Review of Literature
2. Review of Literature

2.1 Rhizosphere

Root systems were initially thought to provide support and uptake and supply of nutrients and water, it can aptly be addressed as chemical factory as they aid in numerous underground interactions and are an integral part of the plant system (Badri et al., 2009). Rhizosphere is the thin zone of soil directly in contact with the root system (Walker et al., 2003), whereas a group of rhizosphere dwelling bacteria aggressively colonizing the root surface are termed as rhizobacteria. Plant roots also produce, accumulate, and secrete myriad of bio compounds (Walker et al., 2003). The compounds produced by plant roots act as chemical magnets for a vast number of heterogeneous rhizobacterial communities. The constitution of these exudates is largely influenced by the physiological state and species of plants and rhizobacteria (Kang et al., 2010). These exudates are also known to encourage the plant-beneficial symbiotic interactions and arrest the growth of the competing plant species as well as phytopathogens (Nardi et al., 2000). Primarily, three different but interacting constituents are categorized in the rhizosphere: the rhizosphere (soil), the rhizoplane, and the root. Out of these, the rhizosphere is the region of soil influenced by roots by the release of substrates that affect the rhizobacterial activity. The rhizoplane, is the root surface to which the soil particles adhere while the root itself is a cardinal component of the system, as several microorganisms thrive in the root tissues (Barea et al., 2005).

2.2 Plant Growth Promoting Rhizobacteria

Different bacterial genera are vital components of soils. They are involved in various biotic activities of the soil ecosystem to make it dynamic for nutrient turn over and sustainable for
crop production. Bacteria lodging around/in the plant roots (rhizobacteria) are more versatile in transforming, mobilizing, solubilising the nutrients compared to those from bulk soils (Hayat et al., 2010). Therefore, the rhizobacteria are the dominant deriving forces in recycling the soil nutrients and consequently, they are crucial for soil fertility (Glick, 1995). The plant growth promoting rhizobacteria (PGPR), are characterized by the following inherent distinctiveness:

i. They must be adept to dwell in the surface of the roots and within the roots

ii. They must sustain, divide and co-exist with other microbes till they reprogram genetic expression pattern of self or host

iii. They must boost plant growth and enhance plant health (Vacheron et al., 2013).

Fig. 1: The underlying mechanisms behind functioning of PGPR
Some common examples of PGPR genera exhibiting plant growth promoting activity are: *Enterobacter, Rhizobium, Erwinia, Azotobacter, Bacillus, Burkholdaria, Mycobacterium, Mesorhizobium, Flavobacterium, Pseudomonas*, etc.

A number of beneficial bacteria attach to roots, multiply and colonize root surface or are found in close association with root and rhizosphere of host plants. This association of bacteria to the root is known as root colonization (Lucy et al., 2004). The soil around the roots is called rhizospheric soil. The effective colonization of the rhizobacteria in the plant rhizosphere and its sustenance is important for the resultant beneficial effects of PGPR on plant growth and development (Chin-A-Woeng and Lugtenberg, 2004). For beneficial processes namely phytostimulation, phyto-remediation and biocontrol etc., root colonization in the rhizospheric region is a primary step (Lugtenberg et al., 2001).

Several parameters influence root colonization root exudates, bacterial strain, living and non-living components of the ecosystem (Benizri et al., 2001). Many other physiological parameters are also important in colonization of the root system, such as attachment to the root cortex and penetration of the root tissues.

The impact of abiotic factors such as pH and phosphorus on root colonization was studied by Baushe et al. (Bauske et al., 1997) and Chabot et al. (Chabot et al., 1998). The effect of temperature on root colonization have been studied by various researchers and the results indicated that rhizobacteria were able to colonize roots at lower soil temperatures (5 °C) more effectively than at higher temperatures (25 °C) (Benizri et al., 2001). Weger et al. (De Weger et al., 1987) stated that motility of soil rhizobacteria is a cardinal feature that helps the rhizobacteria to colonize the root. It was also reported that the mutant strains have restricted
capacity to colonize potato root since they are devoid of flagella. In similar study by Bashan and Holguin (Bashan and Holguin, 1995) was based on the comparative analysis of a non-motile *Azospirillum brasilense* mutant with its parental strain. From the results it can be inferred that the wild-type strain was more efficient in colonizing roots adjoining the site of inoculation in contrast to the mutant. The function of chemosensory pathways and also flagellar motility in detail has been studied by Blair (Blair, 1995).

