CHAPTER ONE

GENERAL INTRODUCTION AND REVIEW OF LITERATURE

1.1. Introduction

In the present era, one of the major global issues is world food hunger. There is a continuous demand of food supply for the world population which is increasing at an alarming rate. The Declaration of the World Summit on Food Security (FAO, 2009) calls for an average annual increase in food production of 44 million metric tons to feed approximately 9 billion people by 2050 (Godfray et al., 2010). It is well known that sustained crop productivity relies on constant supply of nutrients which is mainly applied in the form of inorganic fertilizers containing phosphate, nitrate, ammonium and potassium. In the last five decades, the rate of nitrogen, phosphorus, and potassium (NPK) fertilizer application has increased tremendously. The International Fertilizer Industry Association reported that the three countries with the highest fertilizer use in 2006 were China, India, and USA, consuming 50.15, 21.65, and 20.83 million tons of NPK fertilizer, respectively, compared with consumption in 1961 of 1.01, 0.42, and 7.88 million tons, respectively. Although the application of chemical fertilizers has enhanced soil fertility and crop productivity, it often negatively affect the complex system of biogeochemical cycles, including nitrate leaching into ground water, run-off of nitrogen and phosphorous, and eutrophication of aquatic system (Smolders et al., 2010). Therefore, integrated nutrient management systems are needed to maintain agricultural productivity and protect the environment. Due to the emerging demand for reduced dependence on synthetic chemical products and for the growing necessity of sustainable agriculture within a holistic vision of development and environmental protection, the plant growth promoting rhizobacteria (PGPR) have gained worldwide importance and acceptance for their agricultural benefits.

PGPR are the soil bacteria inhabiting around/on the root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere. The term PGPR includes three types of soil bacteria, depending on their lifestyle: free-living bacteria inhabiting the zone around the root (rhizosphere), bacteria that colonize the root surface (rhizoplane), and endophytic bacteria that live within roots. However, this division is not exclusive, since any individual bacterial strain may adopt any of the three life strategy depending on the soil environment conditions and the host-root partner involved (Alavi et al., 2013; Mitter et al., 2013). PGPR enhance plant growth and development by a number

of mechanisms. They have either direct mechanisms to facilitate uptake of nutrient, availability of nutrient through nitrogen fixation, solubilisation of complex organic nutrients, and production of phytohormones or indirect mechanisms to produce antibiotics, siderophores, hydrogen cyanide (HCN), hydrolytic enzymes etc. (Ahemad and Kibret, 2014). Although the plant growth promotion capacity of PGPR can be more easily determined under controlled conditions using sterile substrates, the inoculated PGPR in soil may compete with other microflora with loss of their positive effects (Sturz and Christie, 1995).

Crop productivity has fundamental dependence on inorganic nitrogen (N) fertilization. During the past five decades, the application of N fertilizers has resulted in greatly increased global food production and decreased world hunger. According to the report of the United Nations Food and Agricultural Organization, the World population under synthetic N-fertilizer is continuously increasing and in 2015, out of the total world population of 7.38 billion, 3.54 billion population were supported by synthetic fertilizers (Fig 1.1).

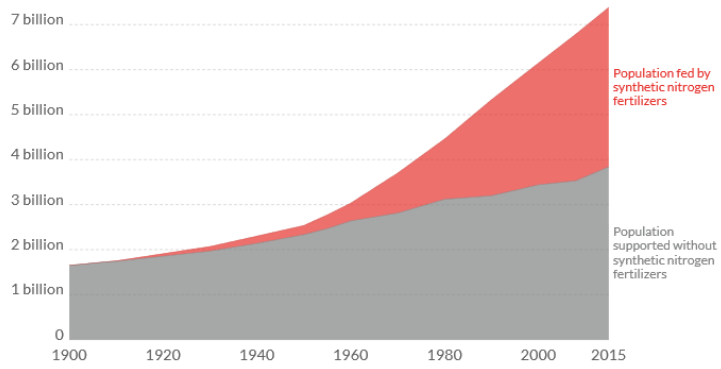

Image courtesy: Max Roser and Hannah Ritchie (2020)

Fig 1.1. World population supported with and without synthetic nitrogen fertilizers

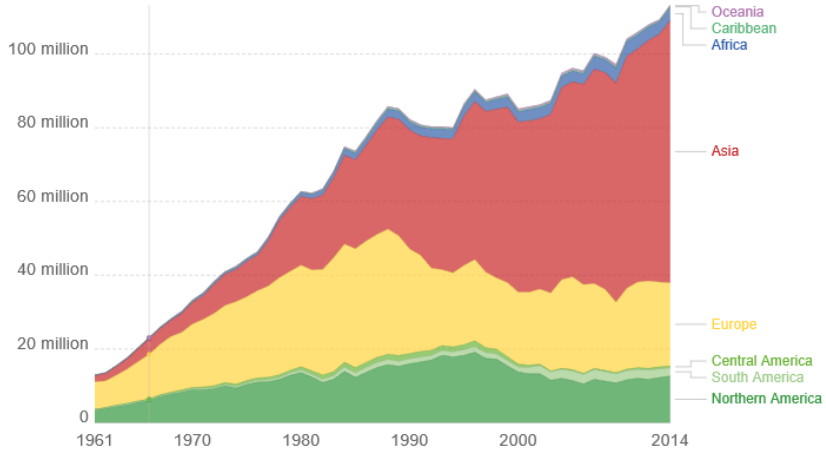

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Fig 1.2. Global Nitrogen fertilizer production (tonnes of nitrogen produced per year)

The global production of N-fertilizers increased from 80 million tonnes per year in 2000 to more than 100 million tonnes per year in 2014 (Fig 1.2). Approximately 100 million metric tons (MMt) of nitrogenous fertilizers are added to soil worldwide annually, which is predicted to increase to 240 MMt by the year 2050 (Tester and Langridge 2010; Sharma et al., 2017). Consequently, world population fed by plants grown on synthetic nitrogenous fertilizers has increased considerably. Currently, the application of N fertilizers in India has reached to more than 100 kg per hectare of cropland (Fig 1.3) (http://www.fertilizer.org/ifa).

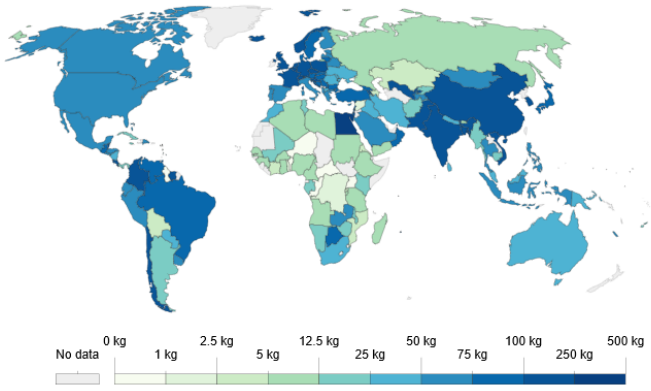

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Fig 1.3. Nitrogen fertilizer use per hectare of cropland, 2017 (Kg of total nutrient per hectare)

The increased application of inorganic nitrogenous fertilizers in agriculture has economic consequence because it is one of the major costs associated with the production of high-yielding crops. Moreover, the excess use of N-fertilizers causes significant environmental damage due to their inefficient uptake by the plants and as a result only 30 to 65% of the applied N being utilized. Excess N compounds released from agricultural systems as N_2, trace gases or leached nitrate threaten the quality of air, water, and soil. Increased soil leaching into drainage water and the release of atmospheric nitrous oxide and reactive N gases (NO*x*) into the troposphere can lead to acidification of soils, eutrophication of waterways and death of aquatic life (Ottman and Pope, 2000; Diaz and Rosenberg 2008; Xu et al. 2012). Moreover, nitrate (NO_3^-**)** that accumulates in harvestable vegetative organs has been considered as a source of potential danger to human health.

