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A Deeper Insight into the Symbiotic Mechanism of *Rhizobium* spp. from the Perspective of Secondary Metabolism

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14.1 Introduction

Growth of an organism is determined by mineral nutrient availability, and among all the mineral nutrients, nitrogen is the most crucial for plant growth as it is a component of proteins, nucleic acids and other cellular constituents. Atmosphere comprises about 10^{15} tonnes of gaseous nitrogen out of which about 1.4×10^8 metric tonnes of nitrogen is fixed biologically all over the globe every year. This accounts for about 90% of the total nitrogen being fixed in terrestrial environment, and the rest 10% is fixed by lightning (Postgate 1982; Zahran 1999). An additional 1.4×10^8 metric tonnes of nitrogen being fixed each year by utilization of nitrogenous fertilizers, fossil fuels and planting of legumes (Vitousek et al. 1997; Gage 2004). The prokaryotes are the so far only known source of biological nitrogen fixation being carried out by 87 species in 38 genera of bacteria, 2 genera of archaea and 20 genera of cyanobacteria (Dixon and wheeler 1986). Nitrogen fixation can be accomplished by both free living (*Clostridium, Azotobacter*, *Beijerinckia*, *Rhodospirillum* and *Chromatium*) and symbiotic nitrogen-fixing bacteria (*Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Azorhizobium* and *Frankia*). Symbiotic nitrogen fixation in Leguminosae family is associated with class alphaproteobacteria, family *Rhizobiaceae*, whereas filamentous, gram-positive actinomycete, *Frankia*, induces nodules on a variety of woody plants from the family Betulaceae, Casuarinaceae, Rosaceae, Myricaceae, Rhamnaceae, Elaeagnaceae, Coriariaceae and Datiscaceae (Benson and Clawson 2000).

Rhizobium is a genus of gram-negative motile bacteria which has the ability to fix atmospheric nitrogen. *Rhizobium* species forms a symbiotic nitrogen-fixing association with roots of leguminous plants such as soybean, pea and alfalfa. An equivalent term used by other researchers is 'root nodule bacteria' (RNB) (Zakhia

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et al. 2004; Howieson and Brockwell 2005). Soil-inhabiting bacteria, *Rhizobium*, form specific root structure, nodules generally of two types, determinate and indeterminate. Differ mainly in that indeterminate nodules are elongated in shape and have persistent meristem that continuously form new nodule (Handberg and Stougaard 1992).

14.2 Historical Perspective of *Rhizobium*

Beijerinck (1888) was the first to isolate and culture microorganism from root nodules of different legume species and named it as *Bacillus radicicola*. Later on, the name *Rhizobium* was proposed by Frank (1889) for nitrogen-fixing bacteria of legumes. The word *Rhizobia* is derived from the Greek word *rhíza*, meaning "root", and *bios*, meaning "life". The term *Rhizobium* is usually used as a singular form of rhizobia. Genera other than *Rhizobium* were identified later; this includes *Sinorhizobium* (Chen et al. 1988), *Bradyrhizobium* (Jordan 1982) and *Mesorhizobium* (Jarvis et al. 1997). Nobbe and Hiltner (1896) developed the technology for inoculation of legume with *Rhizobium* spp. and granted US patent for it (Das et al. 2017). Mass production of *Rhizobium* inoculants began in 1895 in the USA, mostly by employing peat-based inoculants (Roughley and Vincent 1967). Besides peat-based formulation used worldwide, vermiculite, mineral soil, bentonite, perlite and coal are used as rhizobial inoculants (Stephans and Rask 2000; Temprano et al. 2002; Das et al. 2017).

14.3 Rhizobial Genome

Rhizobium has a large and complex multipartite genome with genome size varying from 5.4 to 9.2 Mb and plasmid number ranges from 0 to 7 (MacLean et al. 2007). The genome organization reflects the adaptive potential and the lifestyle of species (MacLean et al. 2007; González et al. 2006). Comparative genomic studies reveal the evolutionary pattern of rhizobia-legume symbiosis. Outcomes of genome comparisons were quite interesting as it revealed that no gene is common and specific to all rhizobia (Amadou et al. 2008; Laranjo et al. 2014).

14.4 Rhizobium: Plant Symbiosis

The bacteria colonize plant cells within root nodules and convert atmospheric nitrogen into ammonia, a process known as nitrogen fixation (O'Gara and Shanmugam 1976). The ammonia is used by the plants as a nitrogen source. In turn the rhizobia are supplied with nutrients (Lodwig and Poole 2003) and are protected inside the nodule structure (van Rhijn and Vanderleyden 1995). However, in ineffective nodules no nitrogen is fixed, yet rhizobia are still supplied with nutrients, and in this case, the rhizobia could be considered parasitic (Denison and Kiers 2004). Other genera of rhizobium such as *Azorhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Bradyrhizobium* have also got the ability to fix nitrogen. The rhizobium-legume association is unique and specific in that each rhizobial strain has definite host range varying from narrow to exceptionally wide (Perret et al. 2000).

14.4.1 Mechanism of Root Nodule Formation

The process of nodule formation involves a complex series of steps (Vincent 1974; Newcomb 1981a, b). Plants of Leguminosae family usually secrete a variety of organic compounds such as amino acids and flavonoids which are recognized by bacterial NodD protein. Rhizobium is generally chemotactic towards the plant roots due to the secretion of such compounds (Bergman et al. 1988; Caetano-Anolles et al. 1988; Kurrey et al. 2016). Nodulation takes place due to specific and complex interaction between the plant and the *Rhizobium*. The initial attachment usually involves a protein called "rhicadhesin" which is found on the surface of all leguminous plants. Upon binding of these compounds with NodD protein, nodulation genes get activated. *Rhizobium* secretes Nod factors, lipochito-oligosaccharides which get recognized by the leguminous plant, and triggers early step of nodulation (Pawlowski and Bisseling 1997; Spaink 1992). Host specificity of rhizobia is determined by terminal sugar residues of lipochito-oligosaccharides secreted by rhizobia (Denarie and Cullimore 1993; Fisher and Long 1992; Stokkermans and Peters 1994). When the root hair of the plant comes in contact with bacterium, the growing root hairs get curled and form a pocket for the particular rhizobia (Mylona et al. 1995). The bacteria invade the plant by forming a new infection thread. The infection threads progress towards the primordium, and the bacteria are released into the cytoplasm of the host cells, surrounded by a plant-derived peribacteroid membrane (PBM) (Verma and Hong 1996). This separation usually occurs to suppress plant defence responses which are likely to harm the bacteria. The bacteria produce cytokinin which facilitates division of plant cells to form nodules and the nodule formation initiates on the root hairs. Afterwards, the nodule primordium develops into a mature nodule. The bacteria differentiate into their endosymbiotic form, which is usually known as bacteroid. Bacteroids, altogether with the surrounding PBMs, are called symbiosomes (Roth and Stacey 1989; Guan et al. 1995).

Rapid cell division starts in the infected tissue. The area of N_2 fixation is usually pink or red in colour due to the presence of "leghaemoglobin" required for active oxygen transport (Appleby 1984; Kannenberg and Brewin 1989). The formed nodule establishes a direct vascular connection with the host for nutrient uptake. In the process of nodule formation, certain genes called *nod* genes are involved and are known as nodulin genes (van Kammen 1984). The "early nodulin genes" encode products which get expressed before the commence of N_2 fixation and are involved in infection and nodule development. However, the "late nodulin genes" interact with the bacterium and aid in metabolic specialization of the nodule (Nap and Bisseling 1990).

14.4.2 The Infection Thread

The invasion of root tissues is initiated by intracellular ‘tunnels’ known as infection threads, which initially arise in root hair cells (Callaham and Torrey 1981). In uninfected root hairs, the nucleus is paired to the tip by microtubules which facilitate new wall material to the growing apex (Lloyd et al. 1987; Ridge 1988). The bacterial infection usually removes the nucleus from the tip and facilitates the pathway for incorporation of wall precursors. Initially, the infection thread develops as an invagination of root hair wall, and the nucleus migrates towards the base of the root hair. The new wall material synthesized is thereafter directed to the tip of the invagination to produce an interior growing cylinder of wall material bounded by a membrane, and the bacteria embedded in a matrix (Gage 2004). Infection thread structures develop subsequently in the underlying cortical cell layers and facilitate the bacteria in the infection thread to spread from one cell to adjacent cell (Libbenga and Harkes 1973). During this process of tissue invasion, the wall of the infection thread limits the rhizobia to the extracellular space, thus preventing its contact with the plant plasma membrane (VandenBosch et al. 1989). Cell invasion can only arise by endocytosis from unwalled infection droplets that evolve from infection threads at a particular stage of development.

