# Chapter 7
# Plant Growth-Promoting *Rhizobium*: Mechanisms and Biotechnological Prospective

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## 7.1 Introduction

The use of microbial agents for improving agriculture productivity depends on soil and plant health. Usually, the rhizospheric soil, inhabited and influenced by plant roots, is rich in nutrients, due to accumulation of amino acids, organic acid, fatty acids, phenols, plant growth regulators, sterols, sugars, and vitamins released as exudates, secretion, and deposition (Gopalakrishnan et al. 2015). The accumulation of simple and complex natural matter results in enrichment of soil (10–100-fold). Microbial flora includes bacteria, fungus, and algae along with protozoa, among which rhizospheric bacteria significantly influenced the plant growth. Rhizospheric bacteria can be further categorized according to their proximity and association with roots: (1) bacteria, which live near to root surfaces (rhizosphere); (2) group of bacteria colonizing the root surfaces (rhizoplane); (3) group of bacteria entering inside and residing in root tissues, inhabiting spaces between cortical cells (endophytes); and (4) group of bacteria living inside cells in specialized root structures known as root nodules.

The bacterial group belonging to these classes are referred to as plant growth-promoting rhizobacteria (PGPR). Bacteria belonging to categories 1–3 are further classified as extracellular plant growth-promoting rhizobacteria (ePGPR) and category 4 as intracellular PGPR (iPGPR). The ePGPR includes the genera *Bacillus, Pseudomonas, Erwinia, Caulobacter, Serratia, Arthrobacter, Micrococcus,*

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A.P. Hansen et al. (eds.), *Rhizobium Biology and Biotechnology*, Soil Biology 50,
DOI 10.1007/978-3-319-64982-5_7

*Flavobacterium, Chromobacterium, Agrobacterium*, and *Hyphomicrobium,* whereas iPGPR includes the genera *rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium*, and *Allorhizobium* (Gopalakrishnan et al. 2015). They are reported as the nonpathogenic soil-borne microorganisms which can promote plant growth, yield, and increased disease resistance. As the plant growth promotion considered being the results of improved and balance nutrient mobilization, along with hormone and metabolite production by plant growth-promoting rhizobia. They are the soil bacteria inhabiting around/on the root surface and are directly or indirectly involved in plant growth promotion in normal and stressed conditions.

The increased disease suppression can occur through microbial antagonistic mechanisms or the induction of systemic resistance (ISR) or systemic acquired resistance (SAR) in plants. Due to the use of PGPR, the global demand and dependence on hazardous agricultural chemicals, which disturbs the agro-ecosystem balance, were reduced drastically. The known species of *Rhizobium* (*Rhizobium, MesoRhizobium, BradyRhizobium, AzoRhizobium, AlloRhizobium*, and *SinoRhizobium*) have been widely used for effective establishment of the nitrogen-fixing symbiosis with leguminous crop plants (Bottomley and Maggard 1990). *Rhizobium* spp. are gram-negative soil bacteria that have a profound scientific and agronomic significance due to their ability to establish nitrogen-fixing symbiosis with leguminous plants, which is of major importance in the maintenance of soil fertility (Somasegaran and Hoben 1994). *Rhizobium* promotes growth by direct and indirect mechanisms (Tables 7.1 and 7.2).

## 7.2 Direct Promotions

### *7.2.1 Nitrogen Fixation*

The various biochemical reactions of BNF occurred through symbiotic association of $N_2$-fixing microorganisms with legumes that convert atmospherical elemental nitrogen ($N_2$) into ammonia ($NH_3$). Rhizobia are soil bacteria that colonize legume roots and induce nodules in which atmospheric nitrogen is converted into plant-available compounds. The number and diversity of indigenous rhizobia in the rhizosphere depend on a number of abiotic and biotic factors and proximity to other organisms (Karas et al. 2015). Various *Rhizobium* species, including *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, and *Sinorhizobium*), are in intimate symbiotic association with leguminous plants due to the chemotactic response to flavonoid metabolites released as signals by host plant. Such behavior results in the induction and expression of nodulation (nod) genes in *Rhizobium* species and leads to specific lipo-chitooligosaccharide signals (LCO), which trigger mitotic cell division and lead to the formation of nodules (Matiru and Dakora 2004). The nodulation on leguminous plants depends upon

**Table 7.1** Rhizobia as plant growth promoters—direct mechanisms

| *Rhizobium* sp./Growth promoting traits | Activity | References |
|---|---|---|
| *$N_2$ fixation* | | |
| *Bradyrhizobium* sp. (vigna)RM8 | Enhanced the nodule numbers, leghemoglobin, yield with high protein content along with shoot, root, and soil nitrogen | Wani et al. (2007a) |
| *Mesorhizobium* sp. RC3 | Higher dry matter accumulation, more number of nodules, yield with high protein content and enhanced shoot, root, and soil nitrogen | Wani et al. (2008) |
| *Rhizobium* sp. RP5 | More dry matter accumulation, more nodule, with high yield and protein content (P) | Wani et al. (2007b) |
| *Rhizobium leguminosarum* strain MRP1 | Enhanced growth, nodulation, and leghemoglobin content, increased N2 and P, high yield and seed protein content | Ahemad and Khan (2010a) |
| *Mesorhizobium* strain MRC4 | Significant increase in nodulation and leghemoglobin content, along with higher shoot and root nitrogen and phosphate content | Ahemad and Khan (2009a, 2010c) |
| *Siderophore production* | | |
| *B. Japonicum* | Siderophore production | Wittenberg et al. (1996) |
| *R. ciceri* | Siderophore production | Berraho et al. (1997) |
| *Rhizobium* BICC 651 | Produced a catechol siderophore to acquire iron under iron-poor condition | Datta and Chakrabartty (2014) |
| *Rhizobium meliloti* | Siderophore-producing strains and act as potential biocontrol agent against *Macrophomina phaseolina* that causes charcoal rot of groundnut<br>Siderophore production from "Stem nodule of *Aeschynomene indica*" (weed legume) | Ghorpade and Gupta (2016) |
| *Rhizobium nepotum* | Siderophore production for plant growth | Naik and Dubey (2011) |
| *Phytohormone production* | | |
| *Mesorhizobium ciceri* | IAA production | Wani et al. (2007c) |
| *Rhizobium leguminosarum* | IAA production | Dey et al. (2004) |
| *Rhizobium leguminosarum* | Cytokinin | Noel et al. (1996) |

(continued)