Present study was undertaken with the aim to reduce the inorganic N input in soil with the aid of PGPR with enhancing or maintained plant yield. The research work involved isolation of PGPR from rhizospheric soil followed by selection of two potent PGPR for application to mustard (*Brassica campestris* L.) plants. For plant applications the nutrient formulations (NF) containing various levels of N and PGPR, were optimised by general method and by the statistical tool, namely, Response Surface Methodology (RSM). The plants treated with the NFs were evaluated for morphological and biochemical and yield parameters. Finally, the RSM optimised NF treated plant with highest yield was subjected to transcriptomic analysis.

1.2. Objectives

The scope of the investigation was confined to the following objectives:

1. To isolate bacteria from rhizospheric soil.
2. To determine plant growth promotion (PGP) traits of rhizobacterial isolates and their morphological and biochemical characterization.
3. To screen the potent plant growth promoting rhizobacteria (PGPR) on the basis of qualitative and quantitative tests of various plant growth promoting (PGP) attributes.
4. Phylogenetic characterization of the potent PGPR.
5. To formulate general and RSM based nutrient formulations containing potent PGPR and various levels of nitrogen and application to mustard plant.
6. To evaluate the performances of the plants under various treatment regimes by morphological, physiological and biochemical parameters.
7. Comparative transcriptomic analysis of mustard plant roots.

1.3. Review of Literature

This chapter is an effort to elucidate the concept of PGPR in the current scenario and their underlying mechanisms of plant growth promotion with recent updates. The latest paradigms of a wide range of applications of these beneficial rhizobacteria in different agro-ecosystems have been presented explicitly to garner broad perspectives regarding their functioning and applicability.

1.3.1. Rhizosphere

Architecture of the root plays a crucial role in plant uptake of water, nutrients and minerals and to provide anchorage in the soil. The root is a dynamic structure with growth and branching depending on the continuous incorporation of internal and environmental factors (Asari et al., 2016). The narrow zone of soil directly surrounding the root system is known as rhizosphere (Walker et al., 2003). It is a nutrient rich habitat that harbours a huge variety of microorganisms having `neutral, beneficial or deleterious effects on the plant. The term 'rhizobacteria' implies a group of rhizospheric bacteria competent in colonizing the root environment (Kloepper et al., 1991). Plant roots synthesize, accumulate, and secrete a diverse array of compounds (Table 1.1) as root exudate, which act as chemical attractants for a vast number of heterogeneous, diverse and actively metabolizing soil microbial communities (Walker et al., 2003). The composition of root exudate is dependent upon the physiological status and species of plants and microorganisms (Kang et al., 2010). The root exudate carry out wide range of chemical and physical modifications to the soil; and acts as chemo-attractant or chemo-repellent and hence, regulate the diversity of actively metabolizing soil microbial communities (Walker et al., 2003) as well as the structure of microbial community in the immediate vicinity of root surface (Dakora and Phillips, 2002). Moreover, these exudates support the beneficial symbiotic interactions of plants and microbes (Nardi et al., 2000). On the other hand, microorganisms present in the rhizosphere determine the plant rooting patterns and nutrient availability, thereby modifying the quality and quantity of root exudates. They also metabolize a fraction of plant-derived small organic molecules in the vicinity as carbon and nitrogen sources, and some microbe-oriented molecules are subsequently absorbed up by plants for growth and development (Kang et al., 2010). In a previous report, Marschner (1995) reported that carbon fluxes of plants are critical determinants of rhizosphere function and approximately 5–21% of photosynthetically fixed carbon is transported to the rhizosphere through root exudation process. Hence, the rhizosphere can be defined as any volume of soil which is influenced by plant roots and/or in association with roots hairs, and plant produced compounds (Dessaux et al., 2009). Furthermore, three large and separate but interacting components are recognized in the rhizosphere namely, the rhizosphere, the rhizoplane, and the root itself. Of these, the rhizosphere is that zone of soil in association of roots that

affect microbial activity by the release of substrates. The rhizoplane, on the other hand, is the root surface including the strongly adhering soil particles while the root itself is a component of the system, because many micro-organisms like endophytes also colonize the root tissues (Barea et al., 2005).Microbial colonization of the rhizoplane and/or root tissues by the microbes is known as root colonization, whereas colonization of the adjacent volume of soil under the influence of the root is known as rhizosphere colonization (Barea et al.,2005; Kloepper et al., 1991; Kloepper, 1994). In a broad aspect, these can be separated into extracellular (ePGPR) existing in the rhizosphere, on the rhizoplane, or in the spaces between cells of the root cortex, and intracellular (iPGPR), which exist inside root cells, generally in specialized nodular structures (Figueiredo et al., 2011). Some examples of ePGPR include A*grobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcous*, *Pseudomonas* and*Serratia* etc. (Bhattacharyya and Jha, 2012). Similarly, some examples of the iPGPR are *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium* belonging to the family Rhizobiaceae.

Table 1.1.
Chemical compounds present in root exudates from various plant species

Types of chemical compounds	Names of the compounds
Sugars	Glucose, Galactose, Fructose, Xylose, Maltose, Rhamnose, Arabinose, Raffinose, Deoxyribose, Ribose
Amino acids	α-Alanine, β-alanine, Glycine, Asparagine, Aspartate, Cystein, Cystine, Glutamate, Isoleucine, Leucine, Lysine, Methionine, Serine, Threonine, Proline, Valine, Tryptophan, Ornithine, Histidine, Arginine, Homoserine, Phenylalanine, γ-Aminobutyric acid, α-Aminoadipic acid
Organic acids	Citric acid, Oxalic cid, Malic acid, Fumaric acid, Succinic acid, Acetic acid, Butyric acid, Valeric acid, Glycolic acid, Piscidic acid, Malonic acid, Formic acid, Aconitic acid, Lactic acid, Glutaric acid, Tetronic acid, Aldonic acid, Erythronic acid, Pyruvic acid,
Vitamins	Biotin, Thiamine, Pantothenate, Riboflavin, Niacin
Enzymes	Acid/alkaline phosphatase, Invertase, Amylase, Protease
Purines /nucleosides	Adenine, Guanine, Cytidine, Uridine
Inorganic ions and gaseous molecules	HCO_3^- , OH^-, H^+ CO_2.H_2, CO_2, H_2O
Secondary metabolites	Phenolics, Flavonoids, Terpenoids, Jasmonicacid, Salicylic acid, Brassinosteroids, Auxins, Cytokinins, Gibberellins, Ethylene and Abscissic acid

1.3.2. Plant Growth Promoting Rhizobacteria (PGPR)

PGPR are the soil bacteria that are found predominantly around or on the root surface and are directly or indirectly involved in promoting plant growth and development via multiple plant growth promoting mechanisms. In 1909, Bottomley firstly reported the use of soil bacteria to promote plant growth apart from in a *Rhizobium*–legume symbiosis. The study showed that a consortium of *Pseudomonas radicicola* and *Azotobacter* sp. increased the the yield of barley (*Hordeum vulgare* L.), growth of oat (*Avena sativa*), and the bulb weight of summer hyacinth (*Galtonia candicans*) (Bottomley, 1909). These effects were ascribed to an increase in nitrogen (N) availability. However, the term PGPR was adopted almost 70 years later, at the Annual Meeting of the American Phytophatological Society (Kloepper and Schroth, 1979), where the mechanism of PGPR function was suggested to be via modification of the soil microflora. A year later, Kloepper et al. (1980) proposed that PGPR produce siderophores, which remove iron from the soil and reduce the growth of deleterious soil microorganisms. Although the definition of PGPR by Martínez-Rodríguez et al. (2014) included non-soil microorganisms that inhabit the aerial parts of the plants, this specific category of bacteria bring benefits to the plant through some positive root-microbe interactions (Zhou et al., 2015). They stimulate plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them. They also improve soil structure and bioremediating the polluted soils as they are capable of sequestering toxic heavy metal species and degrading xenobiotic compounds like pesticides. Indeed, the bacteria inhabiting around/in the plant roots are more efficient in transforming, mobilizing, solubilizing the nutrients than those from bulk soils (Hayat et al., 2010). Therefore, the rhizobacteria are the efficient and dominant driving forces in recycling the soil nutrients and thus are vital for soil fertility (Glick, 2012). Currently, the biological approaches for improving crop production are gaining strong status among agronomists and environmentalists following integrated plant nutrient management system. In this context, rigorous research are ongoing worldwide with greater momentum to explore a wide range of rhizobacteria possessing novel traits like heavy metal detoxifying potentials (Ma et al., 2011; Wani and Khan, 2010) pesticide degradation/tolerance (Ahemad and Khan, 2012), salinity tolerance (Mayak et al., 2004; Tank and Saraf, 2010), biological control of phytopathogens and insects (Joo et al., 2005; Hynes et al., 2008; Russo et al., 2008) along with the normal plant growth promoting properties such as, phytohormone (Ahemad and Khan, 2012; Tank and Saraf, 2010), 1-aminocyclopropane-1-carboxylate deaminase synthesis, hydrogen cyanate (HCN) and ammonia production, siderophore production (Jahanian et al., 2012; Tian et al., 2009), nitrogenase activity (Glick, 2012; Khan, 2005), phosphate solubilisation (Ahemad and Khan, 2012), trace element solubilisation (Kumar et al., 2012) etc. Hence, diverse symbiotic (*Rhizobium, Bradyrhizobium, Mesorhizobium*) and non-symbiotic (*Pseudomonas, Bacillus, Klebsiella, Azotobacter, Azospirillum, Azomonas*), rhizobacteria are now being used worldwide as bio-inoculants to promote plant growth and