As cell divisions in the plant root facilitate the formation of body of the nodule, the infection threads start penetrating individual target cells within the nodule. The bacteriods are released into the plant cytoplasm itself, enveloped in plasma membrane of the plant (Robertson et al. 1978). Thereafter, the bacteria and plant cells differentiate and initiate symbiotic nitrogen fixation and metabolite exchange (Sutton et al. 1981; Verma and Long 1983) (Fig. 14.1).

14.5 Rhizobia as Biocontrol Agent and Biofertilizer

Rhizobium spp. has boosted legume production worldwide by enhanced nitrogen fixation, plant growth promotion and suppression of soilborne pathogens such as *Rhizoctonia solani*, *Pythium* spp., *Fusarium* spp., and *Macrophomina phaseolina* in both legumes and nonlegumes (Table) (Antoun et al. 1978; Malajczuk et al. 1984; Chakraborty and Purkayastha 1984; Ehteshamul-Haque and Ghaffar 1993; Nadia et al. 2007; Das et al. 2017). Ehteshamul-Haque and Ghaffar (1993) deployed biocontrol potential of *Rhizobium leguminosarum*, *Sinorhizobium meliloti* and *Bradyrhizobium japonicum* by soil drenching and seed coating of sunflower, okra, mung bean and soybean. Antimicrobial activity of *Rhizobium* spp. strains ORN 24 and ORN 83 has been exploited against *Pseudomonas savastanoi*, olive knot disease (Maurad et al. 2009). Buonassisi et al. (1986), inoculated seeds of snap bean with *Rhizobium leguminosarum* bv. *phaseoli* (isolated from nodules of commercial snap bean) to control fusarium foot rot of beans caused by *Fusarium solani* f. sp. *phaseoli*. Inoculation of pea and sugar beet seeds with *R. leguminosarum* bv. *vicieae* strain R12 significantly reduced the occurrence of pythium damping-off (Bardin

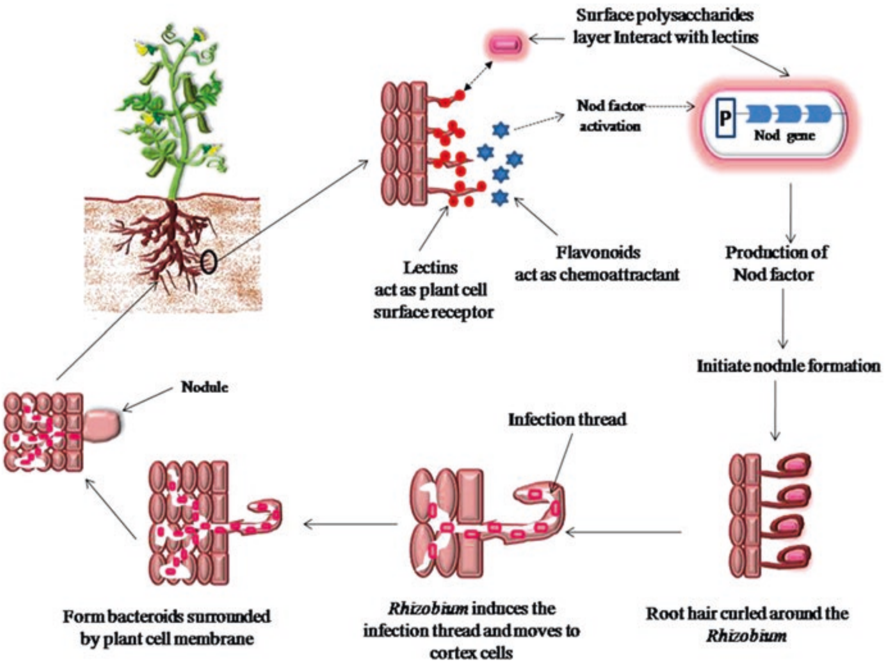


Fig. 14.1 Mechanism of nodule formation in rhizobium-legume symbiosis

et al. 2004). Different strains of *Rhizobium* were reported to reduce incidence of root rot of chickpea, *Rhizoctonia solani*, and increased the nitrogen fixation, phosphorus uptake and plant growth (Hemissi et al. 2011). Seed treatment of chickpea with PGPR + *Mesorhizobium ciceri* provided enhanced plant growth (seedling emergence and shoot length) and reduced fusarium wilt of chickpea significantly over their single treatment (Kumari and Khanna 2014). Co-inoculation of common bean with *Rhizobium* and *Pseudomonas* strains was reported to have increased number of nodules and produce higher yield (Sancheza et al. 2014) (Table 14.1).

14.6 Mechanism of Biological Control by Rhizobia

The mechanism associated with biological control of phytopathogens by rhizobia consists of antibiotic production, siderophore production, HCN production, production of lytic enzymes, phosphate solubilization, competition and induction of plant defence (Arora et al. 2001; Huang and Erickson 2007). Antagonistic activity against a wide range of pathogens is due to its ability to produce wide range of secondary metabolites such as HCN, siderophore, rhizobitoxin, lytic enzymes, IAA production and phosphate solubilization (Antoun et al. 1978; Presmark et al. 1993; Nautiyal, 1997; Biswas et al. 2000; Deshwal et al. 2003; Pandey and Maheshwari 2007).

Table 14.1 Biological control potential of *Rhizobium* spp.

S. N.	Producer	Host	Target plant pathogen	Disease manage	References
1	*Rhizobium japonicum*	*Glycine max*	*Fusarium solani*	Root rot	Al-Ani et al. (2012)
			Macrophomina phaseolina	Charcoal rot	
2	*Rhizobium* sp.	*Cicer arietinum*	*Fusarium oxysporum* f. sp. *ciceris*	Wilt	Arfaoui et al. (2005)
3	*Rhizobium meliloti*	*Arachis hypogaea*	*Macrophomina phaseolina*	Root rot	Arora et al. (2001)
			Pythium sp.	Brown rot of groundnut	Bardin et al. (2004)
			Fusarium solani f. sp. *phaseoli*	Wilt	Buonassisi et al. (1986)
4	*Mesorhizobium loti* MP6	*Brassica juncea*	*Sclerotinia sclerotiorum*	Sclerotinia rot	Chandra et al. (2007)
			Rhizoctonia solani	Root rot	Dubey and Maheshwari (2011)
			Fusarium oxysporum *F. solani*	Wilt	
			Fusarium oxysporum f. sp. *lentis*	Wilt	Essalmani and Lahlou (2002)
5	*Rhizobium* sp.	*Phaseolus vulgaris*	*Fusarium solani* f. sp. *phaseoli*	Wilt	Estevez de Jensen et al. (2002)
6	*Rhizobium* sp.	*Arachis hypogaea*	*Sclerotium rolfsii*	Stem rot	Ganesan et al. (2007)
7	*Rhizobium* sp.	*Glycine max*	*Cylindrocladium parasiticum*	Red crown rot	Gao et al. (2012)
8	*Rhizobium leguminosarum* bv. *viciae*	*Pisum sativum* *Lens culinaris*	*Pythium spp.*	Root rot	Huang and Erickson (2007)
9	*Rhizobium* sp.	*Olea europaea*	*Pseudomonas savastanoi*	Olive knot	Kacem et al. (2009)
10	*Sinorhizobium fredii* KCC5	*Cajanus cajan*	*Fusarium udum*	Wilt	Kumar et al. (2010)
11	*Ensifer meliloti, Rhizobium leguminosarum*	*Trigonella foenum-graecum*	*Fusarium oxysporum*	Wilt	Kumar et al. (2011)
			Phytophthora cinnamomi	Root rot	Malajczuk et al. (1984)
12	*Rhizobium* sp. NBRI9513	*Cicer arietinum*	*Fusarium spp.*	Wilt	Nautiyal (1997)
			Rhizoctonia bataticola	Dry root rot	
			Pythium sp.	Damping-off	