**Table 7.1** (continued)

| *Rhizobium* sp./Growth promoting traits | Activity | References |
|---|---|---|
| *PHB production* | | |
| *B. japonicum* | *nif*H, *nif*DK structural gene responsible for nitrogenase activity to fix and produce massive PHB accumulates | Hahn et al. (1984) |
| *Rhizobium elti, Pseudomonas stutzeri* | Production of poly-β-hydroxybutyric acid (PHB) | Belal (2013) |
| *Sinorhizobium leguminosarum bv. viciae, R. leguminosarum bv. leguminosarum* | Produces polyhydroxy butyrate (PHB) in sludge and in industrial wastewater | Rebah et al. (2009) |
| *Rhizobium* ORS571 | Large amounts of PHB are induced under conditions of oxygen limitation | Stam et al. (1986) |
| *Mesorhizobium* spp. | Exopolysaccharide secretion | Ahemad and Khan (2009a) |
| *Phosphate solubilization* | | |
| *Mesorhizobium mediterraneum* | Enhance growth and phosphate content in chickpea plant | Peix et al. (2001) |
| *Rhizobium* and *Bradyrhizobium* | P solubilization, produce high level of acid phosphatases, reduce pH of medium | Abd-Alla (1994) |
| *R. leguminosarum*<br>*R. meliloti* | Production of 2-ketogluconic acid with P-solubilizing ability | Halder and Chakrabarty (1993) |
| *Heavy metal mobilization* | | |
| (*Rhizobium* RL9) | Increase growth, nodulation, nitrogen, leghemoglobin yield in lentil plant against Pb and Ni metals | Wani and Khan (2012, 2013) |
| *R. leguminosarum* | Enhance plant growth and biomass in maize against Pb | Hadi and Bano (2010) |
| *S. meliloti* | Enhance biomass in black medic against Cu | |
| *ACC deaminase* | | |
| *R. japonicum, B. elkani, M. loti, R. leguminosarum, Sinorhizobium spp.* | Produce high level of ACC deaminase | Subramanium et al. (2015) |
| *R. leguminosarum* bv. *trifolii* SN10 | Produces indole acetic acid and ACC deaminase which enhances rice growth | Philippe et al. (2012) |

diverse factors such as plant–bacterial symbiont compatibility, physical and chemical composition of soil, and presence of differing bioactive molecules, viz., flavonoids, polysaccharides, and hormones associated with them (Hayat et al. 2010). Rhizobial infection occurs when bacteria enter into the root in a host-controlled manner and are then trapped inside the cavity of curling roots (Fig. 7.1).

The $N_2$ fixation process is carried out by enzyme, the nitrogenase complex (Kim and Rees 1994), which is a two-component metalloenzyme consisting of

**Table 7.2** Rhizobia as plant growth promoters—indirect mechanisms

| *Rhizobium* species/Growth promoting traits | Activity | References |
|---|---|---|
| *Biocontrol* | | |
| *R. leguminosarum bv. trifolii, R. leguminosarum bv. viciae, R. meliloti, R. trifolii* | Secretion of antibiotics and cell wall-degrading enzymes that inhibit phytopathogens | Chandra et al. (2007), Siddiqui and Mahmoud (2001), Siddiqui et al. (1998, 2000) |
| *P. fluorescens* and *S. meliloti* | Biocontrol agents to suppress pathogens in Alfalfa | Villacieros et al. (2003) |
| *B. japonicum, R. meliloti, and R. leguminosarum* | Biocontrol against pathogens such as *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* of Okra and sunflower | Ehteshamul-Haque and Ghaffar (1993), Ozkoc and Deliveli (2001), Siddiqui and Shaukat (2003) |
| *Induce systemic resistance* | | |
| *Rhizobial* strain RH 2 | Defense-related enzymes, *viz*., L-phenylalanine ammonia lyase (PAL), peroxidase (POX), and polyphenol oxidase (PPO) level, get increased which decreases the production of 1,3-glucanase and polymethyl galacturonase by the pathogen | Dutta et al. (2008) |
| *Rhizobium leguminosarum* bv. *viceae* FBG05 | Induction of systemic resistance in faba bean (*Vicia faba* L.) against bean yellow mosaic potyvirus (BYMV) | Elbadry et al. (2006) |
| *Rhizobium etli G12* | *Rhizobium* etli G12 reduces early root infection by the potato cyst nematode *Globodera pallida* | Hasky-Gunther et al. (1998) |
| *Rhizobium* strain | Systemic resistance (ISR) is induced in bean (*Phaseolus vulgaris* L.) mediated by rhizobacteria against bean rust caused by *Uromyces appendiculatus* | Osdaghi et al. (2009) |
| *Production of metabolites (volatile and nonvolatile antibiotics)* | | |
| *R. leguminosarum bv. trifolii, R. leguminosarum bv. viciae, R. meliloti, R. trifolii* | Secretion of antibiotics and cell wall-degrading enzymes that inhibit phytopathogens | Chandra et al. (2007), Ozkoc and Deliveli (2001), Siddiqui and Shaukat (2003), Siddiqui and Mahmoud (2001), Siddiqui et al. (1998, 2000) |
| *HCN production* | | |
| *Mesorhizobium* sp. | HCN production | Wani et al. (2008) |
| *Mesorhizobium* loti MP6 | HCN hydrocyanic acid production along with siderophore, IAA, enhances the seed and plant growth | Chandra et al. (2007) |

(continued)

**Table 7.2** (continued)

| *Rhizobium* species/Growth promoting traits | Activity | References |
|---|---|---|
| *Rhizobium species* | HCN production | Abd-Alla (1994), Tank and Saraf (2010) |
| *Bradyrhizobium sp.* | IAA, HCN, ammonia, siderophores, exopolysaccharides | Ahemad and Khan (2011c, d, e, 2012b) |
| *Lytic enzymes* | | |
| *Rhizobium* stain | Produce enzymes including chitinases, cellulases, ß-1,3 glucanases, proteases, and lipases that can lyse a portion of the cell walls of many pathogenic fungi | Frankowski et al. (2001), Kim et al. (2008), Singh et al. (1999) |
| *Abiotic stress* | | |
| *R. tropici co-inoculated with Paenibacillus polymyxa* | Enhancement of plant height, increase in shoot dry weight and nodule number (drought stress) | Figueiredo et al. (2008) |
| *Mesorhizobium spp.* | Overproduction of 60 kDa unknown protein (temperature stress) | Rodrigues et al. (2006) |
| *Rhizobium phaseoli* | Overcome the adverse effect of salinity in the presence of tryptophan, increase nodulation and yield | Zahir et al. (2010) |
| *R. loti* and *Bradyrhizobium* | *R. loti* multiplied at pH 4.5, but *Bradyrhizobium* strains failed to multiply at that pH | Cooper et al. (1985) |
| *R. tropici, R. meliloti, and R. loti* | *R. tropici* and *R. loti* are moderately acid tolerant and *R. meliloti* is very sensitive to acid stress | Vlassak and Vandurleyden (1997), Tiwari et al. (1992), Brockwell et al. (1991) |
| Rhizobial strain | Acid-tolerant alfalfa-nodulating strains of rhizobia, isolated from acidic soils, were able to grow at pH 5.0 and formed nodules in alfalfa with a low rate of nitrogen fixation | Del Papa et al. (1999) |
| *Bradyrhizobium* | The fast-growing strains of rhizobia are less tolerant to acid pH than slow-growing strains of *Bradyrhizobium* | Graham et al. (1994) |
| *R. meliloti* | Mutant strains of *R. meliloti are* competitive with naturalized alfalfa rhizobia and symbiotically effective under drought stress | Athar and Johnson et al. (1996) |

