar estuary. Experimental studies found that the strain PSSG6 was effective in phosphate solubilization with phosphate solubilization efficiency index of 375, followed by the strain PSSG5 with phosphate solubilization efficiency index of 275. Of the 6 strains isolated, the strains PSSG4 and PSSG5 belonged to the genus *Bacillus*, and PSSG1, PSSG2 and PSSG3 were identified as *Citrobacter* sp., *Shigella* sp., and *Klebsiella* sp., respectively and PSSG6 was identified as *Bacillus circulans*.

Genetic manipulation of phosphate-solubilizing bacteria is another way to enhance their ability for plant growth improvement (Rodríguez and Fraga 1999; Rodríguez et al. 2006). The approach may include cloning gene (s) involved in both mineral and organic phosphate solubilization, followed by their expression in selected rhizobacterial strains (Rodríguez et al. 2006). Several attempts have been made to identify and characterize the genes involved for P uptake and its transportation (Rossolini et al. 1998; Shenoy and Kalagudi 2005). Goldstein and Liu (1987) were the first to clone a gene (mps) involved in mineral phosphate solubilization from the Gram negative bacteria *Erwinia herbicola.* Expression of this gene allowed production of GA in *E. Coli* HB101 and conferred the ability to solubilise hydroxyapatite. In another study Babu-Khan et al. (1995) cloned another gene (gabY) involved in GA production and mineral phosphate solubilization from *Pseudomonas cepacia*. Heterologous expression of these genes in agriculturally important bacterial strains would be the next step in programs of improving organic phosphate mineralization in PGPB. The genetic manipulation of bacterial strains to increase their phosphate solubilisation was further reported by Rodríguez et al. (2000). In their study, they constructed the broad host range vector pKT230 and plasmid pMCG898,

which encodes the *Erwinia herbicola* pyrroloquinolinequinone (PQQ) synthase, a gene involved in mineral phosphate solubilization (mps). The construction was transformed and expressed in *Escherichia coli* MC1061, and the recombinant plasmids were transferred to *Burkholderia cepacia* IS-16 and *Pseudomonas sp*. PSS recipient cells by conjugation. Clones containing recombinant plasmids produced higher clearing halos in plates with insoluble phosphate as the unique (P) source. In another work, a bacterial citrate synthase gene was reported to increase exudation of organic acids and P availability to the plant when expressed in tobacco roots (Lo´pez-Bucioet al. 2000). Citrate overproducing plants yielded more leaf and fruit biomass when grown under P-limiting conditions, and required less P-fertilizer to achieve optimal growth. This shows the putative role of organic acid synthesis genes in P uptake in plants. The napA phosphatase gene from the soil bacterium *Morganella morganii* was transferred to *Burkholderia cepacia* IS-16, a strain used as a biofertilizer,using the broad-host range vector pRK293 (Fraga et al. 2001).

Till now the knowledge of the genetics of phosphate solubilization is scanty, some genes involved in mineral and organic phosphate solubilisation have been isolated and characterized. Initial achievements in the manipulation of these genes open a promising perspective for obtaining PGPB strains with enhanced phosphate solubilizing capacity. But, release of genetically modified organisms is controversial. However, studies carried out so far have shown that, genetically modified microorganisms can be applied safely in agriculture (Armarger, 2002; Morrissey et al. 2002). Chromosomal insertion of the genes is one of the tools to avoid horizontal transfer of the introduced genes within the rhizosphere (Rodrı´guezet al. 2006).

2.1.3. Biological Nitrogen Fixation

Nitrogen is an essentially required nutrient for plant development and growth. It is one of the principal plant nutrients, and its low availability due to the high losses by emission or leaching is a limiting factor in agricultural ecosystems, hence bacteria with ability to make atmospheric N available for plants play an important role. The production of chemical fertilizers is a highly energy intensive process using large amounts of fossil energy. Use of excessive amount of manures to achieve high yields has created environmental problems and degradation in natural resources (Sahin et al. 2004). During the past couple of decades, the use of PGPR for sustainable and environment friendly agriculture has been increased tremendously in various parts of

the world (Figueiredo et al. 2008). Increasing and extending the role of bio-fertilizing with PGPR would reduce the need for chemical fertilizers and decrease their adverse environmental effects.

Symbiotic N_2 fixation is the most important mechanism by which most atmospheric N is fixed, but it is limited to legume plant species and various trees and shrubs that form actinorrhizal roots with *Frankia*. This process is carried out in well-defined nodule structures. *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium* and *Mesorhizobium* are the most studied symbiotic bacteria (Zahran, 2001). Rhizobia (species of *Rhizobium, Mesorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium* and *Sinorhizobium*) form intimate symbiotic relationships with legumes by responding chemotactically to flavonoid molecules released as signals by the legume host. These plant compounds induce the expression of nodulation (nod) genes in Rhizobia, which in turn produce lipo-chitooligosaccharide (LCO) signals that trigger mitotic cell division in roots, leading to nodule formation (Dakora, 1995; 2003; Lhuissier et al. 2001; Matiru and Dakora 2004). Co-inoculation of plant growth promoting rhizobacteria (PGPR) with *Bradyrhizobium* has been shown to increase legume nodulation and nitrogen fixation at optimal soil temperatures. Nine rhizobacteria co-inoculated with *Bradyrhizobium japonicum* 532C were tested for their ability to reduce the negative effects of low root zone temperature (RZT) on soybean [*Glycine max* (L.) Merr.] nodulation and nitrogen fixation. At each temperature increased number of nodules formed and the amount of fixed nitrogen also increased when some PGPR strains were co-inoculated with *B. japonicum*, but the stimulatory strains varied with temperatures (Zhang et al. 1995). Rhizobial attachment to roots of asparagus (*Asparagus officinalis* L.), oat (*Avena sativa* L.), rice (*Oryza sativa*), and wheat (*Triticum aestivum*) has also been reported by Terouchi and Syono (1990). Wiehe and Holfich (1995) demonstrated that the strain R39 of *Rhizobium leguminosarum* bv. *trifolii*, multiplied under field conditions in the rhizosphere of host legumes (lupin and pea) as well as non-legumes including corn (*Zea mays*), rape (*Brassica napus* L) and wheat (*Triticum aestivum*). The effect of *Rhizobium leguminosarum* bv. *trifolii* on non-legume plant growth has been reported to be similar to *Pseudomonas fluorescens* as PGPR in its colonization on certain plant roots (Höflich et al. 1994; 1995; Höflich, 2000).

Although the beneficial effects of the symbiotic association of rhizobia with legume plants is known, these bacteria are not considered PGPR, except when associated with non-legume plants (Dobbelaere et al. 2003). Rhizospheric N_2 fixing bacteria have increasingly been used in non legume plants. A range of plant growth promoting rhizobacteria (PGPR) participate in interaction with C3 and C4 plants (e.g., rice, wheat, maize, sugarcane and cotton), and significantly increase their vegetative growth and grain yield (Kennedy et al. 2004). Some important nonsymbiotic nitrogen-fixing bacteria include, *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum*, *Rhodopseudomonas* and *Xanthobacter (*Saxena and Tilak, 1998; Ardakani et al. 2010). Various crops in India have been inoculated with diazotrophs particularly *Azotobacter* and *Azospirillum* (Saxena and Tilak, 1999; Tilak and Saxena, 2001; Bashan and de-Bashan, 2010). In a study Malik et al. (1997) reported that five strains namely *Azospirillum lipoferum* N-4, *Azospirillum brasilense* Wb-3, *Azoarcus* K-1, *Pseudomonas* 96-51, *Zoogloea* Ky-1 were used to inoculate two rice varieties i.e. NIAB-6 and BAS-370 under aseptic laboratory conditions. The nitrogen fixed was quantified using the ^{15}N isotopic dilution methods. Variety BAS-370 had nearly 70% nitrogen derived from atmosphere when inoculated with *Azospirillum* N-4. Similar studies with the mixed inoculum using ^{15}N fertilizer in the micro plots indicated that nearly 29% of plant nitrogen was derived from the atmosphere. Yield of rice (Yanni and El- Fattah 1999), cotton (Anjum et al. 2007), and wheat (Soliman et al. 1995; Hegazi et al. 1998; Barassi et al. 2000) increased with the application of *Azotobacter*. In contrast to *Azotobacter*, Clostridia are obligatory anaerobic heterotrophs only capable of fixing N_2 in the complete absence of oxygen (Kennedy and Tchan, 1992; Kennedy et al. 2004). N-fixation is the first mechanism suggested to promote the growth of plants by *Azospirillum*. This increase in yield is ascribed mainly to an improvement in root development by an increase in water and mineral uptake, and to a lesser extent biological N_2-fixation (Okon and Labandera-Gonzalez 1994; Okon and Itzigsohn, 1995). The majority of evidence collected during the last 3 decades concerning this mechanism has generated controversy (Bashan et al. 2004). At the same time, *Azospirillum* lead the list of PGPR assessed in worldwide experiments (Burdman et al. 2000; Vessey 2003; Lucy et al. 2004; Ramirez and Mellado, 2005). Beneficial effects of inoculation with *Azospirillum* on wheat yields in both