The density of bacterial population in the rhizosphere region is 100 times higher in bulk soil. The previous studies have unravelled that the seed and root exudates attract the rhizobacteria by chemotaxis and this may be the initial process in root and seed colonization (Currier and Strobel, 1976; Heinrich and Hess, 1985; Scher et al., 1985). In the absence of pathogenic microorganisms, many rhizobacteria show similar properties and promote plant growth significantly (Compant et al., 2005; Haas and Defago, 2005).

Free living, symbiotic and endophytic bacteria that colonize root and rhizosphere and that directly or indirectly positively effect the plant growth and development are called plant growth promoting rhizobacteria (PGPR). They directly enhance plant growth by facilitating acquisition of resources or regulating levels of plant hormones (auxin, cytokinin and gibberellin) or indirectly by acting as biocontrol agent or by reducing the effects of pathogenic agents on plant growth. They can be classified into two categories on the basis of their association with the host plant. They may be extracellular (ePGPR) or intracellular (iPGPR) (Martínez-Viveros et al., 2010). The extracellular PGPR include *Azotobacter, Agrobacterium, Arthrobacterium, Azospirillum, Serratia, Burkholderia, Bacillus, Chromobacterium, Pseudomonas and Flavobacterium* (Gray and Smith, 2005). Endophytic bacteria (*Azorhizobium, Allorhizobium, Mesorhizobium, Bradyrhizobium and Rhizobium* etc.)
and *Frankia* species fall in the category of intracellular PGPR. They form symbiotic
association with higher plants and fix atmospheric nitrogen (Gray and Smith, 2005). PGPR
play a key role in agriculture environment and are being employed for sustainable agriculture
practices. They have successfully promoted the growth of many crops like wheat, rice, pea,
lemlent, soybean and canola by producing plant hormones, antibiotics, siderophores, fungal and
bacterial antagonistic substances (Kevin-Vessey, 2003; Bhattacharyya and Jha, 2012). Plant
growth-promoting rhizobacteria (PGPR) assist in plant growth and development *via* direct
and indirect processes. Most PGPR have proved their mettle in enhancing plant growth and
development indirectly (Noel et al., 1996). Direct mechanisms can be explained as
production of plant growth regulators, solublization of mineral materials and fixation of
atmospheric nitrogen. For example, *Bacillus* strains trigger plant response to stress and
secrete various phytohormones for growth and development (Rajendran et al., 2008). Plant
growth promotion also confers antagonistic effect, and protect from harmful impact of
stresses from the environment. Nematicidal capability of few plant growth-promoting
rhizobacteria and their metal adsorbing ability has also been studied (Khan et al., 2008; Wu et
al., 2009).

### 2.3 Relationships of PGPR with their host plants

PGPR form a close association with their host plant and facilitate the availability of nutrients
to the host and enhance plant growth and development. The relationship between the host
plant and PGPR is based on the site where the PGPR colonize the root and rhizosphere of the
host plant. Few rhizobacteria exhibit specificity towards the host plant. Some rhizobacteria
are general root colonizer while others are found endophytically in plant tissues (Bashan and
Levanony, 1990; James et al., 1994). The interaction between soil microorganism and plant
occurs in the aerial part of plant “phyllosphere”, plant internal transport system “endosphere”
and soil surrounding the root “rhizospheres”. A number of endophytic as well as rhizobacteria associated with plants have been reported to promote plant growth and development (Lodewyckx et al., 2002). PGPR attach to plant surface in rhizospheric associations (Andrews and Harris, 2000). The byproducts like amino acids and sugar in the rhizosphere provide nutrition for rhizobacterial growth and eventually increasing bacterial population. A large amount of bacterial population thrives in the rhizospheric region but only small portion of the total root surface is occupied by microorganisms (Compant et al., 2010).