(continued)

Table 14.1 (continued)

S. N.	Producer	Host	Target plant pathogen	Disease manage	References
13	*Rhizobium* sp.	*Glycine max*	*Macrophomina phaseolina*	Charcoal rot	Omar and Abd-Alla (1998)
14	*Bradyrhizobium* sp.	*Helianthus annuus*	*Rhizoctonia solani*	Collar rot	Siddiqui et al. (2000)
15	*Rhizobium* sp.		*Macrophomina phaseolina*	*Charcoal rot*	Romesh Sagolshemcha et al. (2017)
16	*Rhizobium* sp.	*Vicia faba*, *Cicer arietinum*, *Lupinus albus*	*Fusarium oxysporum* *Fusarium solani*,	Wilt	Shaban and El-Bramawy (2011)
			Macrophomina phaseolina	*Charcoal rot*	
			Rhizoctonia solani	*Rot*	
			Sclerotium rolfsii	Collar rot	
17	*Bradyrhizobium japonicum*	*Solanum lycopersicum*	*Macrophomina phaseolina*	Charcoal rot	Siddiqui and Shaukat (2002)
			Fusarium solani	Wilt	
			Rhizoctonia solani	Damping-off, root rot, stem rot and stem canker	
18	*Rhizobium leguminosorum*	*Cicer arietinum*	*Fusarium oxysporum* f. sp. *ciceris*	Wilt	Singh et al. (2010)
19	*Rhizobium sp.* RS12	*Cicer arietinum*	*Macrophomina phaseolina*	Dry root rot	Smitha and Singh (2014)

14.6.1 Antibiotic Production

Antibiotic production is one of the major mechanisms of biological control of phytopathogens. Several workers have reported different rhizobial strains to produce variety of antibiotics (Ligon et al. 2000; Raaijmakers et al. 2002; Deshwal et al. 2003; Bardin et al. 2004; Chandra et al. 2007; Das et al. 2017). Hirsch (1979) reported that 97 strains of *R. leguminosarum* produces bacteriocins, characterized as small and medium based on their size. *R. leguminosarum* plasmid pRL1J1 carries genes for nodulation and bacteriocin production, encodes for medium bacteriocin (Hirsch et al. 1980). *R. leguminosarum* bv. *trifolii* T24 produces a potent antibiotic, trifolitoxin that promote clover nodulation have been reported by Triplett and Barta (1987). Different strains of *R. leguminosarum* bv. *viciae*, *R. leguminosarum* bv. *trifolii*, *R. meliloti*, *B. japonicum* and *S. meliloti* have been reported to secrete diverse group of antibiotics having potential for inhibition of phytopathogens (Chakraborty and Purkayastha 1984; Bardin et al. 2004; Deshwal et al. 2003; Hafeez et al. 2005; Chandra et al. 2007; Gopalakrishnan et al. 2015) (Table 14.2).

14.6.2 Production of Antimicrobial Secondary Metabolites

14.6.2.1 HCN Production

HCN are volatile, secondary metabolite produced during the early stationary phase of rhizobacteria (Rezzonico et al. 2007; Knowles and Bunch 1986). HCN is inhibitor of various metalloenzymes such as cytochrome C oxidases of respiratory electron transport. It disrupts the energy supply to the cell and is highly toxic; even at low concentration, it has deleterious effect on growth and development of aerobic plant pathogens (Corbett 1974; Gehring et al. 1993; Deshwal et al. 2003; Siddiqui et al. 2006; Martínez-Viveros et al. 2010). Beauchamp et al. (1991) and Antoun et al. (1998) have reported that 12.5 and 3% of the total strains of rhizobia screened were HCN producers, respectively. HCN production has also been reported in *Mesorhizobium loti* MP6, retarding the growth and development of *S. sclerotiorum* causing white rot in *Brassica campestris* (Chandra et al. 2007). Six *Rhizobium* spp. strains (an isolate from root nodules of chickpea) has been reported to produce HCN, reducing the incidence of chickpea wilt by *Fusarium oxysporum* f. sp. *ciceris* (Arfaoui et al. 2006).

14.6.2.2 Siderophore Production

Iron is one of the key components of metabolic molecules such as ribonucleotide reductase, cytochromes, etc. (Guerinot 1994). Some microbes are equipped with the ability to produce siderophores, an iron-binding compound of low molecular weight (Matzanke 1991; Andrews et al. 2003). Siderophores scavenges iron (Fe^{3+}) from environment under iron stress condition which in turn determines the colonization of bacteria on plant roots leaving pathogens (Crowley and Gries 1994; Siddiqui 2006; Martínez-Viveros et al. 2010). Rhizobia has been endowed with the ability to produce a range of siderophores varying from catechol and hydroxamate type (Modi et al. 1985; Roy et al. 1994; Persmark et al. 1993), rhizobactin type (Smith et al. 1985), citrate type (Guerinot et al. 1990), phenolate type (Patel et al. 1988), vicibactin type (Carson et al. 1992), anthranilic acid (Rioux et al. 1986) to dihydroxamate type (Carson et al. 2000). Arora et al. (2001) reported that *M. phaseolina* causing charcoal rot of groundnut was inhibited by siderophore-producing strains of *Rhizobium meliloti* under in vitro condition. Seed treatment with hydroxamate siderophore producer, *Mesorhizobium loti* MP6, reduced the occurrence of white rot of *Brassica campestris* (Chandra et al. 2007).

14.6.3 Lytic Enzyme Production

Chitinases, cellulases, β-1,3-glucanase β-1,4-glucanase, β-1,6-glucanase, proteases, pectinase and amylases are some of the lytic enzymes produced by microorganisms

Table 14.2 Representative list of secondary metabolites of important *Rhizobium* species (KEGG database accessed on April 25, 2018)

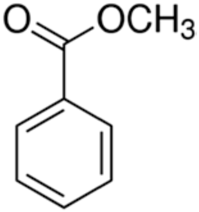
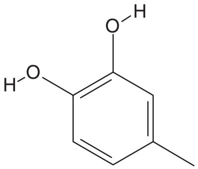
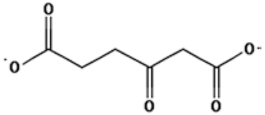
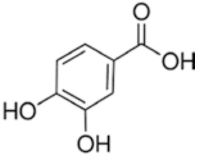
Rhizobium species	Type of secondary metabolite	Biosynthesis pathway	Structural formula
1. *Rhizobium leguminosarum* bv. *viciae* 3841	a) Methylbenzoate	Xylene degradation	
	b) Methylcatechol	Benzoate degradation	
	c) 3-Oxoadipate	Catechol ortho cleavage	
	e) 3,4-Dihydroxybenzoate	Terephthalate degradation	

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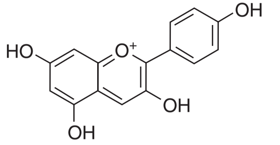
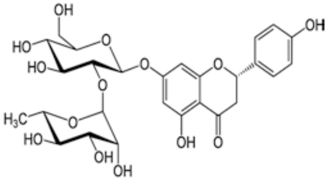
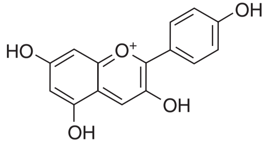
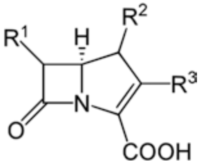
Table 14.2 (continued)

Rhizobium species	Type of secondary metabolite	Biosynthesis pathway	Structural formula
	f) Pelargonidin	Flavonoid biosynthesis	
	g) Naringenin	Flavanone biosynthesis	
	h) Paspaline	Paspaline biosynthesis	
2. *Mesorhizobium opportunistum*	a) Carbapenem	Carbapenem biosynthesis	