greenhouse and field conditions have been reported (Hegazi et al. 1998; El Mohandes, 1999; Ganguly et al. 1999). *Azospirillum* species are aerobic heterotrophs that fix N_2 under microaerobic conditions (Roper and Ladha, 1995) and grow extensively in the rhizosphere of gramineous plants (Kennedy and Tchan, 1992; Kennedy et al. 2004). A field experiment was conducted to evaluate the suitable combination of plant growth promoting rhizobacteria (*Azospirillum* biofertilizer strain BM9 and BM11) along with different nitrogenous fertilizer levels (0, 20, 40, 60, 80 and 100% N) on rice variety Binadhan 4. It has been observed that, a significant increase in growth parameter like plant height, shoot dry weight, root length and dry weights, grain and straw yields, effective tillers/hill and panicle length, and nitrogen, phosphorus and potassium uptake over control under most nitrogen levels (Islam et al. 2012).

Studies confirmed that up to 60–80% of sugarcane plant N (equivalent to over 200 kg N $ha^{-1}year^{-1}$) as derived from BNF and *Azospirillum diazotrophicus* is apparently responsible for much of this BNF (Boddey et al. 1991). The *Acetobacter*-sugarcane system has now become an effective experimental model and the diazotrophic character (nif^{+}) is important component of this system (Lee et al. 2002). Reinhold-Hurek et al. (1993) reported a strain of the endophytic Gram-negative N_2-fixing bacterium *Azoarcus* sp. BH72, originally isolated from Kallar grass (*Leptochloa fusca* (L.) Kunth) growing in the saline-sodic soils typical of Pakistan. *Azoarcus* spp. also colonise grasses, such as rice, in both laboratory and field conditions (Hurek et al. 1994). The genus *Burkholderia* include 67 validly published species, with several of these including *Burkholderia vietnamiensis, B. kururiensis, B. tuberum and B. phynatum* being capable of fixing N_2 (Estrada-delos Station et al. 2001; Vandamme et al. 2002). Tran Vân et al. (2000) reported that inoculation of rice with *B. vietnamiensis,* in a field trial, increased grain yields significantly up to 8 ton ha^{-1}. In field trials, this strain was found capable of saving 25–30 kg N ha^{-1} of fertilizer. *Pseudomonas* and *Bacillus* species (Alam et al. 2001; Cakmakci et al. 2001; Kokalis-Burelle et al. 2002), and the other PGPR and endophytic bacteria, such as *Enterobacter, Klebsiella, Burkholderia,* and *Stenotrophomonas,* have been gaining attention in the recent years, because of their association with important crops and potential to enhance the plant growth (Chelius and Triplett, 2000; Sturz et al. 2001; Dong et al. 2003; Ramirez and Mellado, 2005). N-fixing bacterial strains *Pseudomonas putida* RC06, *Paenibacillus polymyxa* RC05 and RC14, and *Bacillus*

OSU-142 have great potential, and as formulations, they are used as biofertilizers for better yield and the quality of wheat, sugar beet, and spinach growth (Cakmakci et al. 2006; Cakmakci et al. 2007).

2.2. Biological Control of Plant Pathogens

Phytopathogens have a great impact on crop yields. They can reduce the performance of plant and crop quality. Plant-growth promoting rhizobacteria (PGPRs) have been used as good biocontrol agents against soil borne pathogens. Potential biocontrol agents produce antibiotics, siderophore that chelate iron, making it unavailable to pathogens; the ability to synthesize anti-fungal metabolites that cause disease suppression and production of fungal cell wall-lysing enzymes, or hydrogen cyanide, which suppress the growth of fungal pathogens; the ability to successfully compete with pathogens for nutrients or specific niches on the root increase yield of plants. The biological control that results from PGPR are reported to be caused by several mechanisms such as competition, antibiosis, and induced systemic resistance (Kloepper et al. 1980).

2.2.1. Production of Lytic Enzymes

Production of lytic enzymes is one of the most important mechanisms that PGPR use for the control of plant pathogens and thus indirectly promoting the growth of plants. These microbially synthesized enzymatic compounds include defence enzymes, such as chitinase, β-1, 3-glucanase, peroxidase, protease and lipase (Bashan and de-Bashan, 2005; Karthikeyan et al. 2006). Chitinase and β-1, 3-glucanase degrade the fungal cell wall and cause lysis of fungal cell. During the degradation of fungal cell wall chitin and glucan oligomers released, which act as elicitors that elicit various defence mechanisms in plants.

Frankowski et al. (2001) reported that chitinase producing *Serretia plymuthica* C48 inhibited spore germination and germ-tube elongation in *Botrytis cinerea.* Singh et al. (1999) reported the crucial role of extracellular chitinase in suppression of *Fusarium* wilt of cucumber (*Cucumis sativus*) caused by *F. oxysporum f. sp. cucumerinum.* The two chitinolytic strains *Paenibacillus* sp. 300 and *Streptomyces* sp. 385 when used in ratio of 1:1 or 4:1 gave significantly ($P < 0.05$) better control of the disease than each of the strains used individually or than mixtures in other ratios. The SDS analysis revealed the presence of five bands with molecular masses of 65, 62, 59,

55, and 52 kDa in the case of *Paenibacillus* sp. 300; and three bands with molecular masses of 52, 38, and 33 kDa in the case of *Streptomyces* sp. 385. Bashan and de-Bashan (2005) reported that, *Pseudomonas stutzeri*, capable of producing lytic enzymes, have demonstrated the lysis of the pathogen *Fusarium* sp. Numerous species of *Bacillus* had been reported to synthesize chitinase and act as biocontrol agents since they display antagonism against a wide range of plant pathogens that infect several economically important crops (Collins et al. 2003). Solanki et al. (2012) characterized mycolytic enzymes like chitinase, β-1, 3-glucanase and protease from four *Bacillus* strains and they showed elevated defence response during *R. solani* suppression in terms of chitinase, glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase activity and total phenolic content in leaves of tomato under greenhouse conditions. They reported that, *Bacillus megaterium* MB3, *B. subtilis* MB14, *B. subtilis* MB99 and *B. amyloliquefaciens* MB101 were able to produce chitinase, β-1, 3-glucanase and protease in different range with the presence of *Rhizoctonia solani* cell wall as a carbon source. The ability of production of chitinase and β-1, 3-glucanase was also supported by molecular evidence. PCR amplification of chitinase (chiA) gene of 270 bp and β-1, 3-glucanase gene of 415 bp was given supportive evidence antibiosis. Venkadesaperumal et al. (2014) have isolated hydrolytic enzymes producing *Pantoea agglomerans* OM5 and *Exiguobacterium* sp. EM9 from isolated from mud volcano and lime cave of Andaman and Nicobar Islands. Zhang et al. (2014) reported β-1, 3-glucanase mediated inhibition of sapstain fungi. They isolated Sixty-three strains of bacteria from different plants, but 19 strains showed antagonistic activity to four poplar discoloration fungi, viz. *Ceratocystis adiposa* Hz91, *Lasiodiplodia theobromae* YM0737, *L. theobromae* Fx46, and *Fusarium sp*. YM05. *Bacillus* and *Pseudomonas* were the predominant genus, and *Bacillus subtilis* and *B. amyloliquefaciens* were the dominant species in 13 strains. The strains B82 (*B. amyloliquefaciens*) and B37 (*B. subtilis*) showed strongest antagonistic activity against all the pathogens. The strain B37 produced a significant amount of β-1, 3-glucanase, effectively inhibiting the growth of four poplar sapstain fungi. It could produce a small amount of proteases, but chitinase was not found. Recently, Bhattacharya et al. (2016) reported the chitinase production by *Bacillus pumilus* JUBCH08. They have optimized the chitinase production from the isolate. The chitinase was found to be thermostable and alkali-tolerant with maximum activity at pH 8.0 and 70°C for 1 h. The molecular

weight of chitinase was found to be 64 kDa. In dual plate assay, the bacterium showed 45% antagonism against *F. oxysporum*.