(continued)

**Table 7.2** (continued)

| *Rhizobium* species/Growth promoting traits | Activity | References |
|---|---|---|
| *Heavy metal stress* | | |
| *Rhizobium sp.* | Greater accumulation of HM in nodules than roots and shoots | Younis (2007) |
| *Bradyrhizobium RM8* | Enhance growth performance | Wani et al. (2007a, b) |
| *R. leguminosarum* | Enhance plant growth and biomass | Hadi and Bano (2010) |
| *Pesticide tolerance* | | |
| *Rhizobium MRP1* | Enhanced biomass (Herbicide Quizalafo-p-ethyl) | Ahemad and Khan (2010a, b) |
| *Rhizobium MRL3* | Leghemoglobin content, root and shoot N, root and shoot P, seed yield, and seed protein (Herbicide Clodinafop) | Ahemad and Khan (2010a, b) |
| *Rhizobium MRP1* | Concentration-dependent progressive decline in PGP substances except exopolysaccharides Fungicide Hexaconazole) | Ahemad and Khan (2011a, 2012a) |
| *Rhizobium* strain MRL3 | Exploited as a bio-inoculant to augment the efficiency of lentil exposed to insecticide-stressed soil insecticidal) | Ahemad and Khan (2011a, b) |

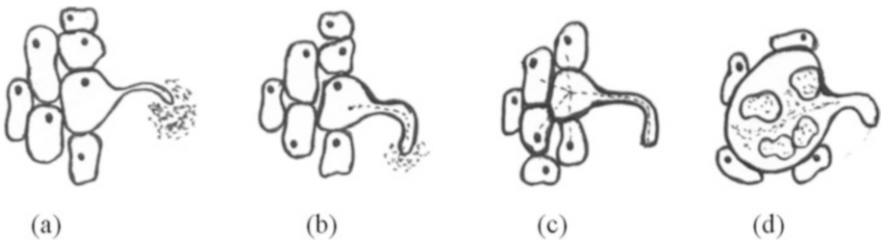


**Fig. 7.1** (**a**) Rhizobial infection to root, (**b**) trapping of bacteria to root curlings, (**c**) formation of infection thread by which *Rhizobium* reaches base of root, and (**d**) development of nodule primordium in the cortex of root into a nodule

(1) dinitrogenase reductase (iron protein) and (2) dinitrogenase (with metal cofactor). Usually, dinitrogenase reductase provides electrons with high reducing powers, while dinitrogenase uses these electrons to reduce $N_2$ to $NH_3$. Further based on metal cofactor, three different N-fixing systems are classified into Mo-nitrogenase, (b) V-nitrogenase, and (c) Fe-nitrogenase.

The *nif* genes responsible for $N_2$ fixation are found in both the symbiotic and free-living systems. The *nif gene* includes the structural genes, involved in the activation of Fe protein, Fe–Mo cofactor synthesis, electron donation, and few

regulatory genes essential for functioning of enzymes. In rhizobia, symbiotic activation of *nif* genes is dependent on the low oxygen level, which is regulated by a set of genes called fix-genes (Kim and Rees 1994). The $N_2$ fixation is a high-energy demanding process, which is supposed to require 16 moles of ATP for each mole of reduced nitrogen (Glick 2012). Thus, if the bacterial carbon resources can be directed toward oxidative phosphorylation, it results in the synthesis of ATP required by legume plants.

### 7.2.2 Siderophore Formation

The bacteria acquire iron by the secretion of low-molecular mass iron chelators referred to as siderophores, which have high association constants for complexing iron. Most of the siderophores are water soluble and can be divided into extracellular and intracellular siderophores. Generally, rhizobacteria differ regarding the siderophore cross-utilizing ability; some are proficient in using siderophores of the same genus (homologous siderophores), while others could utilize those produced by other rhizobacteria of different genera (heterologous siderophores). Plants assimilate iron from bacterial siderophores by different mechanisms, for instance, chelate and release of iron, the direct uptake of siderophore–Fe complexes, or a ligand exchange reaction (Schmidt 1999). Numerous studies of the plant growth promotion vis-a-vis siderophore-mediated Fe uptake as a result of siderophore producing rhizobacterial inoculations have been reported (Rajkumar et al. 2010; Ahemad et al. 2014). Siderophores act as solubilizing agent for iron in limiting conditions and can also form a stable complex with heavy metals, viz., Al, Cd, Pb, Zn along with radionuclides U and Np (Neubauer et al. 2000). Thus, the binding of bacterial siderophores to metal increases its solubility and can make it available to plants, which can help to alleviate the stress.

### 7.2.3 Phytohormone Production

Symbiotic and non-symbiotic bacteria can promote plant growth directly by the production of plant hormones (Dobbelaere et al. 2003). The rhizospheric bacteria possess the ability to synthesize and release auxins as secondary metabolites, which are further used by plants for developmental processes and in defense response (Patten and Glick 1996). *Rhizobium leguminosarum* were reported to produce growth hormone indole-3-acetic acid in rice associated with significant growth-promoting effects as inoculants on rice seedlings (Biswas et al. 2000). *Mesorhizobium loti* MP6 associated with *Brassica* along with IAA was reported to produce chrome-azurol, siderophore, and hydrocyanic acid, enhance germination, and increase vegetative growth and yield (Chandra et al. 2007).

The bacteria belonging to *Rhizobium* have been shown to produce auxins via indole-3-acetamide formation, and genes controlling IAA production have been reported (Ahemad and Khan 2011a, b). However, the synthesis of IAA by *Rhizobium* spp. in the presence and absence of tryptophan has been demonstrated (Wani et al. 2007b). The IAA produced by rhizobacteria increases the root surface area and length, which provides higher access to soil nutrients. In turn, IAA also loosened root walls to facilitate more root exudates, which support the growth of rhizosphere bacteria (Glick 2012). IAA affects plant physiology by plant cell division, extension, rate of xylem development, adventitious root formation, pigment formation, photosynthesis, etc.; thus, rhizobacterial IAA can act as effector molecules in plant–microbial interaction in pathogenesis and phytostimulation (Spaepen and Vanderleyden 2011). Rhizobia influence crop growth and development by changing the physiological status (Glick and Bashan 1997) and morphological characteristics of inoculated roots (Yanni et al. 1997).