**Fig. 2: Impact of PGPR on nutrient acquisition and root functioning**

PGPR can modulate root development and growth through the production of phytohormones, secondary metabolites and enzymes. The most commonly observed effects are a reduction of the growth rate of primary root, and an increase of the number and length of lateral roots and root hair. PGPR also influence plant nutrition via nitrogen-fixation, solubilization of phosphorus, siderophore production, and modify root physiology by changing gene transcription and metabolite biosynthesis in plant cells.
2.4 PGPR benefits the plant

PGPR have positive effects on plants. PGPR promote plant growth by myriad of ways such as increasing rate of germination, root proliferation, yield, chlorophyll content, concentration of nitrogen and magnesium, root and shoot weight, delay leaf senescence and make them resistant to drought. PGPR can also be exploited as biocontrol by providing disease resistance to the plant. PGPR improve water and nutrient uptake by the plant and therefore contribute to improvise plant growth. A number of PGPR have been found in colonized form in the root and rhizosphere of important cereal crops like wheat, rice and maize (James and Olivares, 1998). Shoot weight of rice increased significantly due to inoculation with different PGPR strains (Okon, 1985). Some *Azospirillum* strains inoculated to rice, wheat and maize have shown 10-30% increase in grain and forage yield (Okon and Baker, 1987). PGPR increased the seed emergence, plant weight and yield (Kloepper et al., 1980).

Cakmakci et al (Çakmakçı et al., 2007) reported favourable response in wheat, maize, cucumber, potato and pea due to PGPR application. Increase in nitrogen fixation, nodulation, nutrient uptake and significant increase in the yield of soybean due to inoculation of PGPR was also documented by Zhang et al. (Zhang et al., 1996). It was also reported that inoculation of bacteria enhances biotic and abiotic stress resistance in plants (Malhotra and Srivastava, 2009). The co-culturing of plantlets with PGPR results in the production of more biomass and secondary metabolites.

2.5 Mode of Action of PGPR

PGPR promote plant growth by various direct and indirect mechanisms (Compant et al., 2005; Compant et al., 2010). PGPR directly promote plant growth by secreting biochemical which in turn facilitated nutrient uptake from the soil. They enhance plant growth via a
number of mechanisms such as fixation of nitrogen, active solubilization and uptake of phosphorus and iron. Most of the agricultural land has deficiency of one or more of these compounds and thus plant growth is not upto the desired level. PGPR promote plant growth and development indirectly by removing phytotoxic materials, removal of plant pathogen, production of antibiotics and by chelation of iron. Plant growth is also promoted by synthesizing extracellular enzymes which hydrolyses cell wall of fungi and develop mycorrhizal association thereby acting as a biocontrol. Multiple mechanisms like fixation of nitrogen, solubilization of phosphate, synthesis of plant hormones, chemicals and enzymes for biocontrol of minor pathogens are employed by the rhizobacteria for the enhancement of plant growth.

Fig. 3: Myriad of beneficial effects wielded by PGPRs
2.5.1 Nitrogen fixation by soil microorganisms

The production of ammonia (NH₃) from atmospheric nitrogen by the mechanism of microorganisms using a complex system of enzyme like nitrogenase is involved in Biological Nitrogen Fixation (BNF) (Mantelin and Touraine, 2004). Biological nitrogen fixation is crucial for reducing energy cost and for sustainable agriculture (Mantelin and Touraine, 2004). Nitrogen-fixing rhizobacteria like Azospirillum, Azoarcus, Burkholderia, Enterobacter, Gluconacetobacter and Herbaspirillum are potential nitrogen fixer and are found in partnership with the roots of important crops (Boonjawat et al., 1991).

![Diagram of nodulation process]

**Fig. 4: The steps involved in nodulation process** a) Interaction of rhizobial rhicadhesin with host lectins and rhizobial attachment with root cells. (b) Emission of nod factors by rhizobia causes root hair curling. (c) Rhizobia penetrate root hair and form an infection thread through which they penetrate the cortical cells and form bacteroid state thereby nodules are formed