development under various stresses like salinity (Mayak et al., 2004), herbicides (Ahemad and Khan, 2011; Ahemad and Khan, 2010), insecticides (Ahemad and Khan, 2011), fungicides (Ahemad and Khan, 2012; Ahemad and Khan, 2011), heavy metals (Ma et al., 2011; Wani and Khan, 2010) etc.

1.3.3. Mechanisms of growth promotion of plants by PGPR

The beneficial effect of PGPR on plant growth and yield has been reported in several research works (Bergottini et al., 2015). PGPR employ various direct and indirect mechanisms to stimulate plant growth and development. They have direct mechanisms to facilitate uptake of nutrient, availability of nutrient through nitrogen fixation, solubilisation of complex organic nutrients, and production of phytohormones (Santoro et al., 2015; Grobelak et al., 2015; Gupta et al., 2015). They also indirectly promote plant growth by producing antibiotics, siderophores, HCN, hydrolytic enzymes etc (Fig. 1.4). Hence, the rhizobacteria constitute leading driving forces in revitalizing the soil health and are important for soil fertility (Babalola and Glick, 2012; Ahemad and Kibret, 2014).

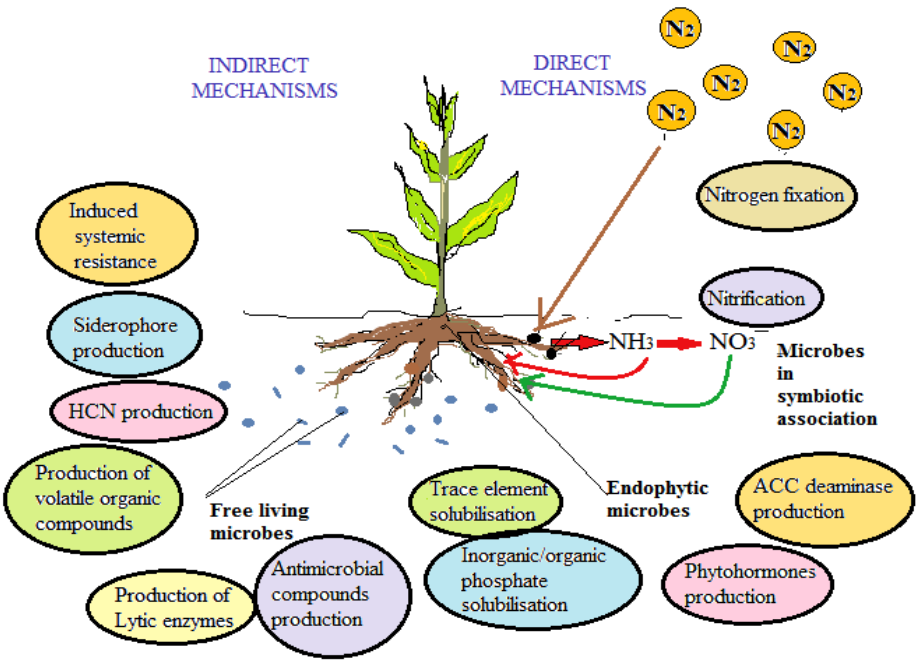

Fig 1.4. Direct and Indirect Mechanisms of action by PGPR on plant growth and development

1.3.3.1 Direct mechanisms of plant growth promotion by PGPR

The direct mechanisms of plant growth promotion by PGPR involve increased nutrient uptake and availability by nitrogen fixation, solubilisation of phosphate, potassium and trace elements, mineralization of organic compounds and phytohormone production.

1.3.3.1.1. Nitrogen fixation

Nitrogen (N) is a fundamental nutrient for plant growth and productivity. The atmospheric N_2 content is about 78%, however, it is unavailable to the growing plants directly. The atmospheric N_2 can be converted into plant utilizable forms by the biological process i.e. nitrogen fixation which involves conversion of atmospheric N_2 to ammonia by N_2-fixing microorganisms (Kim and Rees, 1994). N_2 fixation is a beneficial process which is an economical substitute to chemical fertilizers (Ladha et al., 1997). In the world, approximately two-thirds of the N_2 fixed are produced by biological processes whereas the remaining is synthesized industrially by the Haber-Bosch process (Rubio and Ludden, 2008). N_2-fixers are generally grouped as (a) symbiotic N_2-fixing bacteria and (b) non-symbiotic (free living and endophytes). Symbiotic N_2-fixers forms symbiotic association with leguminous (e.g. *Rhizobium* species) and non-leguminous (e.g. *Frankia*) plants (Ahemad and Khan, 2012; Zahran, 2001). The non-symbiotic N_2-fixing bacteria include cyanobacteria (*Anabaena, Nostoc*), *Azotobacter, Azospirillum, Azocarus, Gluconoacetobacter diazotrophicus* (Bhattacharyya and Jha, 2012); but they can add only a small amount of the fixed N_2compared to the total N requirement of the plant (Glick, 2012). Symbiotic N_2-fixing rhizobia under the family rhizobiaceae establish an infection thread that leads to their symbiotic relationship with the roots of leguminous plants. This symbiosis involves a complex interaction between the host and symbiotic microbes resulting in the formation of the nodules wherein the rhizobia colonize as intracellular symbionts (Fig. 1.5). Diazotrophs are the free living N_2 fixers in non-leguminous plants that are capable of establishing a non-obligate interaction with the host plants (Glick et al., 1999). N_2-fixing microorganisms carry out nitrogen fixation by the help of a complex enzyme system known as nitrogenase (Raymond et al., 2004, Kim and Rees, 1994). The complex structure of nitrogenase reported by Dean and Jacobson (1992) is a two-component metalloenzyme consisting of (i) dinitrogenase reductase which is an iron protein and (ii) dinitrogenase which has a metal cofactor. Dinitrogenase requires the electrons produced by dinitrogenase reductase to reduce N_2 to NH_3. Depending on the metal cofactor, three different N fixing systems have been identified (a) Mo-nitrogenase, (b) V-nitrogenase and (c) Fe-nitrogenase. Structurally, N_2-fixing system varies among different bacterial genera. Most nitrogen fixation is carried out by the activity of the molybdenum nitrogenase, which is found in all diazotrophs (Bishop and Jorerger, 1990). The genes for nitrogen fixation, called nif genes are found in both symbiotic and free living systems (Kim and Rees, 1994). Nitrogen fixation is a very energy demanding process which requires at least 16 mol of ATP for each mole of reduced nitrogen (NH_3).