2.2.2. Antibiotic production

The production of antibiotics is considered one of the most powerful and well-studied biocontrol mechanisms for combating phytopathogens (Martinez-Viveros et al. 2010). The term, antibiotics constitute a wide and heterogeneous group of low molecular weight chemical organic compounds that are produced by a wide variety of microorganisms (Raaijmakers et al. 2002). Under laboratory conditions many different types of antibiotics produced by PGPR have shown to be effective against effective against phytopathogenic agents (Bowen and Rovira, 1999). Some PGPR synthesize antifungal antibiotics, e.g. *P. fluorescens* produces 2, 4-diacetyl phloroglucinol which inhibits growth of phytopathogenic fungi (Nowak-Thompson et al. 1994). To date a number of antibiotic compounds have been characterized chemically and include N-containing heterocycles such as phenazines (Brisbane et al. 1989; Gerber, 1969), pyrrole type antibiotics (Hashimoto and Hattori, 1996). The list of the antibiotics produced by PGPR include: butyrolactones, zwittermycin A, kanosamine, oligomycin A, oomycin A, phenazine-1-carboxylic acid, pyoluteorin, pyrrolnitrin, viscosinamide, xanthobaccin, and 2, 4-diacetyl phloroglucinol (2, 4-DAPG) (Whipps, 2001). The last is one of the most efficient antibiotics in the control of plant pathogens (Fernando et al. 2006) and can be produced by various strains of *Pseudomonas,* one of the most common bacterial species of the rhizosphere (Rezzonico et al. 2007). The 2, 4-DAPG has a wide spectrum of properties in that it is antifungal (Loper and Gross, 2007; Rezzonico et al. 2007), antibacterial (Velusamy et al. 2006) and antihelmintic (Cronin et al. 1997). Someya et al. (2003) reported that *Serratia marcescens* strain B2 is an antagonistic bacterium that produces the red-pigmented antibiotic prodigiosin and suppresses rice sheath blight caused by *Rhizoctonia solani* AG-1 IA. Rice sheath blight disease was suppressed when plants were inoculated with this bacterium an hour before pathogen inoculation but not when plants were treated 4 weeks before pathogen inoculation. Bacteria isolated from rice plants and rhizosphere mediate the suppression of antibiotic production of biological control agents and that such suppression is common under field conditions.

2.2.3. HCN Production

Some rhizobacteria are capable of producing HCN (hydrogen cyanide, also known as cyanide) (Rezzonico et al. 2007). It is a volatile, secondary metabolite that suppresses the development of microorganisms and that also affects negatively the growth and development of plants (Siddiqui et al. 2006). Cyanide is toxic to plants capable of disrupting enzyme activity involved in major metabolic processes, its role as a biocontrol substance is overwhelming (Voisard et al. 1989; Devi et al. 2007). Hydrogen cyanide (HCN) among cyanogenic compounds effectively blocks the cytochromeoxidase pathway and is highly toxic to all aerobic microorganisms at very low concentrations. However, the microbes, which produce the compound, mainly pseudomonads, are reported to be resistant (Bashan and de-Bashan, 2005). HCN is formed from glycine through the action of HCN synthetase enzyme, which is a membrane-bound flavoenzyme that oxidizes glycine, producing HCN and CO_2. HCN does not appear to have a role in primary metabolism and is generally considered a secondary metabolite (Blumerand Haas, 2000). To date many different bacterial genera have shown to be capable of producing HCN, including species of *Alcaligenes, Aeromonas, Bacillus, Pseudomonas* and *Rhizobium* (Devi et al. 2007; Ahmad et al. 2008). HCN production was more common trait of *Pseudomonas* (88.89%) and *Bacillus* (50%) (Ahmad et al. 2010). Chandra et al. (2007) reported that the rhizosphere competent *Mesorhizobium loti* MP6 also produced HCN under normal growth conditions and enhanced the growth of Indian mustard (*Brassica campestris*). Wani et al. (2007) tested the rhizospheric isolates for HCN producing ability *in vitro* to find that most of the isolates produced HCN and helped in the plant growth. The psychrotolerant bacterium *Pseudomonas fragi* CS11RH1 (MTCC 8984), was reported to produce hydrogen cyanide (HCN) and the seed inoculation with the isolate significantly increased the percentage germination, rate of germination, plant biomass and nutrient uptake of wheat seedlings (Selvakumar et al. 2009). Pathma et al. (2011) reported that, fluorescent pseudomonas are capable of biological control phytopathogens by the production of HCN. Various studies attribute a disease protective effect to HCN, e.g. in the suppression of "root-knot" and black rotin tomato and tobacco root caused by the nematodes *Meloidogyne javanica* and *Thielaviopsis basicola*, respectively (Voisard et al. 1989; Siddiqui et al. 2006). The subterranean termite *Odontotermes obesus*, an important pest in agricultural and

forestry crops in India, is also controlled by HCN (Devi et al. 2007). However, there are investigations reporting harmful effects on plants, inhibition of energy metabolism of potato root cells (Bakker and Schippers, 1987), and reduced root growth in lettuce (Alström and Burns, 1989). Likewise, HCN produced by *Pseudomonas* in the rhizosphere inhibits the primary growth of roots in *Arabidopsis* due to the suppression of an auxin responsive gene (Rudrappa et al. 2008). Sandhya et al. (2010) isolated and screened drought tolerant, HCN producing *Pseudomonas* isolates from arid and semiarid crop production systems of India.

2.2.4. Siderophore Production

Iron is essential for almost all form of life, essential for processes such as respiration and DNA synthesis. Despite being one of the most abundant elements in the Earth's crust, the bioavailability of iron in the soil is limited by the very low solubility of the Fe^{3+} ion. This is the predominant state of iron in aqueous, non-acidic, oxygenated environments. It accumulates in common mineral phases such as iron oxides and hydroxides (the minerals that are responsible for red and yellow soil colours) hence cannot be readily utilized by organisms (Kraemer, 2005) 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 et al. 2007), although several are biosynthesised independently (Challis, 2005).

Under iron-limiting conditions PGPR produce low-molecular-weight compounds called siderophores to competitively acquire ferric ion (Whipps, 2001). PGPR deprive pathogenic fungi of this essential element since the fungal siderophores have lower affinity (O'Sullivan, 1992; Loperet al. 1999). Siderophore producing PGPR can prevent the proliferation of pathogenic microorganisms by sequestering Fe^{3+} in the rhizosphere (Siddiqui, 2006). Fe depletion in the rhizosphere does not affect the plant, as the low Fe concentrations occur at microsites of high microbial activity during establishment of the pathogen.

Various workers have isolated siderophore producing bacteria belonging to the *Bacillus* sp. (Chakraborty et al. 2006), *Bradyrhizobium* (Khandelwal et al. 2002), *Ochrobactrum anthropi* (Chakraborty et al. 2009), *Pseudomonas* (Boopathi and Rao, 1999), *Serratia* (Kuffner et al. 2008; Chakraborty et al. 2010) and *Streptomyces*

(Kuffner et al. 2008) which could promote growth of crop plants and suppress disease. However, the growth promotion that occurred may be due to other mechanisms or combinations of mechanisms that increase nutrient availability, suppress pathogens, or affect root growth via hormone production.