*Rhizobium* strains are also reported as the potent producers of cytokinins during their establishment (Senthilkumar et al. 2008), which stimulate cell division and root development and root hair formation (Frankenberger and Arshad 1995). *Rhizobium* as PGPR is supposed to produce gibberellins (Boiero et al. 2007). Gibberellins are phytohormones (GA1–GA89) which are responsible for stem elongation and leaf expansion. It promoted bolting of the plants, parthenocarpy in fruits, increase in fruit size, breaking of tuber dormancy, and sex expression of flowers. *Rhizobium* sp. and *B. japonicum* were reported to produce abscisic acid (Boiero et al. 2007), which stimulates the stomatal closure, inhibits shoot growth, promotes root growth, increases storage proteins, and produces proteinase inhibitors essential to provide pathogen defense and counteract with gibberellins (Mauseth 1991).

### *7.2.4 PHB Production*

The carbon storage polymer poly-β-hydroxybutyrate (PHB) is a potential biodegradable alternative to plastics, which plays a key role in the cellular metabolism of many bacterial species. Most species of rhizobia synthesize PHB, but not all species accumulate it during symbiosis with legumes. The ability to accumulate PHB during symbiosis appears to be dependent on the physiology of the nodule formed by the host plant. Two major types of root nodules are formed in the rhizobia–legume symbiosis: (1) determinate nodules, which do not possess a persistent meristem and instead form a spherical-shaped structure, and (2) indeterminate nodules, which possess a continuous meristem resulting in a long, cylindrical structure (Hadri et al. 1998). *PhbB* and *PhbC* are key enzymes in the anabolic arm of the PHB cycle and are encoded on the *S. meliloti* chromosome. Both *phbB* and *phbC* mutants of *S. meliloti* strain Rm1021 are deficient in the ability to produce succinoglycan, resulting in dry, non-mucoid colonies when grown under carbon-rich conditions; this phenotype is not observed in PHB degradation mutants (Aneja et al. 2004).

### 7.2.5 Phosphate Solubilization

Phosphorus (P), the second important plant growth-limiting nutrient after nitrogen, is abundantly available in soils in both organic and inorganic forms. The P is required for differing metabolic processes, viz., energy transfer, signal transduction, biosynthesis of biomolecules, and plant physiology. Majority of P is unavailable due to its fixation with various elements in soil, thus remaining unavailable to plants. The phosphate-solubilization potential of *Rhizobium* (e.g., *Rhizobium*/*Bradyrhizobium*) was associated with the production of 2-ketogluconic acid and reduction of pH of the medium (Halder and Chakrabarty 1993). The ability of rhizobia to solubilize both organic and inorganic P has been exploited for increasing the yield of plants. The plant absorbs P in soluble forms, monobasic ($H_2O_4$) and diabasic ($HPO_4^{2-}$) ions, which is available by release of mineral dissolving compounds, e.g., organic acids anions, protons, hydroxyl ions, $CO_2$, liberation of extracellular enzymes, and then in turn release of P during substrate degradation (Sharma et al. 2013).

### 7.2.6 Synthesis of ACC Deaminase Enzyme

Usually, ACC deaminase production is reported in rhizospheric bacteria that can colonize the plant root (Belimov et al. 2001). Ethylene is a potent growth regulator in plants, which regulates ripening, promotes adventitious root, and stimulates germination by breaking seed dormancy (Esashi 1991). As higher ethylene concentration is toxic to plants (inhibits root elongation), the PGPR reduces its concentration by the activity of enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyzes ACC, the precursor of ethylene in plants (Yang and Hoffman 1984). The end product of this hydrolysis, ammonia and α-ketobutyrate, can be used by rhizobia/bacterium as sole sources of nitrogen and carbon essential for their growth (Klee et al. 1991; Gopalakrishnan et al. 2015). The various strains of rhizobia, such as *R. Leguminosarum* bv. *viciae*, *R. hedysari, R. japonicum, R. gallicum, B. japonicum, B. elkani, M. Loti*, and *S. meliloti*, were known to produce ACC deaminase (Gopalakrishnan et al. 2015). It has been confirmed that IAA producing bacteria are reported to produce high levels of ACC, which inhibit ethylene levels reported to promote plant growth, enhanced rhizobial nodulation, and mineral uptake (Glick 2012).

## 7.3 Indirect Promotions

The ability of biocontrol bacteria to indirectly promote plant growth has been the source of considerable interest, both in terms of (i) types of mechanisms used by the biocontrol bacteria and (ii) commercial use of such bacteria instead of chemical pesticides. In fact, these two objectives are largely complementary and are environment-friendly approach (Lugtenberg and Kamilova 2009). Besides induced resistance in plants, rhizobacteria are also known to be involved in an indirect mechanism by acting as biocontrol agents (Glick 2012). During their growth in rhizosphere, they compete for nutrients and niche exclusion. Induced systemic resistance (ISR) and antifungal metabolite (antibiotics) production are the predominant methods for controlling pathogenic or nonpathogenic competitors.

Biocontrol is a process through which a living organism limits the growth or propagation of undesired organisms or pathogens. Several rhizobial strains are reported to have the biocontrol properties. Hence, usage of these strains against soil-borne pathogens can lead to potential control. The mechanisms of biocontrol by rhizobia include competition for nutrients (Arora et al. 2001), production of antibiotics (Bardin et al. 2004; Chandra et al. 2007; Deshwal et al. 2003a), production of enzymes to degrade cell walls (Ozkoc and Deliveli 2001), and production of siderophores (Carson et al. 2000; Deshwal et al. 2003b).

### *7.3.1 Competition for Nutrient and Space*

The indigenous rhizobia represent the most vigorous competition encountered by inoculants. *Rhizobium* is an unusual organism in that no resting stage is known, and thus, it is inoculated into soil in its vegetative stage. Consequently, after establishment in soil, *Rhizobium* encounters microbial competition from predators, antagonists, inhibitors, and competitors for space, nutrients, and growth substances produced by host plant or available in soil. *Rhizobium* is a facultative organism. It can survive and multiply in soil in the complete absence of vegetation (Brockwell 1963); it can grow in rhizosphere of many plant species (Rovira 1961; and once inside the nodule, it grows fast and can form population analogous to pure culture due to enormous growth). Once it colonizes the soil, *Rhizobium* can be established as nodulating and permanent strain.