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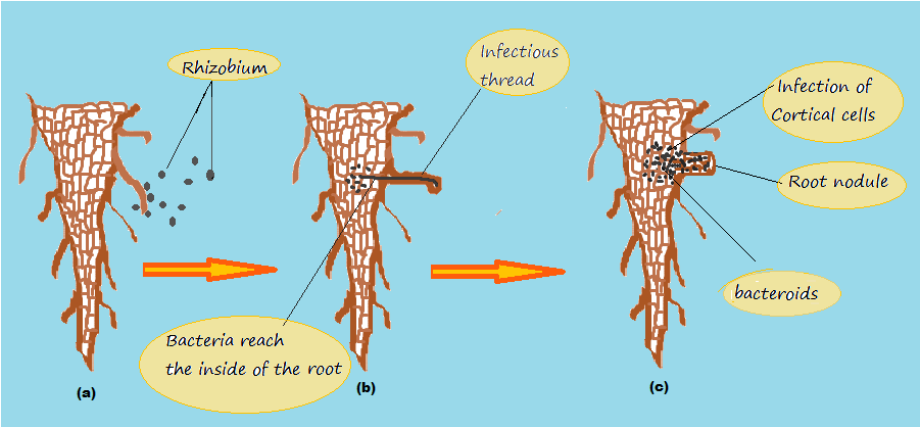

Fig 1.5. The nodulation process (a) Attachment of *Rhizobium* with root cells. (b) Secretion of nod factors by rhizobia causing root hair curling and formation of infectious thread through which the bacteria penetrate the cortical cells and (c) Formation of nodules

1.3.3.1.2. Phosphorous solubilisation

Phosphorus (P), the second important plant growth-limiting nutrient after N, is profusely available in soils in both organic and inorganic forms (Khan et al., 2009). In spite of soil being a large reservoir of P, the amount available to plants is generally low. The insoluble nature of P is an important factor for its unavailability to the plants. The plants absorb P only in two soluble forms, the monobasic (H_2PO_4) and the diabasic (HPO_4^{2-}) ions (Bhattacharyya and Jha, 2012). The insoluble P in soil is present either in the form of inorganic minerals, like apatite or as one of several organic forms, such assoil phytate (inositol phosphate), phosphomonesters and phosphotriesters (Glick, 2012). To overcome the deficiency of P in soils, there are frequent applications of phosphate fertilizers in crop fields. However, regular application of P-fertilizers is expensive and environmentally adverse. Plants can absorb only a fraction of applied P-fertilizers and the rest is rapidly converted into insoluble complexes in the soil (Mckenzie and Roberts, 1990). This problem has led to look for an ecologically safe and cost effective alternative for improving crop production in low P soils. In this context, organisms with phosphate solubilising activity, often termed as phosphate solubilizing microorganisms (PSMs), may provide the available forms of P to the plants and hence, can be a potent substitute to chemical P-fertilizers (Khan et al., 2006). Of the various PSMs inhabiting the rhizosphere, phosphate-solubilizing bacteria (PSB) are considered as promising biofertilizers since they can supply plants with P from sources otherwise poorly available by various mechanisms (Zaidi et al., 2009). The bacterial genera, *Azotobacter, Bacillus, Beijerinckia, Enterobacter, Erwinia, Flavobacterium, Microbacterium, Pseudomonas, Rhizobium* and *Serratia* are reported to be the most significant PSB (Bhattacharyya and Jha, 2012). The

PSB secrete low molecular weight organic acids and carry out solubilisation of insoluble P (Zaidi et al., 2009). On the other hand, the mineralization of organic P occurs through the expression of a variety of different phosphatases by PSB, which catalyse the hydrolysis of phosphoric esters (Glick, 2012). The traits of phosphate solubilization and mineralization can exist altogether in the same bacterial strain (Tao et al., 2008). Though PSB are commonly found in most soil but their presence and performances are strictly affected by environmental factors especially under stress conditions (Ahemad and Khan, 2010; Ahemad and Khan, 2012). There are several reports wherein the effects of inoculation of PSB alone or in consortia are mentioned (Zaidi and Khan, 2005; Poonguzhali et al., 2008; Chen et al., 2008; Ahemad and Khan, 2012). Besides providing P to the plants, the PSB also augment the growth of plants by stimulating the efficiency of biological nitrogen fixers (BNF) and enhancing the availability of other trace elements by synthesizing important plant growth promoting substances (Suman et al., 2001; Ahmad et al., 2008; Zaidi et al., 2009)

1.3.3.1.3. Potassium solubilisation

After N and P, potassium (K) is the most important plant nutrient to play a key role in the plant growth, metabolism and development. K enhances plant resistance to diseases, pests, and abiotic stresses and activates over 80 different enzymes responsible for various plant processes such as energy metabolism, starch synthesis, nitrate reduction, photosynthesis and sugar degradation (Almeida et al., 2015; Cecílio Filho et al., 2015; Gallegos-Cedillo et al., 2016; Mumtaz et al., 2017; White and Karley, 2010; Yang et al., 2015). Depending on soil type, 90 to 98% of soil K is in mineral forms and most of them are not available for plant uptake (Sparks and Huang, 1985). PGPR could solubilize the insoluble K to soluble forms by various mechanisms including production of inorganic and organic acids, acidolysis, chelation and exchange reactions (Etesami et al., 2017). During K solubilization, the major mechanisms involved are the production of the organic acids, inorganic acids and protons (acidolysis mechanism) (Sheng et al., 2008; Maurya et al., 2014; Meena et al., 2014) which are able to convert the insoluble K (in the form of mica, muscovite, and biotite feldspar) to soluble forms that plants can uptake (Hu et al., 2006; Meena et al., 2014; Mo and Lian, 2011). In acidolysis mechanism, the released H^+ can help to dissolve the mineral K resulting in the slow release of readily available exchangeable K. Several organic acids such as oxalic, tartaric, gluconic, 2-ketogluconic, citric, malic, succinic, lactic, propionic, glycolic, malonic, and fumaric acid, have been reported to be produced and secreted by K solubilising bacteria, which are effective in releasing K from insoluble mineral-K (Krishnamurthy, 1989; Hu et al., 2006; Sheng and He, 2006; Liu et al., 2012; Prajapati et al., 2012; Prajapati et al., 2013; Saiyad et al., 2015).

1.3.3.1.4. Trace elements solubilisation

Zinc (Zn) is an essential micronutrient required for optimum plant growth. Plants can uptake Zn in the form of divalent cation (Zn^{2+}) (Kabata-Pendias and Pendias, 2001), however, only small proportion of total Zn is present as soluble form in the soil. Rest of the Zn is present in the form of insoluble complexes and minerals (Alloway, 2008) resulting in reduced availability to plants. Hence, the plant deficiency of Zn happens to be one of the most vital micronutrient deficiency. To overcome Zn deficiency, Zn fertilizers like $ZnSO_4$ (White and Broadly, 2005) or Zn-EDTA (Karak et al., 2005) are applied in agricultural lands. However, their usage has economic and environmental concern. After a week of application of Zn fertilizers, the chemical forms are converted into insoluble forms and remains in the soil (Rattan and Shukla, 1991). Hence, Zn solubilizing bacteria are potential alternatives for Zn supplementation that convert applied insoluble Zn to available forms which can be absorbed by the plants.

There are several PGPR that have been found to be effective Zn solubilizers. These bacteria colonize the rhizosphere and solubilize complex Zn compounds into simpler ones and thus making Zn available to the plants. Zn solubilizers have several mechanisms to solubilise Zn. One of such mechanisms is acidification process in which they produce organic acids to sequester the Zn^{2+} and decrease the pH of the nearby soil (Alexander, 1997). According to another report, the anions can also chelate Zn and enhance Zn solubility (Jones and Darrah, 1994). Zn solubilisation mechanisms involve the production of siderophores and proton (Saravanan et al., 2011). Further, oxidoreductive systems on cell membranes and chelated ligands are also responsible for Zn solubilisation (Chang et al., 2005; Kamran et al., 2017). Various bacterial genera showing enhanced Zn content and growth when applied to plants are *Pseudomonas, Rhizobium* strains (Deepak et al., 2013; Naz et al., 2016), *Bacillus aryabhattai* (Ramesh et al., 2014), *Bacillus* sp. (Hussain et al., 2016), and *Azospirillum*. Under laboratory condition, various bacterial strains viz. *Pseudomonas aeruginosa* (Fasim et al., 2002), *Gluconacetobacter diazotrophicus* (Saravanan et al., 2007), *Serratia liquefaciens, S. marcescens*, and *Bacillus thuringiensis* (Ullah et al., 2015), *Bacillus* sp., *Pseudomonas striata, Pseudomonas fluorescence, Burkholderia cenocepacia* (Pawar et al., 2015) were reported to show Zn solubilisation. These strains have been reported to increase Zn content of straw and grains in soybean and wheat (*Triticum aestivum*), enhancing food efficacy and dealing with deficiency of Zn. Vaid et al. (2014) reported the beneficial effect of inoculation of Zn solubilizing bacteria on rice plant growth and increased Zn nutrition (42.7%) of grains.