Arora et al. (2001) reported that two siderophore-producing strains (RMP3 and RMP5) of *Rhizobium meliloti* isolated from the rhizosphere of the medicinal plant *Mucuna pruriens* were able to inhibit a widely occurring plant pathogen, *Macrophomina phaseolina* that causes charcoal rot in groundnut. Further, there was a marked enhancement in percentage seed germination, seedling biomass, nodule number and nodule fresh weight of *M. phaseolina*-infected groundnut plants inoculated with the strains RMP3 and RMP5, compared to uninoculated and uninfected controls. In another study Sharma and Johri (2004) inoculated maize seeds with siderophore-producing pseudomonads with the goal to develop a system suitable for better iron uptake under iron-stressed conditions. Siderophore production was compared in fluorescent *Pseudomonas* spp. GRP3A, PRS_9 and *P. chlororaphis* ATCC 9446 in standard succinate (SSM) and citrate (SCM) media. Succinate was better suited for siderophore production. Maximum siderophore level (216.23 μg/ml) was observed in strain PRS_9 in deferrated SSM after 72 h of incubation. Bacterization of maize seeds with strains GRP3A and PRS_9 showed significant increase in germination percentage and plant growth. Maximum shoot and root length and dry weight were observed with 10 μM Fe^{3+} along with bacterial inoculants suggesting application of siderophore producing plant growth promoting rhizobacterial strains in crop productivity in calcareous soil system. Wheat isolate *Acinetobacter calcoaceticus* (SCW1), a strong catechol type siderophore producer improved the plant growth in pot as well as field condition. Siderophore mediated antagonism was observed against common phytopathogens viz., *Aspergillus flavus, A. niger, Colletotrichum capsicum* and *Fusarium oxysporum* (Sarode et al. 2008). Sinha and Mukherjee (2008) reported the Cd tolerant *Pseudomonas aeruginosa* can promote the growth of stimulated the growth of mustard and pumpkin plants in Cd-added soil. The bacterial strain could tolerate up to 8 mM of Cd and could accumulate Cd intracellularly. The strain showed Cd-induced siderophore production maximally at 1.75 mM of Cd concentration under culture condition. In another study Sayyed and Chincholkar (2009) reported the *in vitro* phytopathogen suppression activity of siderophoregenic preparations of

Alcaligenes feacalis. Siderophore-rich culture broth, siderophore rich supernatant, and purified siderophore preparation exerted antifungal activity against *Aspergillus niger* NCIM 1025, *A. flavus* NCIM 650, *Fusarium oxysporum* NCIM 1008, and *Alternaria alternata* IARI 715. Siderophore-rich broth showed potent antifungal activity among all the preparations. *Alcaligenes faecalis* isolated from ground nut rhizosphere enhanced the growth of same plant by providing Fe nutrition to plants. The strain may be exploited as sulphur biofertilizer besides its applicability as a bioinoculant for Fe nutrition (Sayyed et al. 2010). Wahyudi et al. (2011) isolated 12 strains *Bacillus* species, capable of producing siderophore, from the rhizosphere of soybean plant. Furthermore, 3 isolates (25%) among them were able to inhibit the growth of *Fusarium oxysporum*, 9 isolates (75%) inhibited the growth of *Rhizoctonia solani*, and 1 isolate (8.3%) of *Bacillus sp*. inhibited the growth of *Sclerotium rolfsii*.

2.2.5. Induced systemic resistance

According to van Loon et al. (1998) nonpathogenic rhizobacteria can induce a systemic resistance (ISR) in plants that is phenotypically similar to pathogen-induced systemic acquired resistance (SAR). SAR develops when plants successfully trigger their defence mechanism following an earlier localized exposure to a pathogen. When SAR induces a hypersensitive reaction through which it becomes limited in a local necrotic lesion of brown, desiccated tissue (van Loon et al. 1998). Though both SAR, ISR is effective against different types of pathogens but differs from SAR in that the induction by PGPB does not cause visible symptoms on the host plant (van Loon et al. 1998). Bacterial determinants of ISR include lipopolysaccharides, siderophores, and salicylic acid (SA). Whereas some of the rhizobacteria induce resistance through the SA-dependent SAR pathway others prefer jasmonic acid and ethylene perception by the plant for ISR to develop. ISR offers a natural mechanism for biological control of plant disease. It has been hypothesised that the inducing rhizobacteria in the plant roots provoke a signal, which spreads systemically within the plant and increases the defensive competence of the distant tissues from the subsequent infection by the pathogens. ISR thus extended the protective action of PGPR from their antagonistic activity against soil-borne pathogens in the rhizosphere to a defence-stimulating effect above the surface of the ground tissues against foliar pathogens (van Loon and Bakker, 2006).

PGPB-elicited ISR was first observed on carnation (*Dianthus caryophillus*) with reduced susceptibility to wilt caused by *Fusarium* sp. The strain, *P. fluorescens* WCS417, active against *Fusarium oxysporum* f. sp. dianthi was tested on carnation and results showed that bacteria, while remaining confined to the plant root system, were still protective when the pathogen was slash-inoculated into the stem (Van Peer et al. 1991) and on cucumber (*Cucumis sativus*) with reduced susceptibility to foliar disease caused by *Colletotrichum orbiculare* (Wei et al. 1991). In another study several strains of PGPR, which applied to roots of cucumber, and the leaves were subsequently challenged inoculation with the anthracnose fungus *Colletotrichum orbiculare* (Gang et al. 1991). Appearance of ISR is dependent on the combination of host plant and bacterial strain (Kilic-Ekici and Yuen, 2004; van Loon et al. 1998). Most reports of PGPB-mediated ISR involve free-living rhizobacterial strains, but endophytic bacteria have also been observed to have ISR activity. For example, ISR was triggered by *P. fluorescens* EP1 against red rot caused by *Colletotrichum falcatum* on sugarcane (Viswanathan and Samiyappan, 1999), *Burkholderia phytofirmans* PsJN against *Botrytis cinerea* on grapevine (Barka et al. 2000 and 2003) and *Verticllium dahlia* on tomato (Sharma and Nowak, 1998), *P. denitrificans* 1-15 and *P. putida* 5-48 against *Ceratocystis fagacearum* on oak (Brooks et al. 1994), *P. fluorescens* 63-28 against *F. oxysporum* f. sp. *radicis-lycopersici* on tomato (M'Piga et al. 1997) and *Pythium ultimum* and *F. oxysporum* f. sp. *pisi*on pea roots (Benhamou et al. 1996a), and *Bacillus pumilus* E34 against *F. oxysporum* f. sp. *pisi*on pea roots (Benhamou et al. 1996b) and *F. Oxysporum* f. sp. *Vasinfectum* on cotton roots (Conn et al.1997).

Braun-Kiewnick et al. (2000) reported that strains of *Pantoea agglomerans* suppressed the development of basal kernel blight of barley, caused by *Pseudomonas syringae* pv. *syringae*, when applied to heads prior to the *Pseudomonas syringae* pv. *syringae* infection window at the soft dough stage of kernel development. Field experiments in 1994 and 1995 revealed 45 to 74% kernel blight disease reduction, whereas glasshouse studies resulted in 50 to 100% disease control depending on the isolate used and barley cultivar screened. The efficacy of biocontrol strains was affected by time and rate of application. Percentage of kernels infected decreased significantly when *P. agglomerans* was applied before pathogen inoculation, but not when co-inoculated. A single *P. agglomerans* application 3 days prior to the pathogen

inoculation was sufficient to provide control since populations of about 10^7 *CFU* per kernel were established consistently, while *Pseudomonas syringae pv. syringae* populations dropped 100-fold to 2.0 × 10(^4) CFU per kernel. An application to the flag leaf at EC 49 (before heading) also reduced kernel infection percentages significantly. Basal blight decreased with increasing concentrations (10(^3) to 10(^7) CFU ml^{-1}) of *P. agglomerans*, with 10(^7) CFU/ml providing the best control. For long-term preservation and marketability, the survival of bacterial antagonists in several wettable powder formulations was tested. Over all formulations tested, the survival declined between 10- to >100-fold over a period of 1.5 years ($r = –0.7$; $P = 0.000$). Although not significant, storage of most formulations at 4°C was better for viability (90 to 93% survival) than was storage at 22°C (73 to 79%). However, long-term preservation had no adverse effect on biocontrol efficacy. *Pantoea agglomerans* PTA-AF1 mediated resistance to *Botrytis cinerea* in grape plants has been reported by Trotel-Aziz et al. (2008). In this study, results from the *in vitro* antifungal experiments revealed that among the seven identified bacteria, only *P. agglomerans* PTA-AF1 and *P. fluorescens* PTACT2 displayed important zones of inhibition, which remained constant over time. This indicates the production by these two strains of antifungal metabolites active against *B. cinerea*. Pusey et al. (2008) reported the role of *P. agglomerans* E325 in the suppression of *Erwinia amylovora* on flower stigmas of apple flower. *P. agglomerans* strain E325 exhibited a capacity to reduce the pH on stigmas to levels that could reduce growth of *E. amylovora*. Conversely, an increase in pH was observed on flowers inoculated with *E. amylovora* alone. A similar trend was indicated in the field on the final sampling date, with mean pH values numerically higher for pathogen-inoculated flowers and lower for the antagonist-inoculated flowers.