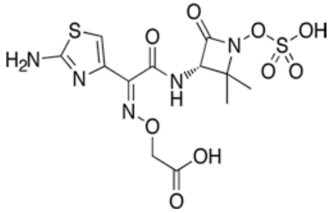
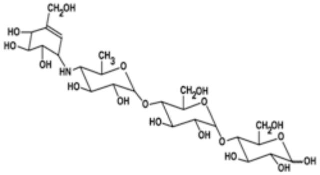
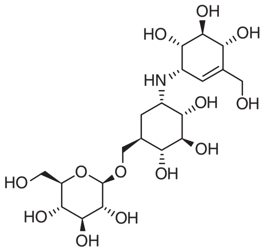
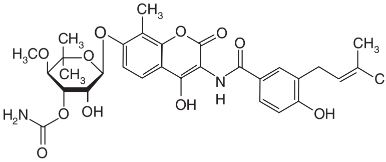
	b) Monobactam	Monobactam biosynthesis	
	c) Acarbose	Acarbose biosynthesis	
	d) Validamycin	Validamycin biosynthesis	
	e) Novobiocin	Novobiocin biosynthesis	

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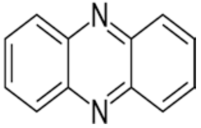
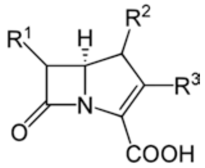
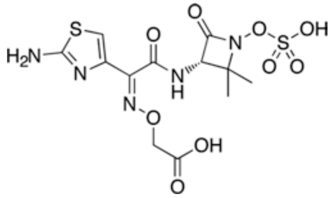
Table 14.2 (continued)

Rhizobium species	Type of secondary metabolite	Biosynthesis pathway	Structural formula
	f) Phenazine	Phenazine biosynthesis	
3. *Azorhizobium caulinodans*	a) Carbapenem	Carbapenem biosynthesis	
	b) Monobactam	Monobactam biosynthesis	

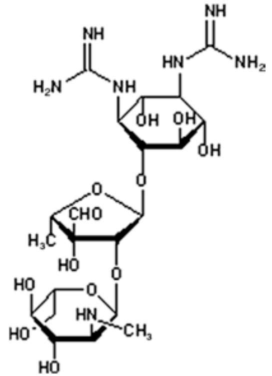
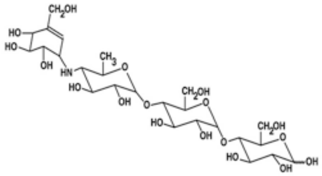
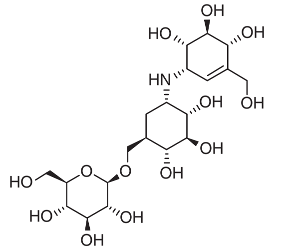
	c) Streptomycin	Streptomycin biosynthesis	
	d) Acarbose	Acarbose biosynthesis	
	d) Validamycin	Validamycin biosynthesis	

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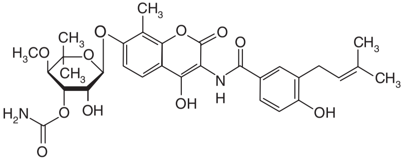
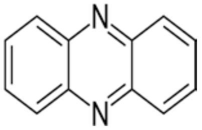
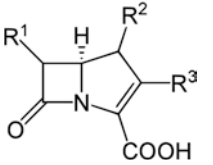
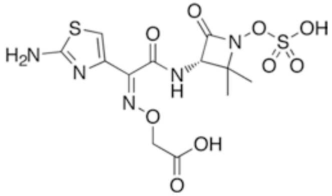
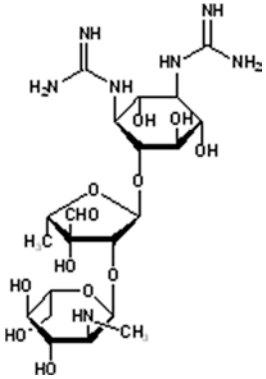
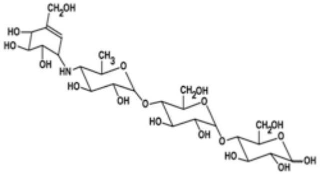
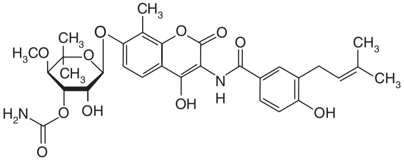
Table 14.2 (continued)

Rhizobium species	Type of secondary metabolite	Biosynthesis pathway	Structural formula
	e) Novobiocin	Novobiocin biosynthesis	
	f) Phenazine	Phenazine biosynthesis	
4. *Sinorhizobium meliloti* 1021	a) Carbapenem	Carbapenem biosynthesis	
	b) Monobactam	Monobactam biosynthesis	

	c) Streptomycin	Streptomycin biosynthesis	
	d) Acarbose	Acarbose biosynthesis	
	e) Novobiocin	Novobiocin biosynthesis	

for disease reduction (Chatterjee et al. 1995; Diby et al. 2005; Gupta et al. 2006; Ruiz Duenas and Martinez 1996; Szekeres et al. 2004). There are reports of rhizobial isolates producing chitinase to inhibit pathogenic microbes (Chernin et al. 1955; Mazen et al. 2008). Mazen et al. (2008) reported that seed treatment with chitinase-producing *Rhizobium* spp. alone or co-inoculated with mycorrhizal fungi leads to reduction of damping-off of fababean. *Rhizobium* strains isolated from *Sesbania sesban* has been reported to be produce chitinase (Sridevi and Mallaiah 2008). *R. leguminosarum* isolate TR2 and *Ensifer meliloti* isolate TR1 and TR4 showed β-1,3-glucanase and chitinase activity, respectively, and inhibited fusarium wilt of fenugreek (Kumar et al. 2011). *Rhizobium* sp. Strain RS12, with chitinase-producing ability, suppresses diseases of chickpea caused by *F. oxysporum*, *S. sclerotiorum* and *M. phaseolina* by preventing mycelia growth and development (Smitha and Singh 2014).

14.6.4 Phosphate Solubilization

Phosphorus is present in soil in immobile for and thus become unavailable to microbe and plant (Gyaneshwar et al. 2002). Group of rhizobia have been reported to be potent phosphate solubilizers, some of them as *R. leguminosarum* mobilizes phosphorus making it available to plant (Rodriguez and Fraga 1999; Mehta and Nautiyal 2001). *Rhizobium* inoculated *P. vulgaris* showed significant difference in acid phosphatase activity in its rhizospheric zone (Makoi et al. 2010). Bradyrhizobium strains that have been reported by Deshwal et al. (2003) for their ability to produce siderophores, phosphate solubilization and IAA, conferring it strong root colonizing, growth promotion and vigorous antagonistic activity against *M. phaseolina* (charcoal rot of peanut). Co-inoculation of *Rhizobium* and phosphate solubilizing bacteria have been reported to have synergistic effect increasing nodulation, shoot and root nitrogen and phosphorus content (Rugheim and Abdelgani 2009).

14.7 Induction of Plant Defence Mechanisms

Systemic resistance in host is induced by up regulating the expression of defence-related genes encoding for antioxidant enzymes, hydrolytic enzymes and pathogenesis-related proteins. Defence-related enzymes such as polyphenol oxidase, L-phenylalanine ammonia lyase, peroxidase, chalcone synthase and isoflavone reductase play crucial role in induction of plant defence to pathogenic attack (Arfaoui et al. 2005; Dutta et al. 2008). Rhizobia have ability to induce defence arsenal by triggering production of plant defensive enzymes, phytoalexins, phenolics and flavonoids (Mavrodi et al. 2001; Yu et al. 2002). Phenolics plays a crucial role in plant defence by activating plant defence genes, acting directly as structural barriers and modulating the pathogenicity, preventing growth and spread of pathogens (Ramos et al. 1997 and Dihazi et al. 2003). Mishra et al. 2006 reported that inoculation of rice with strains of *Rhizobium leguminosarum* bv. *phaseoli* and *R.*