2.5.2 Phytohormones Production

PGPR enhance plant growth by producing hormones or by synthesizing biochemicals that carry out plant essential functions. The important hormones like auxin, ethylene and gibberellins are produced by numerous rhizobacteria (Ahemad and Kibret, 2014). The rhizobacteria synthesise auxins more efficiently that non-rhizosphere bacterial isolates
(Persello-Cartieaux et al., 2003). Indole-acetic-acid (IAA) is the chief naturally occurring auxin and is required for cell division, cell enlargement, root development, phototropism and apical dominance (Ahemad and Kibret, 2014). The quintessential auxin effect is the formation of lateral roots. Ethylenes enhances the root and shoot growth and improvises organ abscission and senescence at high levels (Iqbal et al., 2013). Various strains of PGPR are rich source of enzymes like cyclopropane, carboxalate and deaminase which lowers ethylene level in stressed or developing plants by cleaving the plant ethylene precursor ACC (Iqbal et al., 2013). Ethylene is called for in substantial amounts by the seed inorder to break seed dormancy before it germinates. The high concentration of ethylene after germination inhibits root elongation (Iqbal et al., 2013). Both Gibberellins and Cytokinins trigger shoot development (Ahemad and Kibret, 2014). Cytokinins fall under the aegis of a different class of phytohormones (Persello-Cartieaux et al., 2003). About as much as 90% of rhizobacteria exuberate the ability to produce phytohormones essential for plant metabolism (Barea et al., 1976). Timmusk et al. (Timmusk et al., 1999) had documented the production of cytokinins by single free-living soil bacterium Pseudomonas polymyxa using Immuno Affinity Chromatography (IAC). Salamone et al. (García de Salamone et al., 2001) had also reported higher production of the cytokinins dihydroxyzeatin (DHZR), zeatin riboside (ZR) and isopentenyl adenosine (IPA) by a wild strain Pseudomonas fluorescens in comparison to two mutants, CNT1 and CNT2 strains.

### 2.5.3 Siderophore Production

Iron is an important nutrient for almost all livings forms. Each and every microorganisms known up till now, with certain lactobacilli being the only exception, primarily require iron (Neilands, 1995). Iron occurs primarily as Fe$^{3+}$ in aerobic environmental conditions and has a strong probability to form insoluble hydroxides and oxyhydroxides, and therefore being
normally inaccessible by both plants as well as microorganisms (Rajkumar et al., 2010). Generally, bacteria obtain iron by the production of low-molecular mass iron chelators known as siderophores which have robust association constants for integrating iron. Almost all the siderophores are water-soluble in nature and are categorized into extracellular siderophores and intracellular siderophores. Rhizobacteria in general, vary with respect to the siderophore’s ability of cross-utilization; few rhizobacteria are adept in utilization of siderophores of the same genus (homologous siderophores) on the other hand, several rhizobacteria can also utilize siderophore produced by other rhizobacteria of different genera (heterologous siderophores) (Khan et al., 2009). Both Gram-negative and Gram-positive rhizobacteria, iron (Fe$^{3+}$) in Fe$^{3+}$-siderophore complex present on the rhizobacterial membrane is reduced to Fe$^{2+}$ which is again liberated into the cell from the siderophore through gating mechanism joining the inner and outer membranes. In this reduction step the siderophore is either destroyed or recycled (Neilands, 1995; Rajkumar et al., 2010). Thereby, siderophores play the role of solubilizing agents for iron from minerals or organic compounds under the circumstances of iron inadequacy (Indiragandhi et al., 2008). Siderophores like iron form constant complexes with other heavy metals that are of environmental utility, such as Al, Cd, Cu, Ga, In, Pb and Zn, as well as with radionuclides including U and Np (Kiss and Farkas, 1998; Neubauer et al., 2000). Association of the siderophore to a metal enhances the soluble metal absorption (Rajkumar et al., 2010). Therefore, rhizobacterial siderophores help to lessen the stresses burdened on plants by high concentration of heavy metals. Plant digests iron from rhizobacterial siderophores by employing various mechanisms, for example, chelation and liberation of iron, the direct uptake of siderophore-Fe complexes, or via a ligand exchange reaction (Schmidt, 1999). Various studies pertaining to the plant growth promotion via siderophore-mediated Fe-uptake as a consequence of siderophore producing rhizobacteria have been reported (Rajkumar et al., 2010). To cite an example, Crowley and
Kraemer (Crowley, 2006) discovered a siderophore mediated iron transport system in oat plants and came to the conclusion that siderophores secreted by rhizosphere microorganisms cater iron to oat, which has the proficiency for using Fe-siderophore complexes under iron-limiting conditions. In a similar fashion, the Fe-pyoverdine complex synthesized by *Pseudomonas fluorescens* C7 was uptaken by *Arabidopsis thaliana* plants, resulting in a boost of iron supply inside plant tissues and thereby enhancement of plant growth and plant health (Vansuyt et al., 2007). Of late, Sharma et al. (Sharma et al., 2003) studied the role of the siderophore-producing *Pseudomonas* strain GRP3 on iron nutrition of *Vigna radiate*. After 45 days, the plants exhibited a decline in chlorotic symptoms and iron, chlorophyll-a and chlorophyll-b content augmented considerably in strain GRP3 inoculated plants in comparison to the control.