Ramamoorthy and Samiyappan (2001) tested the efficacy of various *P. fluorescens* isolates for the management of fruit rot of chilli caused by *Colletotrichum capsici*. Among the various isolates tested *P. fluorescens* isolates *viz.* Pf1 and ATR increased the plant growth and produced the maximum amount of indole acetic acid. *P. fluorescens* Pf1 effectively inhibited the mycelial growth of the pathogen under *in vitro* conditions and decreased the fruit rot incidence under greenhouse condition. Seed treatment plus soil application of talc based formulation of *P. fluorescens* isolate Pf1 effectively reduced the disease incidence. Induction of systemic resistance against

C. capsici infection was mediated through the expression of various defense related enzymes and chemicals. Shternshis et al. (2002) tested three commercially available products based on compounds of biological origin for their ability to control the raspberry midge blight in the Siberian region of Russia. *Bacillus thuringiensis* sub sp. *israelensis* (Bacticide) and *Streptomyces avermitilis* metabolites (Phytovern) were used against *Thomasiniana theobaldi* (a general member of the midge blight) and chitinase was used against fungi (mainly *Didymella applanata*) associated with *T. theobaldi*. The Bacticide (0.2%) and Phytoverm (0.2%) sprays caused a two fold decrease in midge blight severity and the same type of result was obtained with chemical insecticides. Four fold decrease in the severity of midge blight observed when sprayed with 1% chitin. In addition, Chitinase and Phytovem caused a significant suppression of the independent spur blight. Bansal et al. (2003) tested the efficacy of *Azotobacter chroococcum* against tomato wilt pathogen (*Fusarium oxysporum* f. sp. *lycopersici*) during rabi 2000-01 and 2001-02 in pot house under artificial inoculum conditions. Tomato seedlings var. local, treated with *A. chroococcum* before transplanting along with soil application of nitrogen @ 60, 80 and 100 kg ha^{-1} showed complete inhibition of plant mortality (7.36%) was also observed when seedlings were treated with *A. chroococcum* only as compared to the seedlings without any treatment (17.35%). It may be attributed to the production of antifungal substances by *A. chroococcum*. Zhang et al. (2004) studied the effect of plant growth-promoting rhizobacteria on plant growth and systemic protection against blue mold disease of tobacco (*Nicotiana tabacum* L.), caused by *Peronospora tabacina*, using five PGPR strains with known plant growth promotion and induced resistance activities in other crops. PGPR strains were applied as seed treatments alone at planting and in combination with root drenches after planting. When PGPR were applied as seed treatments, PGPR strains 90-166, SE34 and C-9 at 10^9 CFU mL^{-1} increased all or most parameters of plant growth 7 weeks after planting (WAP), while 89B-61 and T4 did not enhance any or few parameters. Seed treatments with PGPR strains 90-166 and C-9 at 10^9 CFU mL^{-1} at 13 WAP resulted in significant disease reduction in blue mold severity compared to the non treated control. When PGPR were applied as seed treatments and root drenches, all PGPR strains at 10^9 CFU mL^{-1} enhanced tobacco growth compared to the non-treated control. The time interval between the last PGPR treatment and challenge with *P. tabacina* affected systemic disease protection elicited by some PGPR strains. When the time interval

was 8 weeks, 3 PGPR strains 90-166, SE34 and T4 at 10^9 CFU mL^{-1} reduced disease severity, while treatments with all tested PGPR strains resulted in significantly lower disease compared to the non treated control when it was reduced to 6 weeks. Inoculation of *Pseudomonas fluorescens* isolates PGPR1, PGPR2 and PGPR4 reduced the seedling mortality caused by *Aspergillus niger* (Dey et al. 2004).

Bhatia et al. (2003) observed maximum colony growth inhibition due to *Pseudomonas* PS 2 (74%) as compared to PS 1 (71%) on trypticase soy agar (TSM) plates after 5 days of incubation. Both the light and scanning electron microscope examination showed hyphal coiling, vacuolation and granulation of cytoplasm resulting in lysis of hyphae of *Macrophomina phaseolina* by pseudomonads isolates. They also evaluated the effect of cell free culture filtrates of strains PS1 and PS 2. The cell free culture filtrate of PS 1 and PS 2 at 20% concentration restricted the growth of mycelium of *M. phaseolina* colony by 57 and 61% respectively after 4 days of incubation. Volatile substances produced by PS 1 and PS 2 also inhibited the colony growth of *M. phaseolina* by 25 and 32% respectively. Colony growth of *M. phaseolina* was significantly decreased by PS 1 and PS 2 as compared to control both in iron sufficient and iron deficient conditions. PS 2 showed higher antagonistic activity than PS 1, as evidenced by pronounced colony growth inhibition.

Saravanakumar et al. (2005) evaluated fluorescent pseudomonads based bioformulation for their ability to control *Macrophomina* root rot disease in mung bean (*Vigna mungo*). Under *in vitro* condition isolate Pf1 showed the maximum inhibition in mycelial growth of *Macrophomina phaseolina*. Bioformulation of Pf1 with chitin was effective in reducing the root rot incidence in green gram both under glasshouse and field conditions. The rhizosphere colonization of *P. fluorescens* was observed appreciable with the green gram plants. However, amendment of Pf1 with chitin showed maximum colonization. Furthermore, the application of Pf1 amended with or without chitin and neem induced the accumulation of defence enzymes in host plant. Increased accumulation of phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), chitinase, β-1, 3-glucanse and phenolics were observed in Pf1 bioformulation amended with chitin, pre-treated plants challenge inoculated with *M. phaseolina* under glasshouse conditions. The study revealed that in addition to direct antagonism and plant-growth promotion, PGPR strains amended with chitin bioformulation induced defence-related enzymes

and pathogenesis related (PR) proteins which collectively enhance the resistance in green gram against the infection of *M. phaseolina*.

Bhatia et al. (2005) isolated ten isolates of fluorescent *pseudomonads* from rhizosphere of sunflower, potato, maize and groundnut. Out of the ten strains, *Pseudomonas* PS I and PS II was found most potential. Bacterisation of sunflower seeds with fluorescent *Pseudomonas* PS I and PS II resulted in increased seed germination, root length, shoot length, fresh and dry weight of roots, shoots and yield of sunflower. Seed bacterisation with strains of fluorescent *Pseudomonas* PS I and PS II reduced incidence of collar rot by 69.8% and 56.9% respectively, in *Sclerotium rolfsii*-infested soil, making the organism a potential biocontrol agent against collar rot of the sunflower. Trivedi et al. (2006) isolated *Pseudomonas corrugata* from temperate site of Indian Himalayan Region and examined the antagonistic activities against two phytopathogenic fungi, *Alternaria alternata* and *Fusarium oxysporum*. Although the bacterium did not show inhibition zones due to production of diffusible antifungal metabolites, a reduction in growth between 58% and 49% in both test fungi, *A. alternata* and *F. oxysporum*, was observed in sealed petri plates after 120 h of incubation due to production of volatile antifungal metabolites. Reduction in biomass of *A. alternata* (93.8) and *F. oxysporum* (76.9) in Kings B broth was recorded after 48 h of incubation in dual culture. The antagonism was observed to be affected by growth medium, pH and temperature. The reduction in fungal biomass due to antagonism of bacteria was recorded maximum in the middle of the stationary phase after 21 h of inoculation. The production of siderophore, ammonia, lipase and chitinase in growth medium by *P. corrugata* were considered contributing to the antagonistic activities of the bacterium. Inoculation with *Serratia marcescens* (90-166) induced systemic protection in the aerial parts of cucumber plants against anthracnose pathogen- *Colletotrichum orbiculare*. In green house experiments, *Serratia marcescens* NBR11213 was evaluated for plant growth promotion and biological control of foot and root rot of betelvine caused by *Phytophthora nicotianae* (Lavania et al. 2006). The combined use of PGPR (*Bacillus cereus* strain BS 03 and a *Pseudomonas aeruginosa* strain RRLJ 04) and rhizobia (strain RH 2) were recommended for induction of systemic resistance against fusarial wilt (*Fusarium udum*) in pigeon pea (Dutta et al. 2008). Choudhary et al. (2007) also described induced resistance and its mechanism of action in plants. Plants have the ability to

acquire enhanced level of resistance to pathogens after exposure to biotic stimuli provided by many different PGPRs. Recently, research on mechanisms of biological control by PGPR revealed that several PGPR strains protect plants against pathogen infection through induction of systemic resistance, without provoking any symptoms themselves. Trivedi and Pandey (2008) characterized *Bacillus megaterium* strain B388, isolated from rhizosphere soil of pine of temperate Himalayan region. The plant growth promotion by the bacterium has been evaluated through petridish and broth based assays. The isolate solubilized tricalcium phosphate under *in vitro* conditions; maximum activity (166 µg/ml) was recorded at 28°C after 15 days of incubation. Production of indole acetic acid demonstrated in broth assays was another important plant growth promoting character. The bacterium produced diffusible and volatile compounds that inhibited the growth of two phytopathogens viz. *Alternaria alternata* and *Fusarium oxysporum*. The carrier based formulations of the bacterium resulted in increased plant growth in bioassays. The rhizosphere colonization and the viability of the cells entrapped in alginate beads were greater in comparison to coal or broth based formulations. PGPR mediated induction of ISR has been reported for several other plant-pathogen systems (Choudhary and Johri, 2009). PGPR have attracted much attention in their role in reducing plant diseases. Although their full potential has not yet been reached, the work to date is very promising.