### *7.3.2 Induced Systemic Resistance*

PGPB can trigger a phenomenon in plants known as ISR similar to SAR essential to activate their defense mechanisms in response to infection by a broad range of pathogens and insect herbivores (Pieterse et al. 2009a, b). ISR-positive plants react

faster and more strongly to pathogenic attack by inducing several defense mechanisms. ISR is not targeted toward any specific pathogens, but it is effective at controlling diseases caused by pathogens. Present in rhizosphere, ISR involves the production of jasmonate and ethylene signaling within the plant, which stimulate the host plant's defense responses (Verhagen et al. 2004). Besides ethylene- and jasmonate-induced signals, other bacterial molecules such as the *O*-antigenic side chain of the bacterial outer membrane proteins, lipopolysaccharide, flagellar proteins, pyoverdine, chitin, ββ-glucans, cyclic lipopeptide surfactants, and salicylic acid have all been reported to act as signals for the ISR.

Various rhizobial species are reported to induce systemic resistance in plants by producing bio-stimulatory agents, including *R. etli, R. leguminosarum* bv. *Phaseoli*, and *R. leguminosarum* bv. *trifolii* (Yanni et al. 2001; Peng et al. 2002; Singh et al. 2006; Mishra et al. 2006). Even the individual cellular components of the bacterium rhizobia are reported to induce ISR, viz., lipopolysaccharides, flagella, cyclic lipopeptides, homoserine lactones, acetoin, and butanediol (Lugtenberg and Kamilova 2009). ISR is involved in priming for enhanced defense, rather than direct activation of resistance by systemic immunity elicited by beneficial microbes maintained over prolonged periods. ISR is associated with microbial antagonism in the rhizosphere; altered plant–insect interactions enrich their microbiome that provides protection against diseases that promote plant health. ISR-inducing beneficial microbes must also produce elicitors that are dependable for the onset of systemic immunity. ISR is supposed to be the result of a long-distance signaling mechanism that in rhizobial and mycorrhizal symbiosis is responsible for autoregulating the colonization density of the symbionts (Staehelin et al. 2011; Pieterse et al. 2012) as to balance the costs and benefits of mutualism.

### *7.3.3 Production of Metabolites (Volatile and Nonvolatile)*

Phytopathogenic microorganisms are a major threat to sustainable agriculture which decrease yield and soil health and have adverse effects on environment and harmful effects on human health (Gupta et al. 2015). PGPR's capacity to colonize and inhibit certain root zone microflora suggests that they have great potential for altering the environment of rhizosphere beneficial for plant growth due to secretion of volatile metabolites, viz., antibiotics (Kloepper and Schroth 1981) and diffusible metabolites, i.e., lytic enzymes. The strains including *R. Leguminosarum* bv. *trifolii, R. leguminosarum* bv. *viciae, R. meliloti*, *R. trifolii, S. meliloti*, and *B. japonicum* have been reported to secrete antibiotics and cell wall-degrading enzymes that can inhibit the phytopathogens (Bardin et al. 2004; Siddiqui et al. 2000).

#### 7.3.3.1 Antibiotics

PGPR produces antibiotics inhibiting the growth of "saprophytic pathogens" associated in root zones (Suslow et al. 1980). PGPR can develop resistance to specific antibiotics due to increased use of these strains; thus, combination of biocontrol strains that synthesize one or more antibiotics is recommended (Compant et al. 2005).

Rhizobia produce a narrow-spectrum peptide antibiotic, trifolitoxin (TFX), which assessed microbial diversity changes in the rhizosphere of bean plants (Robleto et al. 1998). The secretion of peptide antibiotic trifolitoxin (TFX) by *R. Leguminosarum* bv. *trifolii* T24 was reported to control disease. *B. Japonicum* reported to produce rhizobiotoxin directly protect soybean crop against *M. Phaseolina* (Chakraborty et al. 1984). Bacteriocin is produced by 13 of 27 strains of *R. japonicum* and 4 of 15 cowpea rhizobia; its in vitro production was highly irregular and depended on culture conditions (Roslycky 1967).

The two types of bacteriocins produced by *R. leguminosarum* are small and medium bacteriocins (Hirsch 1979). The small bacteriocin can diffuse through cellophane and is heat labile and resistant to proteolytic enzymes, whereas the medium one is unable to diffuse through cellophane. It is heat labile and resistant to proteolytic enzymes. Bacteriocins are bactericidal substances produced by bacteria and are active against bacteria of the same or closely related species (Tagg et al. 1976; Salto et al. 1979). Bacteriocins produced by *Rhizobium* spp. have been characterized as phagelike (Schwinghaner et al. 1973), protease-sensitive, or protease-resistant (Schurter et al. 1979) substances. They possess restricted antimicrobial activity. The production and primary characterization of an antimicrobial substance (AMS) with a broad activity spectrum produced by *Rhizobium trifolii* IARI and of a bacteriocin-like substance (BLS) produced by *R. trifolii* Rel-1 (Joseph et al. 1983). These AMS are equally similar to those produced by *R. japonicum* (Gross and Vidaver 1978) and *R. trifolii* (Schwinghamer 1971). These bacteriocins are dialyzable and resistant to heat and proteolytic enzymes.

#### 7.3.3.2 Hydrogen Cyanide Production

HCN, a secondary metabolite produced by several PGPR strains, has deleterious effects on their growth. The rhizospheric microorganisms have been known to protect their host plants by producing HCN, which protects their host but is inhibitory to several phytopathogens. Rhizobia are relatively less efficient in HCH production, as only 12.5 and 3% strains were found to be HCN producers (Beauchamp et al. 1991; Antoun et al. 1998). The production of metabolites such as HCN along with phenazines, pyrrolnitrin, viscoinamide, and tensin by rhizobia has been reported as biocontrol mechanisms (Bhattacharyya and Jha 2012).

As reported, HCN is a powerful inhibitor of metal enzymes, such as copper-containing cytochrome C oxidases, and is highly toxic to all aerobic microorganisms at picomolar concentrations. HCN first inhibits the electron transport and

energy supply and leads to death of the organisms. It seems to inhibit functioning of enzymes and natural receptor's reversible mechanism of inhibition (Corbett 1974), and it is also known to inhibit the action of cytochrome oxidase (Gehring et al. 1993). The different bacterial genera have shown to produce HCN, including species of *Alcaligenes*, *Aeromonas*, *Bacillus*, *Pseudomonas*, and *Rhizobium* (Devi et al. 2007; Ahmad et al. 2008).