Fig. 5: Shuttle iron delivery mechanisms of siderophores

For proper growth and development of plant iron is essential. Iron is generally present in the soil in insoluble form. Therefore, it remains unavailable to the plant. Plant root absorbs iron in reduced ferrous ion (Fe$^{2+}$) form. The soil system generally has ferric (Fe$^{3+}$) ion, which precipitate readily in iron oxide forms. Plant system releases compounds like siderophores
which bind with Fe$^{3+}$ ions. Bound Fe$^{3+}$ is welcomed by the root surface from chelators and reduced to Fe$^{2+}$ ions and absorbed immediately. Siderophores are basically low molecular weight iron chelating molecules, synthesized by numerous rhizobacteria in low iron condition. Production of siderophores by various rhizobacteria and its absorption as the Fe$^{3+}$-siderophore complexes has been reported in a number of plant system (Bar-Ness et al., 1991; Crowley, 2006). The rhizobacteria living in close proximity with plant species or within plant system facilitate active uptake of siderophores by the plant and thereby providing the adequate iron for plant nutrition (Bar-Ness et al., 1992; Crowley, 2006). Marschner and Romheld (Marschner and Römheld, 1994) had reported that rhizobacteria dwelling in root secrete siderophores, which act as a mechanism for iron acquisition by the plant. Many plants like cotton, cucumber, oats, peanut, sorghum and sunflower have exhibited the ability to utilize microbial siderophores as a primary source of iron (Bar-Ness et al., 1992).
2.5.4 Phosphate Solubilization

The second most vital plant growth-limiting nutrient after nitrogen, is Phosphorous (P), which is copiously available in soils in organic as well as inorganic forms (Khan et al., 2009). In spite of large basin of P, the quantity of available forms to plants is normally low. This low accessibility of phosphorous to plants is due to the fact that majority of soil P is available in insoluble forms, and as matter of fact the plants attract it only in two soluble forms, the monobasic \((\text{H}_2\text{PO}_4^-)\) and the diabasic \((\text{H}_2\text{PO}_4^-)\) ions (Bhattacharyya and Jha, 2012). The insoluble P is primarily available as an inorganic mineral such as apatite or as one of several organic forms including inositol phosphate (soil phytate), phosphor-monoesters, and phosphor-triesters (Glick, 1995). In order to surmount phosphate inadequacy in soils,
phosphate laced chemical fertilizers are repeatedly applied in agricultural fields. Plants soak up only small amounts of applied phosphatic fertilizers and the rest is spontaneously transformed into insoluble complexes in the soil. Excess application of phosphate fertilizers is expensive as well as hazardous for the environment. Such adverse effects on the environment have brought an urgent call for ecologically safe and economically sound alternatives for improving crop production in soils which are deficit in phosphorous. In view of this concept, organisms associated with phosphate solubilizing activity, are referred as phosphate solubilizing microorganisms (PSM), which supplement Phosphorous to the plants and hence provide a potential alternative to chemical phosphatic fertilizers (Khan et al., 2009). Out of the various PSM(s) dwelling in the rhizosphere, phosphate-solubilizing bacteria (PSB) are regarded as potential biofertilizers because they can provide plants with P from sources which is otherwise unavailable by several mechanisms (Zaidi et al., 2009). Bacterial genera like *Azotobacter, Bacillus, Beijerinckia, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium and Serratia* are documented as the most crucial phosphate solubilizing bacteria (Bhattacharyya and Jha, 2012).
Classically, the solubilization of unavailable inorganic phosphorus is the outcome of the action of low molecular weight organic acids which are produced by various rhizobacteria (Zaidi et al., 2009). On the contrary, the mineralization process of organic phosphorus takes place through the production of array of different phosphatases, catalyzing the hydrolysis of phosphoric esters. Prominently, phosphate solubilization and mineralization process can take place simultaneously in the same bacterial strain (Tao et al., 2008). Although PSB are usually found in many soil type; their institution and performances are majorly influenced by environmental factors especially under stress conditions (Ahemad and Kibret, 2014). The advantageous effects of the inoculation with PSB used alone (Chen et al., 2008; Poonguzhali et al., 2008) or in combination with other rhizobacteria treatment have been documented (Zaidi and Khan, 2005). In addition to supplement P to the plants, the phosphate solubilizing bacteria also boost the growth of plants by triggering the efficiency of BNF, augmenting the
accessibility of trace elements by producing cardinal plant growth promoting phytochemicals (Suman et al., 2001; Ahmad et al., 2008; Zaidi et al., 2009).