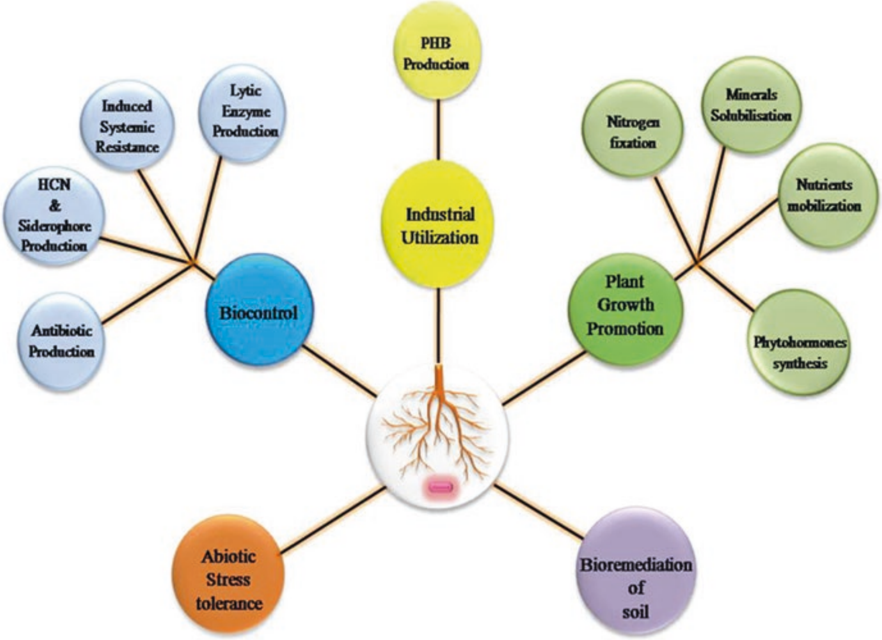


Fig. 14.2 Multifaceted role of *Rhizobium* sp.

leguminosarum bv. *trifolii* induces production of phenolics such as ferulic acid, gallic acid and tannic and cinnamic acids, reducing infection by *Rhizoctonia solani*. Induction and accumulation of phytoalexins such as medicarpin and maackiain in response to *Rhizobium* species in planta, protect it from phytopathogens (Weigand et al. 1986; Weidemann et al. 1991). A phytoalexin, glyceollin have been reported to be produced by *Rhizobium* and *Bradyrhizobium* sp. in soybean, which has antimicrobial activity against plant pathogens (Phillips and Kapulnik 1995) (Fig. 14.2).

14.8 Microbial Secondary Metabolites and Its Importance

Microbial secondary metabolites are low molecular weight compounds, indispensable for growth of producing microbes but play an important role in nutrition, health and economy of the society (Berdy 2005; Ruiz et al. 2010). Microbial secondary metabolites varied widely in its chemical nature from peptides, polyketides, lipids, steroids, terpenoids and carbohydrate to alkaloids (O'Brien and Wright 2011). They include pigments, toxins, antibiotics, pheromones, antitumor agents, enzyme inhibitors, effectors of ecological competition and symbiosis, receptor antagonist and agonists, immunomodulating agents, pesticides, cholesterol-reducing drugs and growth promoters of plants and animals (Demain 1998). These metabolites are not synthesized during logarithmic growth phase but are synthesized during subsequent production stages; stationary phase (idiophase) and metabolites known as idiolites (Demain and Fang 2000; Gonzalez et al. 2003; O'Brien and Wright 2011). Production of secondary metabolites are brought about by addition and biosynthesis of an inducer or exhaustion of nutrients, generate signal which regulate metabolic pathways leading to chemical differentiation (Bibbs 2005; Ruiz et al. 2010). Microbial secondary metabolites are major source of essential agricultural products and contributes to about half of the pharmaceutical market (Demain and Sanchez 2009). In addition to its use as anti-infective drugs, they are used as immunosuppressants to facilitate organ transplantation (Verdine 1996; Barber et al. 2004; Demain and Sachez 2009). Autoinducers of secondary metabolites includes oligopeptides of gram-positive bacteria, N-acylhomoserine lactone of gram-negative bacteria and butanolides of the actinomycetes (Kawaguchi et al. 1988; Demain 1998).

14.9 Rhizobial Formulations

Field applicability of rhizobium for its better exploitation at large scale is determined by a formulation with appropriate inoculum load. Survivability in higher number and for longer period in commercial formulation is major objective of developing an inoculants formulation. Mainly two types of commercial formulation of *Rhizobium* are available in market, they are solid and liquid. Solid inoculants are prepared by blending broth culture with an appropriate carrier material. Selection of carrier material is determined by a number of factors such as survivability of rhizobial cells on carrier material, cost-effectiveness and accessibility, pH buffering moisture absorbing capacity, etc. (Date and Roughley 1977; Brockwell and Bottomley 1995). Peat-based application of rhizobial inoculants is the most widely used method for application of rhizobia worldwide since 1895. A diverse range of carriers such as soil material (peat, clay, charcoal) (Chao and Alexander 1984; Beck 1991; Temprano et al. 2002), perlite (Ronchi et al. 1997; Khavazi et al. 2007), vermiculite (Graham-weis et al. 1987), plant by-products (sawdust, peanut shell, corn cobs) (Sparrow and Ham 1983) and composts (Kostov and Lynch 1998) are used all over the world (Singh et al. 2016; Singh et al. 2017).

Other formulations such as liquid, granular and biofilm-based formulation have been studied, but of all formulations only solid- and liquid-based formulations have been exploited commercially. Liquid formulations are based on broth culture with oil in water suspensions or mineral and organic oil as carriers (Albareda et al. 2008; Bashan 1998). Granular formulations such as peat prills (Fouilleux et al. 1996), peat inoculants coated on sand (Chamber 1983), perlite/alginate beads (Bashan 1986; Hedge and Brahmaprakash 1992) and polymer-coated beads (Brockwell et al. 1980) have been studied. Biofilm-based formulation is latest and efficient one having greater stability under abiotic and biotic stresses. Bacteria may be grown on carrier material to form biofilm or trapped by a fungal matrix (Seneviratne 2003; Seneviratne et al. 2008; Triveni et al. 2013; Prasanna et al. 2013; Jayasinghearachchi and Seneviratne 2004).

14.10 Conclusion and Future Prospects

Currently, there is an increasing threat to agricultural sustainability, soil and groundwater contamination. Biofertilizer and biocontrol agents are used as a highly efficient alternative to chemical fertilizers and chemical pesticides, respectively. *Rhizobium* with promising biofertilization and biocontrol ability can be exploited for increasing legume and nonlegume production. Studies regarding secondary metabolites of *Rhizobium* need to be explored for its greater benefit for agriculture. Genetic engineering approaches can also be used to incorporate genes for secondary metabolites in rhizobial strains lacking it but have potential for biocontrol. Although a number of rhizobial biofertilizer such as solid and liquid formulations are available, better commercial formulations such as polymer and biofilm based need to be urgently introduced in the market.

References

Al-Ani RA, Adhab MA, Mahdi MH, Abood HM (2012) *Rhizobium japonicum* as a biocontrol agent of soybean root rot disease caused by *Fusarium solani* and *Macrophomina phaseolina*. Plant Prot Sci 48:149–155

Albareda M, Rodríguez-Navarro DN, Camacho M, Temprano FJ (2008) Alternatives to peat as a carrier for rhizobia inoculants: solid and liquid formulations. Soil Biol Biochem 40:2771–2779. https://doi.org/10.1016/j.soilbio.2008.07.0210067-y

Amadou C, Pascal G, Mangenot S, Glew M, Bontemps C, Capela D, Carrère S, Cruveiller S, Dossat C, Lajus A, Marchetti M (2008) Genome sequence of the β-rhizobium Cupriavidus taiwanensis and comparative genomics of rhizobia. Genome Res 18(9):1472–1483

Andrews SC, Robinson AK, Rodriguez-Quinones F (2003) Bacterial iron homeostasis. FEMS Microbiol Rev 27(2–3):215–237

Antoun H, Bordeleau LM, Gagnon C (1978) Antagonisme entre *Rhizobium meliloti* at *Fusarium oxysporum* en relation avec lefficacite symbiotique. Can J Plant Sci 58:75–78

Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R (1998) Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus Sativus* L.). Plant Soil 204:57–67

Appleby CA (1984) Leghemoglobin and Rhizobium respiration. Annu Rev Plant Physiol 35(1):443–478

Arfaoui A, Sifi B, El Hassni M, El Hadrami I, Boudabbous A, Chérif M (2005) Biochemical analysis of chickpea protection against Fusarium wilt afforded by two Rhizobium isolates. Plant Pathol J 4:35–42