Reddy et al. (2008) obtained ten isolates of *Pseudomonas fluorescens* from rice rhizosphere and these were tested for antifungal activity against *Magnaporthe grisea*, *Dreschelaria oryzae*, *Rhizoctonia solani* and *Sarocladium oryzae* that are known to attack rice plants. One isolate, *P. fluorescens* 8 effectively inhibited mycelial growth in all these fungi in dual culture tests (50-85%). All the ten isolates of *P. fluorescens* were further tested for the production of siderophore, hydrogen cyanide and salicylic acid. The isolate *P. fluorescens* 8 showed higher production of siderophore, HCN and salicylic acid.

Some PGPR, especially if they are inoculated on seeds before planting, are able to establish themselves on crop roots. They use scarce resources, and thereby prevent or limit the growth of pathogenic microorganisms (Hayat et al. 2010). The *Macrophomina phaseolina* (Tassi) Goid. is the causal agent of charcoal root rot, a devastating pathogen affecting agricultural and forest crops (Shaner et al. 1999), with more than 500 susceptible hosts world wide (Wyllie et al. 1984). Govindappa et al.

(2010) reported that seed treatment with *Trichoderma harizianum* and *Pseudomonas fluorescens* enhanced the seed germination and growth parameters against root-rot disease caused by *Macrophomina phaseolina* and they also induced systemic resistance and/or physiological changes leading to plant defence mechanisms. Singh et al. (2010) isolated ten strains of *Pseudomonas aeruginosa* (PN1 - PN10) from rhizosphere of chir-pine and these were tested for their plant growth promontory properties and antagonistic activities against *Macrophomina phaseolina in vitro* and *in vivo*. The strain PN1 produced siderophore, IAA, cyanogen and solubilized phosphorus, besides producing chitinase and β-1, 3-glucanase. In dual culture, the isolate PN1 caused 69% colony growth inhibition. However, cell free culture filtrate also posed inhibitory effect but to a lesser extent. In pot experiment *P. aeruginosa* PN1 increased plant growth and biomass after 90 days of growth in *M. phaseolina*-infested soil. PN1 showed the strong chemotaxis toward root exudates resulting in effective root colonization. Moreover, increased population in rhizosphere of these bacteria was also recorded after 90 days of treatment. Thus, chemotactic fluorescent *P. aeruginosa* PN1 exhibited strong antagonistic property against *M. phaseolina*, suppressed the disease and improved plant growth of the seedlings of chir-pine proving potential biocontrol agent. Saxena (2010) reported the antagonistic activity of *Pseudomonas fluorescens* BAM-4, *Burkholderia cepacia* BAM-6 and *B. cepacia* BAM-12 isolated from the rhizosphere of moong bean (*Vigna radiata* L.). All three isolates showed significant growth-inhibitory activity against a range of phytopathogenic fungi. Light and scanning electron microscopic (SEM) studies showed morphological abnormalities such as fragmentation, swelling, perforation and lysis of hyphae of pathogens in presence of the antagonists. Two of the strains (BAM-4 and BAM-6) produced siderophore in CAS agar plates, whereas all three strains produced chitinase. Bacterization of seeds of moong bean with pseudomonads has been reported as a potential method for enhancing plant growth and yield, and for providing protection against *Macrophomina phaseolina*. Seed bacterization with these plant growth-promoting rhizobacteria (PGPR) showed a significant increase in seed germination, shoot length, shoot fresh and dry weight, root length, root fresh and dry weight, leaf area and rhizosphere colonization. Yield parameters such as pods, number of seeds, and grain yield per plant also enhanced significantly in comparison to control. In an extensive study, Gopalakrishnan et al. (2011) isolated about 360 bacteria from the rhizosphere of a system of rice

intensification (SRI) fields. On the basis of primary screening of *in vitro* pgpr activities viz. production of siderophore, fluorescence, indole acetic acid (IAA), hydrocyanic acid (HCN) and solubilization of phosphorus, seven most promising isolates (SRI-156, 158, 178, 211, 229, 305 and 360) selected and were screened for their antagonistic potential against *Macrophomina phaseolina* (causes charcoal rot in Sorghum) by dual culture assay, blotter paper assay and in greenhouse. All the seven isolates inhibited the growth of *M. phaseolina*in dual culture assay. The sequences of 16S rDNA gene study revealed the isolates SRI-156, 158, 178, 211, 229, 305 and 360 as *Pseudomonas plecoglossicida*, *Brevibacterium antiquum*, *Bacillus altitudinis*, *Enterobacter ludwigii*, *E. ludwigii*, *Acinetobacter tandoii* and *P. monteilii*, respectively.

Many research studies have shown that induction of ISR by *Bacillus* strains led to a significant reduction in severity or incidence of various diseases on a diversity of hosts under greenhouse or field conditions (Saraf et al. 2014). *Bacillus subtilis* BN1 showed reduction in root rot symptoms caused by *M. phaseolina* in chir pine seedlings along with 43.6% and 93.45% increased root and shoot dry weight, respectively, as compared to control (Singh et al. 2008). Chen et al. (2009) reported surfactin production by *B. amyloliquefaciens* FZB42 not only protects it against other bacteria but also enables it to form biofilms, thus equipping the bacterium with powerful antagonistic advantage during surface colonization. Kumar et al. (2013) showed that the interaction of antagonistic *Bacillus* spp. with potato seeds or vegetative parts show promising antagonism by virtue of producing siderophore and antibiotics against black scurf and stem canker diseases of potato caused by *Rhizoctonia solani*, thereby resulting in increase of potato yield. Dubey et al. (2014) isolated a total of eight motile, aerobic, Gram-positive and straight rod-shaped, endospore forming *Bacillus* spp. from the rhizosphere of chickpea plants collected from different agricultural fields. Phylogenetic study with partial 16S rDNA and comparative analysis of the sequence data confirmed that the isolates belong to distinct phylogenetic lineage corresponding to *Bacillus*. Phenotyping clusters correlate with ARDRA pattern and showed resemblance to partial 16S rDNA sequencing. Among the eight isolates two, *Bacillus* spp. BSK5 and *Bacillus subtilis* BSK17 were the most potent strains for having plant-growth-promoting attributes. These two strains solubilised inorganic phosphate, produced Indole acetic