1.3.3.1.5. Phytohormones production

Phytohormones are the low molecular weight endogenous secondary metabolites which not only activate an effective defence response against both biotic and abiotic stresses but also act as regulators of growth, development and physiological processes of the plants.

PGPR produce several phytohormones such as auxins, cytokinins, giberellin, ethylene and abscissic acid. They add to the pre-existing hormone levels of the plant and have significant effects on overall plant growth and development. Microbial synthesis of the phytohormone auxin (indole-3-acetic acid, IAA) has been known for a long time. It is also known as a signalling molecule in some microorganisms (Bianco et al., 2006; Lui and Nester, 2006; Spaepen et al., 2007). PGPR produce auxins in order to affect host physiological processes for their own benefit (Shih-Yung, 2010). Patten and Glick (1996) reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins. IAA has been responsible in every aspect of plant growth and developmental processes as well as defence mechanisms. Generally, IAA is responsible for plant cell division, extension, and differentiation. It stimulates seed/tuber germination and also increases the rate of xylem and root development. Bacterial IAA provides the plant greater access to soil nutrients by increasing the root surface area and length. In addition as reported by Glick in 2012, rhizobacterial IAA loosens plant cell walls and helps in increasing the amount of root exudation providing an additional nutrients for growth of rhizobacteria. Therefore, rhizobacterial IAA is recognized as an important effector molecule in plant–microbe interactions (Spaepen and Vanderleyden, 2011). This variable range of functions is portrayed by the complexity of IAA biosynthesis, signalling and transport pathways (Santner et al., 2009). Tryptophan is an important amino acid that affects the synthesis of IAA as it is the main precursor for IAA biosynthesis (Zaidi et al., 2009). In one hand tryptophan being a precursor for IAA synthesis and on the other hand anthranilate which is a precursor for tryptophan, reduces IAA synthesis. By this mechanism, IAA biosynthesis is finely regulated by tryptophan with a negative feedback regulation on the enzyme anthranilate synthase and inhibiting anthranilate formation, and ultimately results in an indirect induction of IAA synthesis (Spaepen et al., 2007). Nevertheless, IAA production by most of the rhizobacteria increases when the culture media is supplemented with tryptophan (Spaepen and Vanderleyden, 2011). Cell division, differentiation and vascular bundle formation are essential for nodule formation and as IAA is involved in all these three processes. Hence, it can be concluded that IAA levels on plants are necessary for nodule formation (Spaepen et al., 2007; Glick, 2012). In one of the work by Camerini et al., 2008, inoculation of *Vicia hirsute* with *Rhizobium leguminosarum* bv. viciae wherein the IAA biosynthetic pathway had been introduced, produced root nodules containing 60-fold more IAA than nodules formed by the wild-type strain.

There are other phytohormones involving in the effects of rhizobacteria in plants. These include abscisic acid (ABA), cytokinins (CKs) and gibberellins (GAs). But these are not well studied. The excretion rate of root exudates containing amino acids along with other compounds as presented in table 1.1 is increasing the beneficial effects of CK levels in the plant to the PGPR (Kudoyarova et al., 2014). ABA is known for senescence or ageing of the plants. Although a very little amount is required for the growth as it helps in regulating

the stomata aperture and hence, transpiration rate and CO_2 uptake (Pospisilova, 2003). Some PGPR can reduce the levels of ABA and minimize the ageing process, thereby, increasing the plant growth (Belimov et al., 2014). Sometimes PGPR also increases the level of ABA content of the plant under water deficit conditions and resulting in reduction of water loss (Salomon et al., 2014). Furthermore, in 2014, Porcel et al. reported that when tomato plants deficient in ABA were inoculated with a *Bacillus megaterium* strain, there is a reduction in growth mainly due to an overproduction of ethylene. As a consequence, the positive effects of PGPR depend on the endogenous levels of ABA of the host plant.

1.3.3.1.6. Production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase

Ehtylene is also known as stress hormone other than its role in regulation of plant growth and development. The plant endogenous level of ethylene is considerably increased under stress conditions like drought, salinity, water logging, heavy metal contamination and pathogenicity which affects the overall performance of the plants in a negative way. As for example, the high level of ethylene in plants enhances defoliation and other cellular processes which can cause less crop yield (Saleem et al., 2007; Bhattacharyya and Jha, 2012). Plants treated with PGPR having the enzyme ACC deaminase show increased growth and development. ACC deaminase induces salt tolerance and reduces drought stress in plants by reducing the level of ethylene. (Nadeem et al., 2007; Zahir et al., 2008). Various bacterial genera have been identified with ACC deaminase production trait (activity). These are *Acinetobacter, Achromobacter, Alcaligenes, Azospirillum, Bacillus, Burkholderia, Enterobacter, Pseudomonas, Serratia* and *Rhizobium* etc. (Shaharoona et al., 2007; Nadeem et al., 2007; Zahir et al., 2008; Kang et al., 2010). The mechanism of action of ACC deaminase activity is breaking down ACC, an important precursor for ethylene production, into 2-oxobutanoate and NH_3 (Arshad et al., 2007). Effects of phytopathogens like viruses, bacteria and fungi are reduced by ACC deaminase producers. Not only that, these bacteria also relieve the stress from heavy metals, radiation, polyaromatic hydrocarbons, insect attack, high temperature, high light intensity and water-logging condition (Glick, 2012; Lugtenberg and Kamilova, 2009). The main effects of the seed or root inoculated/treated with ACC deaminase producing PGPR are root elongation, shoot growth, enhancement of root nodulation, mineral uptake and mycorrhizal colonization in many crop plants (Shaharoona et al., 2007; Nadeem et al., 2007; Glick, 2012).

1.3.3.2. Indirect mechanisms

In general, the main mechanisms of biocontrol activity of PGPR are competition for nutrients, niche exclusion, production of antifungal metabolites and siderophores and induced systemic resistance (Lugtenberg and Kamilova, 2009). Many rhizobacteria have been reported to produce antifungal metabolites like, HCN, 2,4-diacetylphloroglucinol, tensin, pyrrolnitrin, pyoluteorin, phenazines and viscosinamide (Bhattacharyya and Jha, 2012). Interaction of some rhizobacteria with the plant roots can leads to plant resistance

against some phytopathogens like bacteria, fungi, and viruses. The phenomenon of gaining resistance in the plant is called induced systemic resistance (ISR) (Lugtenberg and Kamilova, 2009). Furthermore, ISR involving ethylene signalling and jasmonic acid within the plants can stimulate the defence responses of the host plant against a variety of phytopathogens (Glick, 2012). There are various microbial components, such as lipopolysaccharides (LPS), flagella, cyclic lipopeptides, homoserine lactones, 2,4-diacetylphloroglucinol, siderophores and volatiles like 2,3-butanediol and acetoin that can induce ISR (Lugtenberg and Kamilova, 2009).