PGPR increases nutrient supplies to the host plant by conversion of insoluble phosphate to soluble form. (Meunchang et al., 2004) isolated 62 P-solubilizing rhizobacteria from paddy field and drew the conclusion that the rhizobacteria could be promising biofertilizer for rice. (Cattelan et al., 1999) carried out in vitro screening of 116 rhizobacteria isolated from soil for multiple PGPR traits including phosphorous solubilising activity. From their study they concluded that rhizobacteria, which produce ACC deaminase, siderophores and solubilize phosphorus increase prospects for early plant growth.

![Flowchart showing natural processes involved in mineralization and utilization of phosphate](image).

**Fig. 8: Natural processes involved in mineralization and utilization of phosphate**
2.5.5 PGPR as biocontrol of plant pathogens

Biocontrol of plant pathogen is a cardinal PGPR trait. They produce siderophores and synthesize antibiotics and thus defending the plant from phyto-pathogens (Neilands and Leong, 1986). They also synthesize extracellular enzymes like chitinase, protease or lipase and β-1,3-glucanase which break fungal cells. They also produce hydrogen cyanide which exhibits antifungal activities and pose competition with plant pathogen (Dowling and O'Gara, 1994; Loper et al., 1997). PGPR play significant role in inhibiting fungal pathogen and also considerable enhancement of plant growth. Various researchers from different parts of the world have time and again documented that rhizobacteria display rapid growth and compete for carbon and energy source against fungal pathogen and thus acting as biological control (Neilands and Leong, 1986; O'Sullivan and O'Gara, 1992; Dowling and O'Gara, 1994). Reports have also claimed that the entire rhizobacterial populations have the potential of causing fungistasis in rhizosphere (Handelsman and Stabb, 1996). Recent studies have also point out induced systemic resistance, antibiosis and pathogen-antagonist interaction as three important mechanisms for biocontrol of phytopathogens (van Loon et al., 1998; van Loon and Bakker, 2003). Production of antibiotics has been reported from a large number of Pseudomonas strains. These antibiotics have the ability to inhibit pathogenic bacteria, fungi, pathogenicity of higher organisms and in some cases higher organisms (Raaijmakers et al., 2002).

PGPR stimulate the plant protection against pathogens and arrest the pathogenic activity of rhizobacteria through Induced Systemic Resistance mechanisms (ISR) (Van Loon and Bakker, 2006). Induced Systemic Resistance mechanisms have been documented in nearly 15 plant species (van Loon and Bakker, 2006). PGPR also increase the plant growth by Induced Systemic Resistance (Kloeper et al., 2004). The inducible defense mechanisms include
production of pathogenesis proteins, production of antimicrobial phytoalexins and recon-
struction of plant cell wall (Hammond-Kosack and Jones, 1996; Conrath et al., 2006).

2.5.6 Exopolysaccharide

EPS has myriad of functions, which directly or indirectly associated with soil water potential. EPS is instrumental in balancing water potential and is also directly responsible for crop yield. EPS adheres to soil particles and form clusters; which maintains soil moisture and also takes care of plant-microbe interaction. Plants that shelter EPS producing rhizobacteria have stronger chances to withstand adverse conditions. EPS-producing plant growth-promoting rhizobacteria can considerably improve the amount of soil macropores and the rhizospheric soil clusters, eventually resulting in increased water and fertilizer availability to the treated plants. The effect of EPS-producing plant growth-promoting rhizobacteria on clusters of root-adhering soils was studied by Alami et al. (Alami et al., 2000). Bacterial EPS can also be utilised for bioremediation of waste waters (Aguilera et al., 2008).