#### 7.3.3.3 Lytic Enzyme Production

Many microorganisms produce and release *lytic enzymes* that can hydrolyze a wide variety of polymeric compounds, including chitin, proteins, cellulose, hemicellulose, and DNA. Expression and secretion of these enzymes by different microbes result in the suppression of plant pathogen activities directly. The involvement of *Rhizobium* enzymes that degrade plant cell wall polymers is a key step through the infection process in root nodule symbiosis. The production of lytic enzymes such as chitinase, β-1,3 glucanase, protease, and lipase which lyse the pathogenic fungal and bacterial cell walls had been reported in rhizobia (Gopalakrishnan et al. 2015).

*R. leguminosarum biovar trifolii* during infection of white clover roots leading to development of the root nodule symbiosis is the passage of the bacteria across the root hair wall (Sahlman and Fahraeus 1963; Napoli and Hubbell 1975). This rigid assemblage of plant polysaccharides and glycoproteins constitutes a barrier to host specificity (Al-Mallah et al. 1987). Various hypotheses have been proposed to explain how this event occurs: (1) rhizobia redirect growth through the root hair wall from the tip to the localized site of infection and cause invagination rather than penetration of the root hair wall, forming the tubular structure of the infection thread (Nutman 1956); (2) homologous *Rhizobium* strains induce the host plant to produce polygalacturonases, which soften the root hair wall at the site of infection and thus allow the bacteria to penetrate between microfibrils to the cell membrane and initiate an infection thread (Ljunggren and Fahraeus 1961); (3) wall-degrading enzymes produce a localized degradation that completely traverses the root hair wall, allowing direct penetration by the bacteria (Hubbell 1981). The strongest evidence for the involvement of wall hydrolysis in the *R. leguminosarum* bv. *trifolii*–white clover infection process involves wall hydrolysis (Callaham and Torrey 1981). Rhizobial infection of legumes is a delicately balanced process, in which wall-degrading enzymes are involved; their production may be restricted to account for slow, localized penetration without destruction of the root hair and subsequent abortion of the infection process (Hubbell 1981). The role of lytic enzymes in the infection of legumes by *Rhizobium* species has been confirmed to be involved pectinolytic (Prasuna and Ali 1987), cellulolytic (Morales et al. 1984), and hemicellulolytic enzymes.

## 7.4 Abiotic Stress Resistance of Rhizobia

PGPR as stress relievers has been recommended and is the best option for developing stress-tolerant crops with minimized costs and environmental hazards. In the *Rhizobium*–legume symbiosis, the process of $N_2$ fixation is strongly related to the physiological state of the host plant. During BNF, competitive and local rhizobial strain is not expected to express its full capacity due to limiting factors (e.g., salinity, soil pH, nutrient deficiency, mineral toxicity, soil nitrate, soil temperature, heavy metals and biocides temperature extremes, insufficient or excessive soil moisture, inadequate photosynthesis, plant diseases, and grazing) (Thies et al. 1995; Zahran 1999).

The most problematic environments for rhizobia are marginal lands with low rainfall, extremes of temperature, acidic soils of low nutrient status, and poor water-holding capacity. *Rhizobium* and *Bradyrhizobium* species vary in their tolerance to major environmental factors as they possess some key tolerance mechanism/pathways against certain stress factor. The best option for developing stress-tolerant crops with minimized production costs and environmental hazards can be the use of PGP microbes as stress relievers and might therefore open new applications for a sustainable agriculture.

### *7.4.1 Salt and Osmotic Stresses*

The legume–*Rhizobium* symbiosis and nodule formation are sensitive to salt or osmotic stress as it inhibits the initial step's symbioses. Soybean root hairs showed little curling or deformation when inoculated with *B. japonicum* in the presence of 170 mM NaCl, and nodulation was completely suppressed by 210 mM NaCl (Tu 1981). The reduction of $N_2$-fixing activity leads to a reduction in respiration of the nodules, and a reduction in cytosolic protein production, specifically leghemoglobin, by nodules, leads to the decline of dry weight and $N_2$ content in the shoot (Cordovilla et al. 1995). The salt-induced distortions in nodule structure could also be reasons behind the decline of the N2 fixation rate and photosynthetic activity under salt stress (Georgiev and Atkias 1993).

The genera *Rhizobium* and *Bradyrhizobium* are more salt tolerant than their legume hosts; they show marked variation in salt tolerance. Growth of *R. meliloti* was tolerant of 300–700 mM NaCl (Sauvage et al. 1983). Strains of *R. leguminosarum* have been reported to be tolerant to NaCl concentrations up to 350 mM NaCl in broth culture (Breedveld et al. 1991). *Rhizobium* strains from *Vigna unguiculata* were tolerant to NaCl up to 5.5%, which is equivalent to about 450 mM NaCl (Mpepereki et al. 1997).

Rhizobia utilized the mechanism of osmotic adaptation in which intracellular accumulation of low-molecular-weight organic solutes called osmolytes, which counteract the dehydration effect of low water activity through the medium but

not to interfere with macromolecular structure or function. In the presence of high levels of salt (up to 300–400 mM NaCl), the levels of intracellular free glutamate and/or K1 were greatly increased (sometimes up to sixfold in a few minutes) in cells of *R. Meliloti*, *R. fredii*, *Sinorhizobium fredii*, and rhizobia from the woody legume *Leucaena leucocephala*. K1 strictly controls Mg21 flux during osmotic shock (Zahran 1999).

An osmolyte, *N*-acetylglutaminyl-glutamine amide, accumulates in cells of *R. meliloti* dependent upon the level of osmotic stress (Smith et al. 1994). The disaccharide trehalose plays a role in osmoregulation higher levels in cells of *R. leguminosarum* (Breedveld et al. 1991) and peanut rhizobia (Ghittoni and Bueno 1996) under the increasing osmotic pressure of hyper salinity. The disaccharides sucrose and ectoine act as energy source/chemical mediators and were used as osmoprotectants for *Sinorhizobium meliloti* (Gouffi et al. 1999). The intracellular accumulation glycine betaine increases more in the salt-tolerant strains of *R. meliloti* than in sensitive strains (Smith et al. 1988). These osmoprotective substances may play a significant role for the maintenance of nitrogenase activity in bacteroids under salt stress. When externally provided, glycine betaine and choline enhance the growth of *Rhizobium tropici*, *S. meliloti*, *S. fredii*, *R. galegae*, and *Mesorhizobium loti* (Boncompagini et al. 1999). The content of polyamines, e.g., homospermidine, increases in salt-tolerant cells and acid-tolerant strains of *R. fredii* (Fujihara and Yoneyama 1993) and is supposed to maintain the intracellular pH and repair the ionic imbalance caused by osmotic stress.