Arfaoui A, Sifi B, Boudabous A, El Hadrami I, Cherif M (2006) Identification of *Rhizobium* isolates possessing antagonistic activity against *Fusarium oxysporum* f.sp *ciceris*, the causal agent of Fusarium wilt of chickpea. J Plant Pathol 88:67–75

Arora N, Kang S, Maheshwari D (2001) Isolation of siderophore producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. Curr Sci 81:673–677

Barber MS, Giesecke U, Reichert A, Minas W (2004) Industrial enzymatic production of cephalosporin-based b-lactams. Adv Biochem Engin/Biotechnol 88:179–216

Bardin SD, Huang H-C, Pinto J, Amundsen EJ, Erickson RS (2004) Biological control of Pythium damping-off of pea and sugar beet by *Rhizobium leguminosarum* bv. *viceae*. Can J Botany 82:291–296

Bashan Y (1986) Alginate beads as synthetic inoculant carriers for slow release of bacteria that affect plant growth. Appl Environ Microbiol 51(5):1089–1098

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotech adv 16(4):729–770

Beauchamp CJ, Dion P, Kloepper JW, Antoun H (1991) Physiological characterization of opine-utilizing rhizobacteria for traits related to plant growth-promoting activity. Plant Soil 132:273–279. https://doi.org/10.1007/bf00010408

Beck DP (1991) Suitability of charcoal-amended mineral soil as carrier for Rhizobium inoculants. Soil Biol Biochem 23:41–44

Beijerinck MW (1888) Cultur des *Bacillus radicicola* aus den Kno¨llchen. Bot Ztg 46:740–750

Benson DR, Clawson ML (2000) Evolution of the actinorhizal plant nitrogen-fixing symbiosis. In: Triplett E (ed) Prokaryotic nitrogen fixation: a model system for the analysis of a biological process. Horizon Scientific Press, Wymondham, pp 207–224

Berdy J (2005) Bioactive microbial metabolites a personal view. J Antibiot 58:1–26

Bergman K, Gulash-Hofee M, Hovestadt RE, Larosiliere RC, Ronco PG, Su L (1988) Physiology of behavioral mutants of *Rhizobium meliloti*: evidence for a dual chemotaxis pathway. J Bacteriol 170:3249–3254

Bibb MJ (2005) Regulation of secondary metabolism in streptomycetes. Curr Opin Microbiol 8:208–215

Biswas JC, Ladha JK, Dazzo FB (2000) Rhizobia inoculation improves nutrient uptake and growth of lowland rice. Soil Sci Soc Am J 64:1644–1650. https://doi.org/10.2136/sssaj2000.6451644x

Brockwell J, Bottomley PJ (1995) Recent advances in inoculant technology and prospects for the future. Soil Biol Biochem 27:683–697. https://doi.org/10.1016/0038-0717(95)98649-9

Brockwell J, Gault RR, Chase DL, Hely FW, Zorin M, Corbin EJ (1980) An appraisal of practical alternatives to legume seed inoculation: field experiments on seed bed inoculation with solid and liquid inoculants. Aust J Agri Res 31(1):47–60

Buonassisi AJ, Copeman RJ, Pepin HS, Eaton GW (1986) Effect of Rhizobium spp. on *Fusarium solani* f. sp. *phaseoli*. Can J Plant Pathol 8:140–146

Caetano-Anolles G, Crist-Estes DK, Bauer WD (1988) Chemotaxis of *Rhizobium meliloti* to the plant flavone luteolin requires functional nodulation genes. J Bacterial 170:3164–3169

Callaham DA, Torrey JG (1981) The structural basis for infection of root hairs of *Trifolium repens* by *Rhizobium*. Can J Bot 59:1647–1664

Carson KC, Holliday S, Glenn AR, Dilworth MJ (1992) Siderophore and organic acid production in root nodule bacteria. Arch Microbiol 157:264–271

Carson KC, Meyer J-M, Dilworth MJ (2000) Hydroxamate siderophores of root nodule bacteria. Soil Biol Biochem 32:11–21. https://doi.org/10.1016/S0038-0717(99)00107-8

Chakraborty U, Purkayastha RP (1984) Role of rhizobitoxine in protecting soybean roots from *Macrophomina phaseolina*. Can J Microbiol 30:285–289

Chamber MA (1983) Influence of several methods for rhizobial inoculation on nodulation and yield of soybeans. Plant Soi1 74:203–209

Chandra S, Choure K, Dubey RC, Maheshwari DK (2007) Rhizosphere competent *Mesorhizobium loti* MP6 induces root hair curling, inhibits *Sclerotinia sclerotiorum* and enhances growth of Indian mustard (*Brassica campestris*). Braz J Microbiol 38:124–130

Chao WL, Alexander M (1984) Mineral soils as carriers for Rhizobium inoculants. Appl Environ Microbiol 47:94–97

Chatterjee A, Cui Y, Liu Y, Dumenyo CK, Chatterjee AK (1995) Inactivation of *rsm*A leads to overproduction of extracellular pectinases, cellulases, and proteases in *Erwinia carotovora* subsp. *carotovora* in the absence of the starvation/cell density-sensing signal, N-(3-oxohexanoyl)-L-homoserine lactone. Appl Environ Microbiol 61:1959–1967

Chen WX, Yan GH, Li JL (1988) Numerical taxonomic study of fast-growing soybean rhizobia and a proposal that *Rhizobium fredii* be assigned to *Sinorhizobium* gen. nov. Int J Syst Bacteriol 38(4):392–397

Chernin L, Ismailov Z, Haran S, Chet I (1955) Chitinolytic *Enterobacter agglomerans* antagonistic to fungal plant pathogens. Appl Environ Microbiol 61(5):1720–1726

Corbett JR (1974) Pesticide design. In: The biochemical mode of action of pesticides. Academic, London, pp 44–86

Crowley DE, Gries D (1994) Modeling of iron availability in the plant rhizosphere. In: Biochemistry of metal micronutrients in the rhizosphere. Lewis Publishers, Boca Raton, pp 199–224

Das K, Prasanna R, Saxena AK (2017) Rhizobia: a potential biocontrol agent for soilborne fungal pathogens. Folia Microbiol 62(5):425–435

Date R, Roughley R (1977) Preparation of legume seed inoculants a treatise on dinitrogen fixation section IV agronomy and ecology. Wiley, New York, pp 243–275

Demain AL (1998) Induction of microbial secondary metabolism. Int Microbiol 1:259–264

Demain AL, Fang A (2000) The natural functions of secondary metabolites. In: Fietcher AI (ed) Advances in biochemical engineering/biotechnology: history of modern biotechnology, vol 2. Springer, Berlin, p 39

Demain AL, Sanchez S (2009) Microbial drug discovery: 80 years of progress. J Antibiot 62:5–16

Dénarié J, Cullimore J (1993) Lipo-oligosaccharide nodulation factors: a new class of signalling molecules mediating recognition and morphogenesis. Cell 74:951–954

Denison RF, Kiers ET (2004) Why are most rhizobia beneficial to their plant hosts, rather than parasitic? Microbes Infect 6(13):1235–1239

Deshwal VK, Pandey P, Kang SC, Maheshwari DK (2003) Rhizobia as a biological control agent against soil borne plant pathogenic fungi. Ind J Exp Biol 41:1160–1164

Diby P, Anandaraj M, Kumar A, Sarma YR (2005) Antagonistic mechanisms of fluorescent pseudomonads against *Phytophthora capsici* in black pepper (*Piper nigrum* L.). J Spices Aromatic Crops 14(2):122–129

Dihazi A, Jaiti F, Zouine J, Hassni ME, Hadrami IE (2003) Effect of salicylic acid on phenolic compounds related to date palm resistance to *Fusarium oxysporum* f. sp. *albedinis*. Phytopathol Mediterr 42:9–16

Dixon ROD, Wheeler CT (1986) Nitrogen fixation in plants. Blackie and Son, Glasgow. https://doi.org/10.1111/j.1439-0434.1997.tb00355.x

Dubey RC, Maheshwari DK (2011) Role of PGPR in integrated nutrient management of oil seed crops. In: Bacteria in agrobiology: plant nutrient management. Springer, Berlin/Heidelberg, pp 1–15