1.3.3.2.1. Siderophore production

Iron is an important nutrient for almost all forms of life. Almost all microorganisms essentially require iron except lactobacilli (Neilands, 1995). Under aerobic condition, iron occurs mainly in the form of Fe^{3+}, which is likely to form insoluble hydroxides and oxyhydroxides making it unavailable to both plants and microorganisms (Rajkumar at al., 2010). PGPR obtain iron by secreting siderophores which are low molecular weight iron-chelating ligands having high affinity for iron produced under low iron stress (Verbon et al., 2017; Kumar et al., 2016). Production of siderophore has dual role, firstly, it helps in iron nutrition by acting as a solubilising agents for insoluble iron complexes and secondly, it inhibits phytopathogen. Siderophore producing PGPR compete for Fe^{3+} with the pathogens and thus create iron deficiency leading to the death of pathogen (Khurana and Sharma, 2000; Sharma and Kaur, 2010; Schiessl et al., 2017; Shaikh et al., 2016). Siderophores are usually water-soluble and can be divided into two types viz. extracellular siderophores and intracellular siderophores. Based on the ability of rhizobacteria for cross utilization of siderophore, there may be homologous siderophore (rhizobacteria proficient in using siderophores of the same genus) or heterologous siderophores (rhizobacteria with the ability to utilize siderophores produced by different genera) (Khan et al., 2009). Iron is present in the form of Fe^{3+} siderophore complex with both gram positive and gram negative bacterial membrane. The reduction of Fe^{3+} to Fe^{2+} lead to release of iron from the complex into the cell via a gating process which linked the bacterial inner and outer membranes. The reduction process either destroys the siderophore or may recycle it to capture more iron (Neilands, 1995; Rajkumar et al., 2010). Siderophores is not only form stable complex with Fe but also form complexes with other heavy metals like Al, Cd, Cu, Ga, In, Pb and Zn (Kiss and Farkas, 1998; Neubauer et al., 2000). There are different mechanisms by which plants assimilate iron from bacterial siderophores, for example chelation and release of iron, uptake of siderophore-Fe complexes directly, or by a ligand exchange reaction (Schmidt, 1999). There are numerous studies on plant growth promotion via siderophore-mediated Fe-uptake, obtained by inoculations of siderophore-producing rhizobacteria (Rajkumar et al., 2010). For example, a siderophore mediated iron transport system is reported in oat plants and rhizobacteria producing siderophores which delivers iron to the plant under iron-limited conditions (Crowley and Kraemer, 2007). Similarly, *Pseudomonas*

fluorescens C7 synthesised Fe-pyoverdine complex that was taken up by *Arabidopsis thaliana*. This led to an increase in plant iron content and improved plant growth (Vansuyt et al., 2007).

1.3.3.2.2. Production of Hydrogen cyanide (HCN)

HCN is produced during the initial stationary growth phase of bacteria. Though it does not play a role in growth, storage of energy or primary metabolism, but is generally play a significant ecological role and a selective advantage is conferred on the HCN producing strains (Vining, 1990). About 90% of *Pseudomonas* sp. have a common trait of HCN production (Ahmad et al., 2008). Cyanide is considered as one of the typical features of deleterious rhizobacteria as it is a phytotoxic agent capable of inhibiting the main enzymes involving in vital metabolic processes (Bakker and Schippers, 1987). However, its application as biocontrol agent is increasing (Devi et al. 2007). HCN produced in the rhizosphere of seedlings by selected rhizobacteria can be an effective mechanism of biocontrol. Therefore, HCN producing rhizobacteria have potential as ecofriendly way to control weeds and minimize adverse effects on the growth of the crop plants (Kremer and Souissi, 2001).

1.3.3.2.3. Production of protective enzymes

PGPR produce a number of metabolites that control phytopathogenic agents and promote plant growth (Meena et al., 2016). They produce enzymes such as chitinase, β-1,3-glucanase, and ACC-deaminase which are generally involved in degradation of cell walls and neutralizing pathogens (Goswami et al., 2016). Almost all fungal cell walls are constituted of β-1,4-N-acetyl-glucosamine and chitin and therefore, β-1,3-glucanase and chitinase-producing bacteria control fungal growth. *Fusarium oxysporum* and *Fusarium udum* causing fusarium wilt can be inhibited by *Sinorhizobium fredii* KCC5 and *Pseudomonas fluorescens* LPK2 which can produce beta-glucanases and chitinase (Ramadan et al., 2016). Islam et al. (2016) reported that *Phytophthora capsici* and *Rhizoctonia solani*, the most disastrous crop pathogens in the world, are also inhibited by PGPR.

1.3.3.2.4. Disease resistance antibiosis

For sustainable agriculture use of microbes producing antagonistic compounds against phytopathogens has been recommended as an alternative to chemical pesticides. PGPR, like *Bacillus* sp. and *Pseudomonas* sp., capable of producing antibiotics play a significant role in inhibiting pathogenic microorganisms. Over the past 20 years, antibiotic synthesis by PGPR against several phytopathogens has become one of the most studied and effective mechanism of biocontrol (Ulloa-Ogaz et al., 2015). It is reported that most of the species of *Pseudomonas* genera produce a wide variety of (a) antifungal antibiotics viz. phenazines, phenazine-1-carboxamide, phenazine-1-carboxylic acid, 2,4-diacetylphloroglucinol,

pyrrolnitrin, rhamnolipids, pyoluteorin, cepaciamide A, viscosinamide, oomycin A, ecomycins, butyrolactones, N-butylbenzenesulfonamide, pyocyanin (b) bacterial antibiotics like azomycin and pseudomonic acid (c) antitumor antibiotics (e.g. cepafungins and FR901463) and (d) antiviral antibiotics (Karalicine) (Ramadan et al., 2016). Further, the antibiotics can be grouped into volatile compounds including aldehydes, alcohols, ketones, hydrogencyanide, sulfides, etc., and non-volatile compounds, including aminopolyols, cycliclipopeptides, phenylpyrrole, polyketides, heterocyclic nitrogenous compounds etc. (Fouzia et al., 2015). *Bacillus* species are also capable of producing a wide variety of antifungal and antibacterial lipopeptide antibiotics, like surfactin, iturins, and bacillomycin (Wang et al., 2015).

1.3.3.2.5. Induced systemic resistance

Induced systemic resistance (ISR) is the term to define the physiological state of improved defensive capacity evoked in plants in response to a particular environmental stimulus. Induced systemic resistance in many plants is raised by PGPR against several environmental stresses (Prathap and Ranjitha, 2015) especially during phytopathogen invasion. During pathogenic invasion, signal molecules are produced and a defense mechanism is activated via the vascular system resulting in the activation of a vast number of defense enzymes viz. β-1,3-glucanase, chitinase, peroxidase, phenylalanine ammonia lyase, polyphenol oxidase, lipoxygenase, superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidise (APX) along with some proteinase inhibitors. Kamal et al. (2014) reported that ISR functions in a broadway and is not specific against a particular pathogen. ISR involves the signalling of ethylene hormone within the plant that induces the defense responses of a host plant against a variety of phytopathogens. A variety of individual bacterial components also induce ISR, such as cyclic lipopeptides, homoserine lactones, lipopolysaccharides, siderophores, 2,4-diacetylphloroglucinol, and volatiles, like acetoin and 2, 3-butanediol (Berendsen et al., 2015).