### *7.4.2 Extremes of Temperature (Hot/Cold)*

The rhizobia, for which the optimum temperature range for growth is 28–31 °C, and many are unable to grow at 37 °C (Zahran 1999). Temperature affects root hair infection, bacteroid differentiation, nodule structure, and nitrogen fixation. These processes usually function over a range of ~5 °C, but this differs between legumes and is obviously dependent on the environment the rhizobia naturally occupy (Zahran 1999).

Temperature stress is generally divided into two classes: heat shock and cold shock. The heat-shock response is very similar to the acid stress response. Heat shock proteins (HSPs), viz., chaperones and proteases formed, contribute to heat tolerance by conferring heat protection on the bacteria but do not alter the internal temperature on the cell (Yura et al. 2000). The rhizobia possess so many HSPs in comparison to other bacteria; it may be, so they can bring about an immediate response in times of heat stress, minimizing damage caused *R. leguminosarum* which contains at least three copies of the HSP gene cpn60 that encode for Cpn60 (or GroEL) (Wallington and Lund 1994). The Cpn60 protein interacts with another protein called Cpn10 (or GroES) encoded by cpn10, and a copy of a cpn10 gene is upstream of at least two of the cpn60 genes. A superfamily of at least six small

HSPs, one of which is essential for symbiosis, has also been located throughout the *Rhizobium,* though initially in *B. japonicum* (Natera et al. 2000).

Cold shock effects with a loss of membrane and cytosol fluidity and with the stabilization of secondary structures of RNA/DNA lead to a decrease in the efficiency of central dogma followed by low-temperature adaptation that allows continued growth at low temperatures (Panoff et al. 1997). Cold shock response also leads to the production of many cold shock proteins (CSPs) mainly chaperones and proteases (Phadtare et al. 2000). The CSP chaperones are primarily used to bind to RNA/DNA to prevent stabilization and allow translation and transcription to proceed as usual (Phadtare et al. 2000). A CspA homologue is present in *S. meliloti* and is induced following a temperature downshift from 30 to 15 °C, along with the three rRNA (rrn) operons. Both HSPs and CSPs have been shown to be induced by other stresses, as part of a cross-protection, and by the NolR regulator, which is more associated with the nodulation process (Chen et al. 2000).

### *7.4.3 pH Stress*

*Rhizobium* displays varying degrees of pH resistance as measured by its ability to grow in neutral or slightly acidic soil (Zahran 1999). Some mutants of *R. leguminosarum* have been reported to be able to grow at a pH 18 and as low as 4.5. *S. meliloti* are viable only down to pH 5.5 (Foster 2000); *S. fredii* can grow well between pH 4 and 9.5 and able to successfully nodulate in legumes (Richardson and Simpson 1989).

*Rhizobium* contributed to acid tolerance by producing acid shock proteins (ASPs) which do not alter the internal pH of the cell (Foster 1993). There are two main types of ASPs: chaperones and proteases. Chaperones are proteins that either bind to other proteins, preventing them from misfolding, or can also repair proteins that have already misfolded as a result of the acidic conditions (Foster 2000). Proteases break down any misfolded proteins that the chaperones cannot save (Foster 2000). About 20 genes have been identified in *R. leguminosarum* that are specific to the acid stress response in rhizobia and are termed act genes (acid tolerance) (Kurchak et al. 2001).

In *S. meliloti*, genes actR and actS encode for the regulator and sensor in acid shock response (Tiwari et al. 1996b). ActS is the membrane-bound product of acts that, on detection of external acidity, activates ActR (product of actR) via phosphorylation. ActR then goes on to activate the transcription of other acid response genes within the bacterium (Tiwari et al. 1996b), and research on *S. meliloti* has shown that calcium (Tiwari et al. 1996a) and in *R. tropici* glutathione (Riccillo et al. 2000) can also play a key role in acid tolerance. The thiol forms a complex with the reactive protonated species, thus removing their effect over the bacterial cells. Acid shock has also been shown to induce the pH-regulated repressor (PhrR) protein (Reeve et al. 1998). *Rhizobium* that produces greater amounts of exopolysaccharides (EPS) is able to survive in acidic conditions more successfully

(Cunningham and Munns 1984). *R. leguminosarum* bv. *trifolii* has been reported to colonize soil and produce nodulation at a higher frequency in alkaline conditions up to pH 11.5 (Zahran 1999). Homospermidine, a polyamine, accumulates in *B. japonicum* in alkaline conditions, although its function is unknown (Fujihara and Yoneyama 1993).

### 7.4.4 Oxidative Stress

The stress is caused by increased levels of superoxide anions ($O_2^-$), hydrogen peroxide ($H_2O_2$), or hydroxyl radicals ($HO^\bullet$). These reactive species, which can be generated by exposure to radiation, metals, and redox-active drugs, can lead to the damage of all cellular components (Storz and Zheng 2000). *Rhizobium* overcomes this stress in order to undergo symbiosis with legumes (Santos et al. 2001). *S. meliloti* contains three genes that encode for catalases, katA, katB, and katC (Sigaud et al. 1999). KatA is involved in protecting free-living cells from oxidative stress, while KatB and KatC are required for cells to successfully bypass plant defense systems and undergo the nodulation process (Jamet et al. 2003). Oxidative shock has also been shown to induce the PhrR repressor protein (Reeve et al. 1998). *Rhizobium* cells have been shown to be resistant to oxidative shock as part of a cross-protection and by the NolR regulator (Chen et al. 2000). Glutathione has also been shown to contribute to the oxidative stress response in *R. tropici*, in the same way as it does in acid tolerance, though it is unknown how (Riccillo et al. 2000). Perhaps the thiol forms a complex with the reactive oxygen species, thus removing their effect over the bacterial cells.

### 7.4.5 Metal Stress

Metal ions usually cause oxidative stress by Fenton's reaction in bacterial cell and lead to expression of genes to a specific metal, such as nickel (Singh et al. 2001). The response in terms of high intercellular carbohydrates and large cell inclusions increases the resistance of *R. leguminosarum* to cadmium, copper, nickel, and zinc (Zahran 1999). The production of thiols counteracts against the heavy metal-induced oxidation and is supposed to bind to the metal ions, forming a complex, and prevents cell damage by inactivating the ion's redox potential in toxicity caused by cadmium, gold, mercury, and lead (Singh et al. 2001).

## 7.5 Rhizobia Association with Non-legumes

Report suggests the beneficial PGPR of rhizobia beneficial for legumes and non-legumes (Antoun et al. 1998; Yanni et al. 1997). The rhizobial association with non-leguminous plants such as maize, rice, wheat, lettuce, and radishes may be strong or weak; these associations may be at rhizosphere, inside plant tissue (endophytic), and upper plant part (phyllospheric).