Dutta S, Mishra A, Kumar BD (2008) Induction of systemic resistance against fusarial wilt in pigeon pea through interaction of plant growth promoting rhizobacteria and rhizobia. Soil Biol Biochem 40:452–461

Ehteshamul-Haque S, Ghaffar A (1993) Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mungbean. J Phytopathol 138:157–163

Essalmani H, Lahlou H (2002) In vitro antagonistic activity of some microorganisms towards *Fusarium oxysporum* f. sp. *lentis* (french). Crypto Mycol 23:221–234

Estevez de Jensen C, Percich JA, Graham PH (2002) Integrated management strategies of bean root rot with *Bacillus subtilis* and *Rhizobium* in Minnesota. Field Crops Res 74:107–115. https://doi.org/10.1016/S0378-4290(01)00200-3

Fisher RF, Long SR (1992) *Rhizobium*-plmt signal exchange. Nature 357:655–660

Fouilleux G, Revellin C, Hartmann A, Catroux G (1996) Increase of *Bradyrhizobium japonicum* numbers in soils and enhanced nodulation of soybean (Glycine max (L) merr.) using granular inoculants amended with nutrients. FEMS Microbiol Ecol 20(3):173–183

Frank B (1889) Ueber die Pilzsymbiose der Leguminosen. Ber Deut Bot Ges 7:332–346

Gage DJ (2004) Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. Microbiol Mol Biol Rev 68(2):280–300

Ganesan S, Kuppusamy RG, Sekar R (2007) Integrated management of stem rot disease (*Sclerotium rolfsii*) of groundnut (*Arachis hypogaea* L.) using *Rhizobium* and *Trichoderma harzianum* (ITCC-4572). Turk J Agric For 31:103–108

Gao X, Lu X, Wu M, Zang H, Pan R, Tian J, Li S, Liao H (2012) Coinoculation with rhizobia and AMF inhibited soybean red crown rot: from field study to plant defense-related gene expression analysis. PLoS One 7:e33977

Gehring PJ, Mohan RJ, Watamare PG (1993) Solvents, fumigants and related compounds. In: Hayes WJ, Laws ER (eds) Handbook of pesticide toxiocology, vol 2. Academic, San Diego, pp 646–649

Gonzalez JB, Fernandez FJ, Tomasini A (2003) Microbial secondary metabolites production and strain improvement. Indian J Biotechnol 2:322–333

González V, Santamaría RI, Bustos P, Hernández-González I, Medrano-Soto A, Moreno-Hagelsieb G, Dávila G (2006) The partitioned *Rhizobium etli* genome: genetic and metabolic redundancy in seven interacting replicons. Proc Natl Acad Sci USA 103(10):3834–3839

Gopalakrishnan S, Sathya A, Vijayabharathi R, Varshney RK, Gowda CLL, Krishnamurthy L (2015) Plant growth promoting rhizobia: challenges and opportunities. Biotech 5:355–377. https://doi.org/10.1007/s13205-014-0241-x

Graham-Weis L, Bennet ML, Paau AS (1987) Production of bacterial inoculants by direct fermentation on nutrient-supplemented vermiculite. Appl Environ Microbiol 53:2138–2140

Guan C, Pawlowski K, Bisseling T (1995) Nodulation in legumes and Actinorhizal plants. In: Tikhonovich IA, Provorov NA, Romanov VI, Newton WE (eds) Nitrogen fixation: fundamentals and applications. Current plant science and biotechnology in agriculture. Springer, Dordrecht, pp 49–59

Guerinot ML (1994) Microbial iron transport. Annu Rev Microbiol 48(1):743–772

Guerinot ML, Meidl EJ, Plessner O (1990) Citrate as a siderophore in *Bradyrhizobium japonicum*. J Bacteriol 172:3298–3303

Gupta CP, Kumar B, Dubey RC, Maheshwari DK (2006) Chitinase mediated destructive antagonistic potential of *Pseudomonas aeruginosa* GRC1 against *Sclerotinia sclerotiorum* causing charcoal rot of peanut. BioControl 51:821–835

Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS (2002) Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245:83–93

Hafeez FY, Naeem FI, Naeem R, Zaidi AH, Malik KA (2005) Symbiotic effectiveness and bacteriocin production by *Rhizobium leguminosarum* bv. *viciae* isolated from agriculture soils in Faisalabad. Environ Exp Bot 54:142–147. https://doi.org/10.1016/j.envexpbot.2004.06.008

Handberg K, Stougaard JS (1992) *Lotus japonicus*, an autogamous, diploid legume species for classical and molecular genetics. Plant J 2:487–496

Hegde SV, Brahmaprakash GP (1992) A dry granular inoculant of Rhizobium for soil application. Plant Soil 144(2):309–311

Hemissi I, Mabrouk Y, Abdi N, Bouraoui M, Saidi M, Sifi B (2011) Effects of some Rhizobium strains on chickpea growth and biological control of *Rhizoctonia solani*. Afr J Microbiol Res 5:4080–4090

Hirsch PR (1979) Plasmid-determined bacteriocin production by *Rhizobium leguminosarum*. Microbiology 113:219–228. https://doi.org/10.1099/00221287-113-2-219

Hirsch PR, Van Montagu M, Johnston AWB, Brewin NJ, Schell J (1980) Physical identification of bacteriocinogenic, nodulation and other plasmids in strains of *Rhizobium leguminosarum*. Microbiology 120:403–412. https://doi.org/10.1099/00221287-120-2-403

Howieson JG, Brockwell J (2005) Nomenclature of legume root nodule bacteria in 2005 and implications for collection of strains from the field. In: Brockwell J (ed) 14th Australian nitrogen fixation conference. The Australian Society for Nitrogen Fixation, Katoomba, pp 17–23

Huang HC, Erickson RS (2007) Effect of seed treatment with *Rhizobium leguminosarum* on Pythium damping-off, seedling height, root nodulation, root biomass, shoot biomass, and seed yield of pea and lentil. J Phytopathol 155:31–37. https://doi.org/10.1111/j.1439-0434.2006.01189.x

Jarvis BDW, van Berkum P, Chen WX, Nour SM, Fernandez MP, Cleyet-Marel JC, Gillis M (1997) Transfer of *Rhizobium loti*, *Rhizobium huakuii*, *Rhizobium ciceri*, *Rhizobium mediterraneum*, and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. Int J Syst Evo microbiol 47(3):895–898

Jayasinghearachchi HS, Seneviratne G (2004) A bradyrhizobial-Penicillium spp. biofilm with nitrogenase activity improves N2 fixing symbiosis of soybean. Biol Fert Soils 40:432–434. https://doi.org/10.1007/s00374-004-0796-5

Jordan DC (1982) Transfer of *Rhizobium japonicum* Buchanan 1980 to Bradyrhizobium gen. nov., a genus of slow-growing, root nodule bacteria from leguminous plants. Int J Syst Bacteriol 32(1):136–139

Kacem M, Kazouz F, Merabet C, Rezki M, de Lajudie P, Bekki A (2009) Antimicrobial activity of *Rhizobium* sp. strains against *Pseudomonas savastanoi*, the agent responsible for the olive knot disease in Algeria. Grasas Aceites 60(2):139–146

Kannenberg EL, Brewin NJ (1989) Expression of a cell surface antigen from *Rhizobium leguminosarum* 3841 is regulated by oxygen and pH. J Bacteriol 171(9):4543–4548

Kawaguchi T, Azuma M, Horinouchi S, Beppu T (1988) Effect of B-factor and its analogues on rifamycin biosynthesis in *Nocardia* sp. J Antibiot 41:360–365

Khavazi K, Rejali F, Seguin P, Miransari M (2007) Effects of carrier, sterilisation method, and incubation on survival of *Bradyrhizobium japonicum* in soybean (*Glycine max* L.) inoculants. Enzym Microb Technol 41:780–784

Knowles CJ, Bunch AW (1986) Microbial cyanide metabolism. Adv Microbiol Physiol 27:73–111

Kostov O, Lynch JM (1998) Composted sawdust as a carrier for *Bradyrhizobium*, *Rhizobium* and *Azospirillum* in crop inoculation. World J Microbiol Biotechnol 14(3):389–397