Table 1.2.
Effect of PGPR on various plants

Organisms	IAA production	Phosphate solubilization	Phytase production	Application on crops	Result on crops	References
Bacillus Amyloliquefaciens FZB45	D.A	D.A	0.37 U mL^{-1}	*Zea mays* cv. Elita	Shoot and root wt increased, root length increased, support plant growth at low phosphate cond.	Idriss et al., 2002
Pseudomonas	D.A	24.7-44 mg $100ml^{-1}$	D.A	Chinese cabbage	D.A	Poonguzhali et al., 2008
Bacillus	17 µg mL^{-1}	23 mg mL^{-1} TCP 20 mg mL^{-1} DCP 19 mg mL^{-1} ZP	D.A	D.A	D.A	Pankaj et al., 2012
B.subtilis	7 µg mL^{-1}	D.A	D.A	D.A	D.A	Mohite, 2013
Lactobacillus casei	2 µg mL^{-1}	D.A	D.A	D.A	D.A	Mohite, 2013
B.cereus	6 µg mL^{-1}	D.A	D.A	D.A	D.A	Mohite, 2013
Pseudomonas fluroscens	15.38±0.537	D.A	D.A	Onion	D.A	Reetha et al. 2014
Bacillus subtilis	12.67±0.325	D.A	D.A	onion	D.A	Reetha et al., 2014
Bacillus	50.3 µg mL^{-1}	9.77 mg 100 mL^{-1}	D.A	Mungbean	D.A	Kaur and Sharma, 2013
Pseudomonas	55.6 µg mL^{-1}	9.14 mg 100 mL^{-1}	D.A	Mungbean	D.A	Kaur and Sharma, 2013
Azotobacter	52.6 µg mL^{-1}	8.98 mg 100 mL^{-1}	D.A	Mungbean	D.A	Kaur and Sharma, 2013
Advenella sp. PB6	25 µg mL^{-1}	~620 µg mL^{-1}	0.174 U mL^{-1}	*Brassica*	Increased root length	Singh et al., 2014
*Advenella*sp. PB10	35 µg mL^{-1}	~650 µg mL^{-1}	0.161	*Brassica*	Increased shoot length	Singh et al., 2014
*Cellulosimicrobim*s p. Pb9	10 µg mL^{-1}	746 µg mL^{-1}	0.129 U mL^{-1}	*Brassica*		Singh et al., 2014
Streptomyces cellulosae mhcr0816	136.5 mg L^{-1}	1916 mg L^{-1}	0.68 U mL^{-1}	*Triticum aestivum*	improved plant growth, biomass (33 %) and mineral (Fe, Mn, P) content	Jog et al., 2014
Pseudomonas. aeroginosa	3.6 mg L^{-1}	D.A	D.A	*Zea mays* L.	D.A	Kumar, 2015
Azotobacter chrooccum	3 mg L^{-1}	D.A	D.A	*Zea mays* L.	D.A	Kumar, 2015
Azospirillum brasilense	2.3 mg L^{-1}	D.A	D.A	*Zea mays* L.	D.A	Kumar, 2015
Streptomyces sp.	1.9 mg L^{-1}	D.A	D.A	*Zea mays* L.	D.A	Kumar, 2015

Enterobacter cloacae	8.98±0.46 mg L^{-1}	D.A	D.A	D.A	D.A	Haiyambo, 2015
Stenotrophomonasmaltophilia	3.6 mg L^{-1}	D.A	D.A	D.A	D.A	Haiyambo, 2015
Pseudomonas veronii	4.5 mg L^{-1}	D.A	D.A	D.A	D.A	Haiyambo, 2015
P. validus	7.0 mg L^{-1}	D.A	D.A	D.A	D.A	Haiyambo, 2015
Baciliussubtilis	7.2 mg L^{-1}	D.A	D.A	D.A	D.A	Haiyambo, 2015
Bacillus licheniformis	7.2 mg L^{-1}	D.A	D.A	D.A	D.A	Haiyambo, 2015
Bacillusamyloliquefaciens	5.5 mg L^{-1}	D.A	D.A	D.A	D.A	Haiyambo, 2015
Pseudomonas fluorescens L228	D.A	1312 mg L^{-1}	D.A	*Pisum sativum*	Improved plant growth	Oteino et al., 2015
Aneurinibacillusaneurinilyticus CKMV1	8.1 µg mL-1	230 mg/l	D.A	tomato	Shoot and root length increased, Dry wt of shoot and root increased	Chauhan et al., 2017
MRS34	28.41 µg mL^{-1}	23.9 µg mL^{-1}	D.A	*Zea mays*	D.A	Manzoor et al., 2016
Enterobacter cloacae	12.125 µg mL^{-1}	D.A	D.A	*Triticum aestivum*	contributed to increase lengths of roots and shoots	Kamran et al., 2017
Bacillus sp.	D.A	D.A	60 µmol L^{-1}	*Zea mays* cv Jidan3	Increased plant height and biomass	Liu et al., 2018
Pseudomonas koreensis MS16	25.6±1.40 µg mL^{-1}	0.5 mg mL^{-1}	D.A	*Triticum aestivum*	Increased plant biomass and grain yield	Suleman et al., 2018
Enterobacter cloacae MS32	28.1±1.23 µg mL^{-1}	0.270 mg mL^{-1}	D.A	*Triticum aestivum*	Increased plant biomass and grain yield	Suleman et al., 2018

D.A= Data absent

1.3.3.2.6. Production of volatile organic compounds

Volatile Organic Compounds (VOCs) produced by rhizobacteria are low molecular weight (MW) compounds with < 300 g mol^{-1} MW and high vapour pressure. These include aldehydes, alcohol, ketones, acids, terpenes and hydrocarobons (Bhattacharyya et al., 2017; Fincheira and Quiroz, 2018). There is a direct relation between VOCs with ISR (Shafi et al., 2017). There are other types of VOCs like indole, 2,3-butanediol, 3-hydroxy-2-butanone (acetoin), cyclohexane, 2-(benzyloxy) ethanamine, benzene, benzene(1-methylnonadecyl), methyl, 1-chlorooctadecane, decane, 1-(N-phenylcarbamyl)- 2-morpholinocyclohexene, dodecane, 2,6,10-trimethyl dotriacontane, 11-decyldocosane, tetradecane, mixture of volatile compounds including Caryophyllene, which can promote the growth of plants, although the identity and quantity of the VOCs emitted vary among species (Ryu et al., 2003, Minerdi et al., 2011; Bailly and Weisskopf, 2012; Kanchiswamy et al., 2015). However, in the absence of pathogens they can also promote plant growth and confer tolerance against abiotic stresses (Bhattacharyya et al., 2015). In 2003, Ryu et al. confirmed the effect of plant promotion by the VOCs by using bacterial mutants which are unable to produce these VOCs or by the application of the pure compounds. VOCs like 2-pentylfuran was shown to increase fresh weight of *Arabidopsis. thaliana*, with an optimum dose of 10 g (Zou et al.,2010). Bailly and Weisskopf (2012) proposed that most of the studies dealing with the effect of VOCs on plant have been carried out using *A. thaliana* as a target for bacterial volatile compounds. Some of the compounds have been proven to promote growth but the actual reason is not clear whether it is due to a specific VOC. Further, many VOCs has inhibitory effects on the growth of plants at high dose and even some are toxic also (Bailly and Weisskopf, 2012). Therefore, it has become important to elucidate the signalling cascades and subsequent metabolic changes that are triggered in the plant by VOCs or VOCs producing PGPR (Ahemad and Kibret, 2014). Several bacterial species from diverse genera producing VOCs include *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Serratia* and *Stenotrophomonas* that enhance plant growth. In 2016, Santoro et al. mentioned that 2, 3-Butanediol and acetoin produced by *Bacillus* sp. are most effective for inhibiting fungal growth and promoting growth of the plants. It has been reported by Sharifi and Ryu, (2016) that bacterial VOCs are important determinants for eliciting plant ISR.

1.3.4. Nitrogen Use Efficiency of plants

The deleterious effect of excess N in the environment is not an unknown fact. Rather there should be improvement in the Nitrogen Use efficiency (NUE) of the plants which has become a challenge nowadays. There are several interacting genetic and environmental factors and so NUE is inherently a complex entity. The definition of NUE itself is also complex, and the term can mean different things in different contexts, including N use efficiency (NUE), N uptake efficiency (NUpE), N utilization (assimilation) efficiency (NUtE). NUtE is defined as total seed yield relative to total shoot N content (Moll et al.,

1982) and is affected by several physiological factors, including N uptake, metabolism, allocation, and remobilization (Girondé et al., 2015). Apparent N recovery rate (ANR), agronomy efficiency of fertilizer N (AE), N physiological use efficiency (NpUE), N transport efficiency (NTE), and N remobilization efficiency (NRE). Table 1.3 shows the definitions of all the terms. Mainly two plant physiological components: NUpE and NUtE that contribute to plant NUE.