EPS-producing plant growth promoting rhizobacteria binds to the cations including Na$^+$, therefore, resulting in an increase in the population of EPS-producing bacteria in and around the rhizosphere which decreases the content of Na$^+$ available for plant uptake, and thereby mitigate stress in plants. However, relatively very less information is available is about the influence of EPS-producing rhizobacteria and their plant growth-promoting effects. EPS secreted by the alfalfa rhizobacteria *Sinorhizobium meliloti* acts as a signaling molecule that stimulates a developmental response in the plant or activates host defense responses (Mendrygal and Gonzalez, 2000). Likewise, EPS produced by the root-associated rhizobacterium *Pantoea agglomerans* YAS34 has been linked with sunflower’s plant growth (Alami et al., 2000). Moreover, EPS from a plant pathogenic *Pantoea agglomerans* has been
demonstrated to exhibit random synthesis of active oxygen species in tobacco, parsley, wheat, and rice cell culture (Ortmann et al., 2006). *Burkholderia gladioli* IN-26, a strain of plant growth-promoting rhizobacteria (PGPR) effectively checks bacterial speck disease on tomato plants and gives protection to tobacco plant against wild fire, caused by *Pseudomonas syringae pv. tabaci*, and this process has been shown to be linked to activation of the PR-1a gene (Park and Kloepper, 2000). Moreover, Isolated EPS from PGPR strain *B. gladioli* IN26 induced systemic resistance in cucumber against *Colletotrichum orbiculare* (Park et al., 2008).

Additionally, out of the innumerable benefits which EPS confers, emulsification of oils-hydrocarbons, organic solvents and uptake of heavy metals are of great significance and relevance in soils contaminated with petroleum fuels, solvents and heavy metals. EPS producing bacteria not only beat contaminants but also treat them efficiently until they are transformed into non-toxic products (Hullebusch et al., 2005; Pal and Paul, 2008). EPS has a crucial role in hydrocarbon degradation which attaches to fat molecule, disrupts or emulsify it to smaller nuclei to be degraded. Alternative method is to decrease surface/interfacial tension, increase the cell surface hydrophobicity so that hydrocarbon attaches to EPS matrix which lies outside bacterial cell. EPS guards the rhizobacteria from harmful effect of hydrocarbons or heavy metals (Hullebusch et al., 2005; Pal and Paul, 2008). The structural components of EPS play a pivotal role and degrade hydrocarbons.

**2.5.7 Phytoremediation of heavy metals by PGPR**

PGPR can also be exploited for the phytoremediation of heavy metals. Metal contamination is due to industrialization and agricultural activities. Soil contamination with heavy metals leads to blocking of molecules and disruption of structure and function of protein/enzymes.
Heavy metals also hamper biochemical processes like photosynthesis and respiration resulting stunting of plant growth. The solubility of metals is minimal in the soil and roots of the plants are unable to uptake metals from the soil. The availability of metals is directly related to the properties of soil and the metabolites (organic acid, siderophores and phytohormones) released by rhizobacteria (Zhuang et al., 2007). Rhizobacteria modify the bioavailability of plant and directly influence plant growth dynamics. Plant growth promoting rhizobacteria also play indirect role in chelation, acidification, immobilization, or precipitation of heavy metals in the soil. They are instrumental in phytoremediation of metal contaminated soils (Glick, 2003). The supplementation of metal tolerant rhizobacteria to degraded soil which is devoid of essential nutrients and thereby affecting the soil health increases the accessibility of vital plant nutrients and uptake of heavy metals from contaminated soil. This symbiotic relationship between plants and rhizobacteria or endophytic fungi enable phytoremediation process and thereby acting as decontaminators and checking soil contamination. These reframe texture and fertility of soil. PGPR another very significant function is that they aid in phytostimulation process. They have the property of disintegrating the contaminant and biologically convert pollutants into volatile compounds that are liberated into the environment. They have the innate ability to transform heavy metals into inactive compounds through immobilization, mobilization or transformation or upliftment of heavy metals (Nies, 1999). They alter the solubility, accessibility and translocation and uptake of heavy metals by decreasing the soil pH and by production and liberation of chelators (Ma et al., 2011).

2.6 Biofertilizers

Fertilizers provide the plant with adequate nutrients. N₂- fixing bacteria such as *Rhizobium* and *Bradyrhizobium* form nodules on leguminous plants roots such as soybean, pea, peanut,
and alfalfa, which fixes atmospheric nitrogen (van Rhijn and Vanderleyden, 1995). *Azospirillum* is a nitrogen fixer that forms symbiotic relationship with wheat, sorghum, and maize. The yield increase is attributed to inoculation by *Azospirillum* primarily due to increase root development and thus to increased rates of water and mineral uptake (Ben et al., 1997). Inadequate levels of available phosphate can inhibit plant growth. Plant-growth promoting bacteria solubilize phosphate from organic or inorganic phosphates, which aids plant growth (Lipton et al., 1987; Vassilev et al., 2006).