acid, siderophore, Hydrocyanic acid and 5 secreted extracellular chitinase and β-1, 3-glucanase which antagonised and caused mycelial deformities in two phytopathogens- *Macrophomina phaseolina* and *Fusarium oxysporum* in dual culture and by culture filtrate. Chithrashree et al. (2011) isolated seven promising *Bacillus* strains from the rice rhizosphere and were evaluated for growth promotion and induced systemic resistance in rice against *Xanthomonas oryzae* pv. oryzae (Xoo). Seed treatments with fresh suspension of strain SE34 gave 71% protection, followed by *B. subtilis* GBO3 and *B. pumilus* T4 with 58% and 52% protection, respectively, compared to the untreated controls. Seed treatments with talc based formulation gave little beat less protection. Talk formulation of SE34 gave 66% protection, while GBO3 and T4 resulted in 52% and 50% protection, respectively, with similar formulation. Nine bacterial strains viz. *Bacillus pumilus* SB 21, *Bacillus megaterium* HiB 9, *Bacillus subtilis* BCB 19, *Pseudomonas plecoglossicida* SRI156, *Brevibacterium antiquum* SRI158, *B. pumilus* INR 7, *P. fluorescens* UOM SAR 80, *P. fluorescens* UOM SAR 14 and *B. pumilus* SE 34 were tested to induce systemic resistance in sorghum cultivars 296B and Bulk Y against the highly pathogenic grain mould pathogens- *Curvularia lunata* and *Fusarium proliferatum*, respectively. The bacterial isolates were effective in inducing resistance in sorghum. Among the strains tested, SRI 158 was found highly effective in reducing grain mould severity in both the genotypes (Nithya et al. 2013). The potentiality of PGPR isolate in the management of bacterial wilt (BW) caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff) of common bean (*Phaseolus vulgaris*) was studied by Martins et al. (2013) in Brazil. The study was aimed at determining the potential of selected PGPR on the biological control of bacterial wilt through seed treatment, growth promotion and induced resistance. The disease control ranged from 42% to 76%, respectively, for *Bacillus subtilis* UFLA285 and ALB629 compared to the untreated control. Plants were assessed for seedling emergence (SE), speed emergence index (SEI), relative growth index (RGI), root dry weight (RDW), shoot dry weight (SDW), as well as biochemical plant responses in the presence or absence of Cff. PGPR treatments also increased RGI, SDW, and RDW. Upon Cff inoculation, UFLA285 increased phenolics' content and ALB629 in the lignin accumulation compared to the untreated control. Without the pathogen inoculation, both PGPR promoted an increase in phenylalanine ammonia lyase activity and total phenolics content and UFLA285 in the lignin accumulation. Son et al. (2014) investigated the effects of plant growth-

promoting rhizobacteria (PGPR) isolated from Dokdo Island for growth promotion of pepper and biological control activity against a gray leaf spot disease pathogen, *Stemphylium lycopersici*. Based on the results of the plant growth promotion assays, nine isolates were selected for further experimentation. The selected isolates were *Arthrobacter globiformis* KUDC1703, *Brevibacterium iodinum* KUDC1716, *Bacillus megaterium* KUDC1728, *Bacillus pumilus* KUDC1732, *Kluyvera cryocrescens* KUDC1771, *Enterobacter ludwigii* KUDC1772, *Pantoea agglomerans* KUDC1793, *Pseudomonas putida* KUDC1807, and *Pseudoxanthomonas dokdonensis* KUDC1809. All selected isolates were able to produce IAA, siderophores, and solubilize phosphates. The results showed that all treatments significantly improved plant growth based on all parameters. Among the nine isolates, *K. cryocrescens* KUDC1771 showed the strongest plant growth-promoting activity, followed by *P. agglomerans* KUDC1793 and *A. globiformis* KUDC1703. Among the nine selected bacterial isolates, four isolates (*B. iodinum* KUDC1716, *B. megaterium* KUDC1728, *B. pumilus* KUDC1732, and *P. putida* KUDC1809) were able to suppress gray leaf spot disease in pepper compared with the negative control. Moreover, RT-PCR studies showed that KUDC1716 enhanced expression of pathogenesis-related (PR) protein genes including CaPR4 and CaChi2 in the absence of pathogen. Dubey et al. (2015) developed an integrated management strategy involving fungal (*Trichoderma harzianum*) and bacterial (*Pseudomonas fluorescen*s and *Bacillus* species) antagonists, rhizobacterium and a fungicide for the management of chickpea wilt caused by *Fusarium oxysporum* f. sp. ciceris (Foc). PGPR strain *P. fluorescens* 80 (Pf 80) and *Bacillus* species (Bskm 5) caused the highest mycelial growth inhibition. Pf 80 was found to be compatible with *T. harzianum* and *Mesorhizobium ciceri*. The fungicides Vitavax, Topsin M, Thiram, Ridomil MZ 72, Captaf and Indofil M 45 inhibited growth of Foc and were found to be compatible with *T. harzianum*. Pf 80 and *M. ciceri* were insensitive to the fungicides including Vitavax power. The combination of seed dressing formulation Pusa 5SD developed from *T. harzianum*, Pf 80, *M. ciceri* and Vitavax power provided maximum protection to emerging seedlings. The seeds treated with Pusa 5SD + Pf 80 +*M. ciceri* + Vitavax power provided the highest germination, grain yield and the lowest wilt incidence in pot and field experiments. Ahmed et al. (2014) isolated a total of 112 bacterial isolates from the rhizosphere of eleven medicinal plants viz., *Ocimum basilicum, Marrubium vulgare, Melissa officinals, Origanum syriacum, Quisqualis indica, Solidago*

virgaurea, Melilotus officinalis, Cymbopogon citratus, Matricaria chamomilla, Thymus vulgaris and *Majorana hortensis*. All the isolates were screened for IAA, phosphate, potassium solubilization, chitinase activities and hydrogen cyanide production capabilities. 36 bacterial isolates showed IAA production, 25 HCN production, 57 chitinase activities, 39 phosphate and 105 potassium solubilization capacity. Eleven bacterial isolates were selected which showed highest plant growth promoting activities and further subjected to estimate siderophores and phenols produced in liquid culture along with antifungal activity against two phytopathogenic fungi. The most potent isolates were identified on the basis of 16S rRNA gene sequence as *Bacillus thuringiensis* C110, *Pseudomonas fluorescens* Th98 and *Pseudomonas poae* Th75. Xu and Kim (2014) reported the biocontrol of *Fusarium* crown and root rot (FCRR) by *Paenibacillus* strains in tomato. The study was carried out to evaluate the efficacy of seven *Paenibacillus* strains in the reduction of FCRR disease and the promotion of growth in tomato under greenhouse conditions. Results showed that all tested strains significantly reduced the severity of disease. Strain SC09-21 reduced the disease severity of FCRR by > 80.0%, whereas strains SC02-09 and SG09-02 resulted in disease reduction of > 60.0%. This protection of tomato plants could be due to the action of antagonistic strains on the growth of plant pathogens.

An extensive study was conducted by Zhang et al. (2015) with the aim to detect and characterize broad-spectrum antipathogen activity of indigenous bacterial isolates obtained from potato soil and soya bean leaves for their potential to be developed as biofungicides to control soilborne diseases such as Fusarium crown and root rot of tomato (FCRR) caused by *Fusarium oxysporum* f. sp. radicis-lycopersici (Forl). Thirteen bacterial isolates *Bacillus amyloliquefaciens* (four isolates), *Paenibacillus polymyxa* (three isolates), *Pseudomonas chlororaphis* (two isolates), *Pseudomonas fluorescens* (two isolates), *Bacillus subtilis* (one isolate) and *Pseudomonas* sp. (one isolate) or their volatiles showed antagonistic activity against most of the 10 plant pathogens in plate assays. Cell-free culture filtrates (CF) of five isolates or 1-butanol extracts of CFs also inhibited the growth of most pathogen mycelia in plate assays. Presence of most antibiotic biosynthetic genes such as*phlD, phzFA, prnD* and *pltC* in most *Pseudomonas* isolates and *bmyB, bacA, ituD, srfAA* and *fenD* in most *Bacillus* isolates was confirmed by PCR studies. These bacterial isolates inhibited the mycelia

growth of most pathogens. However, the inhibition effect of each isolate varied depending on the test pathogen. For instance, the two *Pseudomonas* isolates (*Ps. fluorescens* PEF-5 #18 and *Ps. chlororaphis* SL5) inhibited the growth of all pathogens in dual-culture plate assays. Similarly, *Ps. chlororaphis* 1B-26 and *B. amyloliquefaciens* 9A-31, their volatiles, and CFs inhibited the mycelial growth of most fungal pathogens.Three antagonistic bacterial isolates *Paenibacillus polymyxa* #53, *Ps. chlororaphis* SL5 and *Ps. fluorescens* PEF-5 #18, *Ps. fluorescens* 9A-14, *B. subtilis* 8B-1 and *Pseudomonas* sp. 8D-45 were evaluated for the suppression of FCRR disease and promotion of plant growth of tomato in greenhouse pot experiments. In both potting mix and field soil, all six isolates when applied to tomato roots as irradiated peat formulations significantly decreased FCRR severity and increased plant height, root lengths, and total fresh and dry plant biomass compared to the infected control.