Table 1.3.
Definitions of various terms related to plant's efficiency of N use

Terms	Meanings and formula
N use efficiency (NUE)	The total biomass or grain yield produced per unit of applied fertilizer N; it is an integration of NUpE and NUtE NUE= NUpE+NUtE
N uptake efficiency (NUpE)	The capacity of plant roots to acquire N from the soil (commonly referred to as the percentage of fertilizer N acquired by plant) NUpE= % of fertilizer N acquired by plant
N utilization (assimilation) efficiency (NUtE)	The fraction of plant acquired N to be converted to total plant biomass or grain yield NUtE=total plant biomass or grain yield
Apparent N recovery rate (ANR)	The ratio of net increased total N uptake by the plant with and without N fertilization to total amount of fertilizer N ANR= (N uptake by plant with N fertilization)-(N uptake w/o N fertilization) / Total amount of fertilizer N
Agronomy efficiency of fertilizer N (AE)	The ratio of net increased grain weight of the plant with and without N fertilization to total amount of fertilizer N AE= (Grain weight of the plant with fertilization)-(Grain weight of the plant w/o N fertilization) / Total amount of fertilizer N
N physiological use efficiency (NpUE)	The ratio of net increased grain weight to net increased N uptake with and without application of fertilizer N NpUE=Grain weight of the plant with fertilization -Grain weight of the plant w/o N fertilization / (N uptake by plant with N fertilization)- (N uptake w/o N fertilization) =ANR/AE
N transport efficiency (NTE)	The ratio of total N transported into the above ground parts to total N in the whole plant NTE = Total N transported into the above ground parts / Total N in the whole plant
N remobilization efficiency (NRE)	The ratio of N remobilization from source or senescent leaves to that of sink leaves or developing grains (seeds) NRE= N remobilization from source or senescent leaves / N of sink leaves or seeds

1.3.5. Plant nitrogen acquisition

Nitrate is the chief form of inorganic N in aerobic soil whereas ammonium being the major form in flooded field or acidic soils. The redox potential of the soil is influenced by the release of oxygen from root, which in turn accomplish the interconversion of soil N forms, including those derived from fertilizer. The concentration of nitrate and ammonium in the soil is very dynamic and heterogenous. The range varies from 100 µM to 10 mM. To adapt with the fluctuative situation, plant roots have uptake systems with different affinities for nitrate and ammonium. The uptake system consists of a number of membrane proteins that participate in the acquisition of N involving nitrate uptake, compartmentation, translocation, and remobilization by the plants (Dechorgnat, 2011). Each high and low-affinity nitrate transport system is composed of constitutive and nitrate-inducible components (Forde, 2000). The acquisition of N by the roots is affected by the root architecture, concentration of nitrate and ammonium in the soil, transporters systems as well as temperature fluctuations.

1.3.6. Nitrogen assimilation

Acquisition of N is followed by the assimilation process. In some plants, a very small amount of nitrate is assilmilated into the roots but larger portion of the absorbed N is assimilated in the shoot parts of the plants. Nitrate assimilation is carried out by the cytosolic nitrate reductase that catalyses the reduction of nitrate to nitrite which is further reduced to ammonia by nitrite reductase in the plastids. The ammonium derived either from nitrate or directly absorbed through ammonium transporters (AMTs) is further assimilated into amino acids via the GS/glutamine-2-oxoglutarate aminotransferase (GOGAT) cycle (Pinheiro and Chaves, 2011). The predominant GS/GOGAT isoenzymes are chloroplastic GS2 and Fd-GOGAT and cytosolic GS1 and NADH-GOGAT.

1.3.7. Nitrogen Transportation and Remobilization

The transportation of nitrate to different parts of the plants is conducted by the nitrate (NO_3^-) transporters systems. In *Arabidopsis,* there are two closely related low affinity nitrate transporters (NRT 1s) viz. AtNRT1.5 and AtNRT1.8 which are responsible for the loading and unloading of the nitrate into the root stele or from the shoot. The loading of nitrate into the root phloem is facilitated by another type of transporter AtNRT1.9 present in the companion cells which enhances downward transport of nitrate in roots. During vegetative stage of the plants, N is excessively present in the leaves. As the plant matures and proceeds towards senescence this N is remobilized to the developing seeds. A less intensive study has been done on the uptake of ammonium (NH_4^+) than nitrate uptake. Many species of plants using nitrate transporters systems can also have an efficient system for absorption of ammonium ions. This transporter systems are known as AMT (AtAMT in *Arabidopsis*) and express constitutively at high level of NH_4^+ (Yuan et al., 2007).

AMT can transport N either in the form hydrophobic NH_3 or charged ammonium. Glass et al. in 2002 reported that AMT1 family of high-affinity NH_4^+ transporters contains five members. Out of which AtAMT1.1, AtAMT1.2, and AtAMT1.3 have been extensively studied. Plant species and the type of transporters are important factors upon which the locations of the expression of transporter genes take place. According to various studies, in roots AtAMT1.2, AtAMT1.3 and AtAMT1.5 genes are expressed while only AtATM1.1 is expressed in leaf and root tissues (Forde and Clarkson, 1999; Glass et al., 2002; Khademi et al., 2004). Also, the AtAMT transporters are found in different types of root tissues. According to Ludewlg et al. (2007), AtAMT1.1, AtAMT1.3, and AtAMT1.5 are localized in the cell membrane of rhizodermis cells, while AtAMT1.2 is present in the plasma membrane of cortical and endodermal cells.

According to Calvo (2019), AMT1.1, AMT1.2, AMT1.3, and AMT1.5 genes showed high transcript levels in *A. thaliana* plants treated with three different PGPR mixtures, which demonstrates that PGPR affect ammonium transport in different root tissues. The relative contribution of these transporters to nitrate uptake depends on the developmental stage of the root and the N status of the plant (Wang et al., 2012).

1.3.8. Role of PGPR in Nitrogen status of Plants

PGPR play significant role in promoting the nitrogen status in plants. In 1906, J.F. Breazeale demonstrated that wheat plants that were starved for nitrogen for the first 15 days after germination subsequently showed much higher capacities for absorbing nitrate than plants that had received sufficient N (Breazeale, 1906). Glutamine, the product of the first step in the pathway of N assimilation in bacteria and fungi as well as in plants (Lea and Miflin, 2018), is the organic form of N that has been generally considered to be a key effector in the sensing of the intracellular N status in many organisms. In microbes like *Aspergillus nidulans* and other filamentous fungi, when glutamine levels are high, pathways that are responsible for assimilating N sources such as nitrate are down-regulated through a process named 'nitrogen metabolite repression' (Crawford and Arst, 1993, Siverio, 2002, Pfannmüller et al., 2017). Plant growth is enhanced by PGP under the effect of multigenic processes, including nitrate (NO_3^-) and ammonium (NH_4^+) uptake genes, which could potentially describe the upgrade in plant nutrition and plant growth. In 2000, Bertrand et al. showed that a strain of *Achromobacter* spp. improved the rate of nitrate (NO_3^-) uptake of *Brassica napus* roots.

1.3.9. Response Surface Methodology (RSM)

Response surface methodology (RSM) is a collection of mathematical and statistical techniques that are useful for modelling and analysis in applications where a response of interest is influenced by several variables and the objective is to optimize this response (Montgomery and Runger, 2011). RSM is proved to a suitable mathematical and statistical

tool. The main aim of this approach is to optimize the response with minimum number of experiment. The response is called the dependable variables whereas the parameters that affect the responses are called the independent variable. The interaction effect between the independent variables are being analysed under optimal operating condition for a model or system to optimize the output or response variables (Azargohar et al., 2005). The two main experimental design which are used in RSM are Box-Behnken designs (BBD) and central composite design (CCD) (Bezerra et al., 2008; Zolgharnein et al., 2013). In very recently Central composite rotatable design (CCRD) with axial points and face central composite design (FCCD) has been used in RSM to give the model much flexibility and better optimize the response (Wang et al., 2018).The experimental data were analysed using the several statistical equation viz. Linear, Quadratic, Cubic or 2FI (two factor interaction) to fit in and generation in the model. Models are being generated with constant coefficient term such as liner coefficients for input variables (A, B, C etc.), interaction coefficient term (AB, AC, BC), quadratic coefficient term (A^2, B^2 and C^2). Coefficient of determinant (R^2) close to 1, adjusted R2>0.8, adequate precision (>4), model level of significance ($p<0.05$) and model lack of fit ($p>0.05$) are desirable to validate the model (Saha and Ghosh, 2014). Once the model is validated it can be used to predict the values of input variables in which optimum response will be achievable. Therefore, with minimum number of experiment in RSM as compared to quite large number of experiment in conventional approach, RSM help to reduce disadvantages associated with conventional method.