### 2.7 Rhizoremediators

Degradation of soil contaminants by rhizobacteria is very effective in the laboratory, but are not very effective in bulk soil, as their chief metabolism is dependent on disruption of the contaminant. They waste away soon after inoculation and then become ineffective in contaminant degradation (Bottiglieri and Keel, 2006). A hopeful strategy to diffuse this predicament is to uncouple the energy needed for prime metabolism, from the energy required for contaminant disruption. Kuiper et al. (Kuiper et al., 2001) developed a system called rhizoremediation (Kuiper et al., 2004). The plan included, choosing a rhizobacteria that thrive in and around the roots and are contaminant degrading to develop a system to competently augment such rhizobacteria by utilizing a crude mixture of bacteria from grass roots and PQQ: Pyrrolquinoline quinine IAA: indole-3-acetic acid or auxin flashing for selecting for growth on the contaminant naphthalene and selecting for resourceful colonization of grass roots (Kuiper et al., 2001). Consequently, *P. putida* PCL1444, successfully utilized root exudates, disrupted naphthalene around the root, protected seeds from being destroyed by naphthalene, and allowed the plant to grow.
2.8 Phytostimulators

Rhizobacteria produce biochemicals that trigger the growth of plants. Plant hormones such as indole acetic acid and cofactor pyrrolquinoline quinone (PQQ) stimulate plant growth. The root-growth-promoting hormone auxin, is always produced from the amino acid tryptophan which is the precursor for indole acetic acid production. The concentration of tryptophan differs from plant to plant (Kravchenko et al., 2004). Treatment of seeds with the auxin-generating P. fluorescens WCS365 resulted considerably in an increase in the root or shoot weight of cucumber, sweet pepper, or tomato, and results in considerable increase in the root weight of radish. Azotobacter paspali, the nitrogen fixing bacterium isolated from a subtropical grass species, increases growth of innumerous dicotyledonous and monocotyledonous host plants. Experiments were carried to understand nitrogen fixation mechanisms which brought to light the fact that production of phytohormones such as IAA, gibberellins, and cytokinins, are the main contributors to plant growth and cannot be attributed to mere nitrogen fixation (Ben et al., 1997). Few rhizobacteria, such as strains from B. subtilis, B. amyloliquefaciens, and Enterobacter cloacae, also contribute to plant growth by liberating volatiles (Ryu et al., 2003). Zhang et al. (Zhang et al., 2008) found that B. subtilis GB03 improves the photosynthetic efficiency and chlorophyll content of A. thaliana via alteration of signaling of glucose and abscisic acid. It could also be inferred that the rhizobacteria plays a cardinal role in the acquisition of energy for the host plant. The cofactor PQQ can be attributed for plant growth promotion (Choi et al., 2008). Cofactor PQQ promotes growth and physiological development of tomato and cucumber plants. The studies propose that PQQ also acts as an antioxidant. It can be very conveniently concluded that the effect of PQQ is meandering because PQQ is a cofactor of innumerable enzymes, e.g., involved in antifungal activity and initiation of systemic resistance.
2.9 Stress Controllers

Plant-growth-promoting rhizobacteria, having enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, aid in plant growth and development, by considerably reducing the ethylene levels. Rhizobacteria which take up the ethylene precursor ACC, transform it into 2-oxobutanoate, releasing ammonia. Different kinds of stress are mitigated by ACC deaminase producers, such as impacts of phytopathogenic fungi and stress from polyaromatic hydrocarbons, uptake of heavy metals such as Ca$^{2+}$ and Ni$^{2+}$, and from salinity and drought (Glick et al., 2007).

![Fig. 9: Impact of PGPRs against drought, salt and fertility stresses](image)

The indirect progression of plant physiological development and growth enhancement occurs when PGPR suppress completely or partially the undesirable effects of one or more phytopathogenic microbes and is able to withstand any stress condition. The underlying principle is secreting antagonistic compounds or by conferring competition to pathogens (Glick, 1995). PGPR, act as biocontrol agents, by employing several mechanisms, irrespective of their use in direct growth promotion like synthesis of auxin phytohormone, indirectly lowering plant ethylene levels (Glick et al., 2007) and fixing nitrogen for roots.
PGPR and their association with plants are milked commercially and are poised as very convincing strategy for sustainable agriculture.