Bacillus subtilis (Cf 60) mediated inhibition of *M. phaseolina* in *Coleus forskohlii* has been reported by Malleswari et al. (2015). The strain strongly inhibited the growth of *M. phaseolina* and enhanced growth parameters of *Coleus* and also suppressed root rot disease. Li et al. (2015) exhibited that biocontrol agent *Bacillus amyloliquefaciens* LJ02 induces systemic resistance against cucurbits powdery mildew. Greenhouse trials with the fermentation broth of strain LJ02 showed that it can effectively reduce the occurrence of cucurbits powdery mildew. When treated with LJ02FB, cucumber seedlings produced significantly elevated production of superoxide dismutase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase as compared to that of the control. Moreover, the inoculation with LJ02FB induced the elevated production of free salicylic acid (SA) and expression of one pathogenesis-related (PR) gene PR-1 in cucumber leaves, suggesting SA mediated defence stimulation. Further, LJ02FB-treated cucumber leaves could secrete resistance-related substances into rhizosphere that inhibit the germination of fungi spores and the growth of pathogens. Planchamp et al. (2015) studied the colonization behaviour of *Pseudomonas putida* KT2440 following application to maize seedlings and transcription alanalysis of stress- and defense-related genes as well as metabolite profiling of local and systemic tissues of KT2440-inoculated maize plants were performed. In their study they have observed pronounced changes were in roots compared to leaves. Early in the interaction roots responded via jasmonic acid- and

abscisic acid-dependent signalling but during later steps, the salicylic acid pathway was suppressed. Metabolite profiling revealed the importance of plant phospholipids in KT2440-maize interactions. An additional important maize secondary metabolite, a form of benzoxazinone, was also found to be differently abundant in roots 3 days after KT2440 inoculation. However, the transcriptional and metabolic changes observed in bacterized plants early during the interaction were minor and became even less pronounced with time, indicating an accommodation state of the plant to the presence of KT2440. Since the maize plants reacted to the presence of KT2440 in the rhizosphere, they also investigated the ability of these bacteria to trigger induced systemic resistance (ISR) against the maize anthracnose fungus *Colletotrichum graminicola*. They observed, resistance was expressed as strongly reduced leaf necrosis and fungal growth in infected bacterized plants compared to non-bacterized controls, showing the potential of KT2440 to act as resistance inducers. Hassan et al. (2015) showed the potentiality of *Bacillus* sp. inhabiting plant rhizosphere can protect the plants from multiple pathogens of sugarcane. Two antagonistic strains *Bacillus subtilis* NH-100 and *Bacillus* sp. NH-217 which can sustain their population in sugarcane filter cake at 9.0 log CFU g-1 up to nine months, were used for the biocontrol of red rot of sugarcane. Field experiments showed the efficacy of the formulation in reducing the disease incidence. Surfactin producing antagonistic *Bacillus* sp. can survive for a longer time in non-sterilized sugarcane filter cake, thereby improving the shelf life of formulation and control the red rot disease of sugarcane effectively in the field. Ghosh et al. (2016) reported the inhibition of leaf spot pathogen *Alternaria alternata* of *Aloe vera* by two rhizobacterial isolates *Burkholderia cenocepacia* VBC7 and *Pseudomonas poae* VBK1. The isolates were able to produce prominent zones of inhibition against *Alternaria alternata* in dual culture overlay plates.

The cell free supernatant of VBK1 and VBC7 could reduce conidial germination 89.3 ± 1.22% and 81.5 ± 2.67% respectively. Radial growth assay also suggested prominent growth inhibition by both the biocontrol strains. The mycelial breakage of pathogen in presence of isolates was evidenced by scanning electron micrographs. Greenhouse experiment also suggested excellent capabilities of biocontrol agents to reduce disease severity in good measure even after exposure to high concentration

Table 2 Biological control by PGPR against phytopathogens in different crops

PGPR	Crop	Disease/Pathogen	References
Serratia marcescens B2	Cyclamen	*Rhizoctonia solani*	Someya et al. (2000)
Pseudomonas aeruginosa *Bacillus subtilis*	Mung bean	root rot, root knot	Siddiqui et al. (2001)
Pseudomonas PMZ2 *Bradyrhizobium japonicum*	Soybean	*Fusarium oxysporium*	Zaidi (2003)
Pseudomonas flurescence	Pea nut	Collar rot	Dey et al. (2004)
Paenibacillus polymyxa E681	Sesame	Fungal disease	Ryu et al. (2006)
Mesorhizobium loti MP6,	Mustard *Brassica campestris*	White rot *Sclerotinia sclerotiorum*	Chandra et al. (2007)
Pseudomonas fluorescens	Tea (*Camellia sinensis*)	Blister blight	Saravanakumar et al. (2007)
Paenibacillus lentimorbus GBR158	Tomato	*Fusarium oxysporum f. sp. lycopersici*	Son et al. (2008)
Paenibacillus polymyxa GBR-462	Chili pepper	*Phytopthora capsici*	Kim et al. (2009)
Ochrobactrum anthropi TRS-2	*Camellia sinensis*	brown root rot disease	Chakraborty et al. (2009)
Serratia marcescens TRS-1	*Camellia sinensis*	*Fomes lamaoensis*	Chakraborty et al. (2010)
Bacillus pumilus	Tomato	*Fusarium oxysporum f. sp. lycopersici*	Heidarzadeh & Baghaee-Ravari (2015)
Bacillus amyloliquefaciens	*Solanum lycopersicum* L.	*Ralstonia solanacearum* (Smith)	Singh et al. (2016)

(3.1×10^4 conidia/ml) of pathogenic spores. During *in vivo* field experiments 54.25 ± 3.55% disease severity was observed for untreated plants, whereas only 11.69 ± 1.25% and 15.22 ± 2.64% disease severities were noticed in plants treated with VBK1 and VBC7 respectively. Shrestha et al. (2016) in their study isolated 29 rice-associated bacteria (RAB) out of which twenty six showed promising antimicrobial activity. 16S rDNA sequencing study revealed that, 12 of the 26 antagonistic RABs were closest to *Bacillus amyloliquefaciens*, while seven RAB were to *B. methylotrophicus*. These isolates were observed to inhibit the sclerotial germination of *Rhizoctonia solani* on potato dextrose agar and the lesion development on detached rice leaves by artificial inoculation of *R. solani*.

In a recent study, Shahzad et al. (2017) reported the suppression of pathogenic *Fusarium oxysporum* f. sp. *lycopersici by Bacillus amyloliquefaciens* RWL-1. When tomato plants were inoculated with *B. amyloliquefaciens* RWL-1 and *F. oxysporum* f. sp. *lycopersici* in the root zone, growth attributes and biomass were significantly enhanced by bacterium-inoculation during disease incidence as compared to *F. oxysporum* f. sp. *lycopersici* infected plants. Under pathogenic infection, *B. amyloliquefaciens* applied plants showed increased amino acid metabolism aspartic acid, glutamic acid, serine and proline as compared to diseased plants. In case of endogenous phytohormones, significantly lower amount of jasmonic acid and higher amount of salicylic acid content was recorded in *B. amyloliquefaciens* treated diseased plants. Higher accumulation of defense related PR proteins was found when plant was treated with the bacterium and challenged inoculation with *F. oxysporum* f. sp. *lycopersici*. Vinodkumar et al. (2017) reported biocontrol efficacy of *Bacillus* sps. Isolated from rhizosphere of various plants viz., carnations, cotton, turmeric, and bananas. Among the isolates, *B. amyloliquefaciens* strain VB7 was much effective in inhibiting mycelial growth (45% inhibition of over control) as well as sclerotial production (100%). GC/MS analysis of crude metabolites of *B. amyloliquefaciens* strains VB7 and VB2 revealed the presence of antifungal compounds viz. chloroxylenol, pentadecenoicacid, heptadecenoicacid, octadecenoicacid, pyrrolo, and hexadecenoicacid. The strains also showed promising activity when applied as root dip. Minimal percent disease incidence (4.6%) and maximum plant growth promotion was observed in the plants treated with *B. amyloliquefaciens* (VB7).

The mechanism of action of PGPR to promote plant growth and to induce systemic resistance against plant pathogens to provide an alternative to hazardous chemical fertilizers have been focused in this review. It is evident that several bacterial isolates viz. *Bacillus*, *Serratia*, *Pseudomonas*, *Pantoea*, *Rhizobium*, *Azotobacter*, *Burkholderia*, *Enterobacter* are capable of promoting plant growth both in green house and field condition by variety of mechanisms, which include both direct and indirect mechanisms. Among which biological nitrogen fixation, solubilization of insoluble phosphates, production of phytohormones, increased expression of defense enzymes, activation of genes for PR proteins, increased uptake of nutrients of roots by the PGPR strains have been documented. The use of PGPR strains for improvement of crop yield or suppression of pests and disease may be used as an alternative to make a balance with the ever increasing demand of agricultural productivity and environmental health.