These rhizobia are capable of colonizing the roots of non-legumes; this interaction produces phytohormones, siderophores, and HCN. For the better symbiotic association, both non-legumes exude amino acids, vitamins, organic acids, enzymes, nucleotides, sugars, and plant metabolites (Rovira 1956), whereas rhizobia exudate nutrient sources and perform PGPR activity. In cereals–legumes crop rotation systems, inoculation of the preceding cereal crop with *Rhizobia* and *Bradyrhizobia* increases nodule volume, the dry weight of shoots, number of pods, and the final yield. *B. japonicum, R. leguminosarum, S. meliloti*, and *Arctic rhizobia* are some of the examples of bacterial species, which participate with legumes and non-legume plants. A number of reports available suggest that rhizobia can colonize roots of non-leguminous plants and are able to survive in the internal tissue system.

## 7.6 Plant Tissue Culture and *Rhizobium* Symbiosis

*Rhizobium* is always one of the foremost examples of nitrogen-fixing bacteria in natural conditions. Nowadays, new approaches are arising looking toward the *Rhizobium* applications in tissue culture, including legume symbiosis; these required best conditions for effective rhizobial infection with callus for organogenesis (Holsten et al. 1971). This interaction provides a test system for studying various facts related to legume symbiosis with minimal inference from plant influence. Since the last 3–4 decades, different works have done on *Rhizobium* role in tissue culture study. The presence of *Rhizobium* considered for the similar activity as to supply nitrogen to the growing plants in plant tissue culture. Nitrogenase activity has been studied with respect to root, stem, and leaf through culture using different strains of *Rhizobia*. The medium free from supplements and hormones like nitrate, 2, 4-D, kinetin, etc., shows the rooting from the *Rhizobia*-infected callus, whereas untreated plants remained undifferentiated (Rao 1976). The morphological changes which accompany the onset of nitrogenase activity in callus tissue were found to parallel closely the changes observed in intact nodule systems.

### 7.6.1 Establishment of Symbiosis

This symbiotic association can be established *in vitro* between *Rhizobium* and cultured plant cell or tissue. For the successful association plant, cultured cells are grown on the solid media surface provided with the low level of inorganic nitrogen and then inoculated with *Rhizobium* at log phase. The whole system is cost-effective and provides multiple replicates of the samples. The same medium is to be used throughout the experiment to avoid disturbances in the growth of cells. The change in acetylene level confirms the association of plant and bacteria, which can be done by nitrogenase assay. This assay can be recorded within 3 weeks.

Relatively little is known regarding the factors controlling infection or the development of nitrogenase in the nitrogen-fixing symbiosis between leguminous plants and bacteria. The sensitive acetylene reduction assay technique for the detection of nitrogenase activity (Hardy et al. 1968) and the demonstration that symbiosis can be established between *Rhizobium* and plant cell tissue cultures in vitro (Holsten et al. 1971) allow a novel approach to study these problems.

Various attempts have been shown in plant tissue culture derived from legumes and non-legumes of successful induction of nitrogenase activity in *Rhizobium* (Child and Larue 1974; Child 1975). Some experiments are shown below: (1) Fusion of legumes and non-legumes protoplast, and the hybrid plants have the ability to associate with *Rhizobium*; (2) forced association of $N_2$-fixing bacteria with non-legume tissue culture, and possible regeneration; (3) induced transfer of nitrogen-fixing bacteria into protoplast; and (4) transfer of *nif* gene in non-legumes and plant regeneration. The infection process took place in a liquid nutrient medium containing growth promoters. After some days, the cells were transferred to a similar medium lacking with growth promoters, to allow the establishment of nitrogenase activity. As it is very difficult to form symbiosis between *Rhizobium* and suspension culture cells in conventional vessels, the first established symbiosis in callus culture on solid medium is reported on Gamborg's B5 and B5c media (Gamborg 1970).

## 7.7 Genetic Engineering of Nif

*Nif* gene is present in symbiotic *Rhizobia* species and free-living bacteria like *Klebsiella pneumonia*. Cloning of Nif gene has been achieved in various examples. *Nif* of *K. Pneumonia* contains seven operons, including cluster of 15 genes working together. However, the gene technology can be used to obtain more efficient *Rhizobium*–legume symbiosis, which is of agro-industrial use.

Transfer of *Nif* gene (isolated from *K. pneumonia*) in non-nitrogen-fixing organism, including bacteria and cereals and other plants, is also now possible (Hardy and Havelka 1975; Dixon et al. 1979). In this way, recombinant plasmid containing *Nif* gene can be transformed, and these protoplasts containing *Nif* gene will

regenerate in new plant which will be able to fix atmospheric nitrogen. Another interesting method by phage-mediated gene transfer in plants is also explained (Doy et al. 1973).

Induction of tumor in plants using *A. tumefaciens* is a well-established method (Lippincott and Lippincott 1975; Kado 1976); this tumor is resultant of transfer of T-DNA of *A. tumefaciens* in the plants (Schell et al. 1976). Ti plasmid can be manipulated in *Rhizobium* thus. The bacteria get the ability to induce tumor in plants without losing their ability to induce tumor (Van Larebeke et al. 1977). One recent study explains the intergenic transfer of Ti plasmid and nodulating plasmid between *A. tumefaciens* and *Rhizobium* (Brenner et al. 2005).

*Rhizobium* possesses large plasmids; it is explained that *Nif* gene might be present on these plasmids; thus, the possibilities arise of transferring *Nif* gene from *Rhizobium* to *A. tumefaciens*, which may lead to transfer of *Nif* gene in Dicots as well. *Agrobacterium* and *Rhizobium* possessed closed relationship that is already confirmed by 16s rRNA analysis (Fred et al. 2007).

In recent studies, it is found that *A. tumefaciens* as a natural genetic engineer is now available for transfer of gene in plants. Rhizobia have an open source, better, safer, more environmental friendly, and fewer restrictions in plant biotechnology as compared to *A. tumefaciens*. Now new transgenic can be generated using binary vector carried by rhizobia. Several methods have been available for transfer of plasmid DNA in rhizobia, including conjugation and electroporation; transformation and transduction are used to transfer DNA into rhizobia species. It has been suggested that some species of *Rhizobium,* including *SinoRhizobiumm meliloti*, have sufficient transformation efficiency tested on monocots and dicots (Broothaerts et al. 2005). Now researchers are looking toward interaction between host plants and Rhizobia for more exploration to these fields, i.e., "Rhizobia-mediated transformation" (Patel and Sinha 2011).

**Acknowledgement** The authors would like to thank the Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati (M.S), India, for providing the research facilities and Ms. Grishma Shinde for the drawing included in chapter.

**Conflict of Interest** The author has no conflict of interest.

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