Kumar H, Bajpai VK, Dubey RC, Maheshwari DK, Kang SC (2010) Wilt disease management and enhancement of growth and yield of *Cajanus cajan* (L) var. Manak by bacterial combinations amended with chemical fertilizer. Crop Prot 29:591–598. https://doi.org/10.1016/j.cropro.2010.01.002

Kumar H, Dubey RC, Maheshwari DK (2011) Effect of plant growth promoting rhizobia on seed germination, growth promotion and suppression of Fusarium wilt of fenugreek (*Trigonella foenumgraecum* L.). Crop Prot 30:1396–1403. https://doi.org/10.1016/j.cropro.2011.05.001

Kumari S, Khanna V (2014) Effect of antagonistic Rhizobacteria coinoculated with *Mesorhizobium ciceris* on control of fusarium wilt in chickpea (*Cicer arietinum* L.). Afr J Microbiol Res 8:1255–1265

Kurrey D, Lakpale R, Rajput RS (2016) Growth behavior, nodulation and Rhizobium population, as affected by combined application of herbicide and insecticide in soybean (*Glycine max* L.). J Pure Appl Microbio 10(4):2931–2936

Laranjo M, Alexandre A, Oliveira S (2014) Legume growth-promoting rhizobia: an overview on the *Mesorhizobium* genus. Microbiol Res 169(1):2–17

Libbenga KR, Harkes PAA (1973) Initial proliferation of cortical cells in the formation of root nodules in *Pisum sativum* L. Planta 114:17–28

Ligon JM, Hill DS, Hammer PE, Torkewitz NR, Hofmann D, Kempf HJ, Pée KHV (2000) Natural products with antifungal activity from pseudomonas biocontrol bacteria. Pest Manag Sci 56:688–695

Lloyd CW, Pearce KJ, Rawlins DJ, Ridge RW, Shaw PJ (1987) Endoplasmic microtubules connect the advancing nucleus to the tip of legume root hairs, but F-actin is involved in basipetal migration. CellMot Cytoskel 8:27–36

Lodwig EM, Poole PS (2003) Metabolism of *Rhizobium* bacteroids. Crit Rev Plant Sci 22(1):37–38

MacLean AM, Finan TM, Sadowsky MJ (2007) Genomes of the symbiotic nitrogen-fixing bacteria of legumes. Plant Physiol 144(2):615–622

Makoi JHJR, Bambara S, Ndakidemi PA (2010) Rhizosphere phosphatase enzyme activities and secondary metabolites in plants as affected by the supply of *Rhizobium*, lime and molybdenum in *Phaseolus vulgaris* L. Aust J Crop Sci 4(8):590–597

Malajczuk N, Pearce M, Litchfield RT (1984) Interactions between *Phytophthora cinnamomi* and *Rhizobium* isolates. Trans Br Mycol Soc 82:491–500. https://doi.org/10.1016/S00071536(84)80014-5

Martínez-Viveros O, Jorquera MA, Crowley DE, Gajardo G, Mora ML (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J Soil Sci Plant Nutr 10:293–319

Matzanke BF (1991) Structures, coordination chemistry and functions of microbial iron chelates. In: Winkelmann G (ed) Handbook of microbial iron chelates. CRC Press, Boca Raton, pp 15–64

Mavrodi DV, Bonsall RF, Delaney SM, Soule MJ, Phillips G, Thomashow LS (2001) Functional analysis of genes for biosynthesis of Pyocyanin and phenazine-1-Carboxamide from *Pseudomonas aeruginosa* PAO1. J Bacteriol 183:6454–6465. https://doi.org/10.1128/jb.183.21.6454-6465.2001

Mazen MM, El-Batanony NH, Abd El-Monium MM, Massoud ON (2008) Cultural filtrate of *Rhizobium* spp. and arbuscular mycorrhiza are potential biological control agents against root rot fungal diseases of Faba bean. Global J Biotechnol Biochem 3(1):32–41

Mehta S, Nautiyal SC (2001) An efficient method for qualitative screening of phosphate solubilizing bacteria. Curr Microbiol 43:51–56

Mishra RP, Singh RK, Jaiswal HK, Kumar V, Maurya S (2006) Rhizobium-mediated induction of phenolics and plant growth promotion in rice (*Oryza sativa* L.). Curr Microbiol 52:383–389

Modi M, Shah KS, Modi VV (1985) Isolation and characterisation of catechol-like siderophore from cowpea Rhizobium RA-1. Arch Microbiol 141:156–158. https://doi.org/10.1007/bf00423277

Mourad K, Fadhila K, Chahinez M, Meriem R, Philippe DL, Abdelkader B (2009) Antimicrobial activities of *Rhizobium* sp. strains against *Pseudomonas savastanoi*, the agent responsible for the olive knot disease in Algeria. Grasas Aceites 60(2):139–146

Mylona P, Pawlowski K, Bisseling T (1995) Symbiotic nitrogen fixation. Plant Cell 7:869–885

Nadia H, Massoud O, Mazen M, El-Monium MA (2007) The inhibitory effects of cultural filtrates of some wild *Rhizobium* spp. on some faba bean root rot pathogens and their antimicrobial synergetic effect when combined with Arbuscular mycorrhiza (AM). World J Agric Sci 3:721–730

Nap JP, Bisseling T (1990) Nodulin function and nodulin gene regulation in root nodule development. In: Gresshoff PM (ed) The molecular biology of symbiotic nitrogen fixation. CRC Press, Boca Raton, pp 181–229. ISBN 0-8493-6188-5

Nautiyal CS (1997) Rhizosphere competence of *Pseudomonas* sp. NBRI9926 and *Rhizobium* sp. NBRI9513 involved in the suppression of chickpea (*Cicer arietinum* L.) pathogenic fungi. FEMS Microbiol Ecol 23:145–158. https://doi.org/10.1111/j.1574-6941.1997.tb00398.x

Newcomb W (1981a) Nodule morphogenesis and differentiation [Rhizobium]. Int Rev Cytol Suppl 13:247–298

Newcomb W (1981b) Nodule morphogenesis. In Bourne GH, Danielli JF (eds) Int Rev Cytology, Supplement 13. Academic, New York pp 246–298

Nobbe F, Hiltner L (1896) Inoculation of the soil for cultivating leguminous plants. US patent 570:813

O'Brien J, Wright GD (2011) An ecological perspective of microbial secondary metabolism. Curr Opin Biotechnol 22(4):552–558

O'Gara F, Shanmugam KT (1976) Regulation of nitrogen fixation by Rhizobia. Export of fixed N_2 as NH_4 $^+$. Biochim Biophys Acta 437(2):313–321

Omar SA, Abd-Alla MH (1998) Biocontrol of fungal root rot diseases of crop plants by the use of rhizobia and bradyrhizobia. Folia Microbiol 43:431–437. https://doi.org/10.1007/bf02818587

Pandey P, Maheshwari DK (2007) Two-species microbial consortium for growth promotion of Cajanus cajan. Curr Sci 92:1137–1142

Patel HN, Chakraborty RN, Desai SB (1988) Isolation and partial characterization of phenolate siderophore from *Rhizobium leguminosarum* IARI 102. FEMS Microbiol Let 56(2):131–134

Pawlowski K, Bisseling T (1997) Legume and actinorhizal root nodule formation. In: Plant roots-from cells to systems. Springer, Dordrecht, pp 137–142

Perret X, Staehelin C, Broughton WJ (2000) Molecular basis of symbiotic promiscuity. Microbiol Mol Biol Rev 64:180–201

Persmark M, Pittman P, Buyer JS, Schwyn B, Gill PR, Neilands JB (1993) J Am Chem Soc 115:3950–3956

Phillips DA, Kapulnik Y (1995) Plant isoflavonoids, pathogens and symbionts. Trends Microbiol 3(2):58–66

Postgate JR (1982) The fundamentals of nitrogen fixation. Cambridge University Press, Cambridge/New York

Prasanna R, Kumar A, Babu S, Chawla G, Chaudhary V, Singh S, Gupta V, Nain L, Saxena AK (2013) Deciphering the biochemical spectrum of novel cyanobacterium-based biofilms for use as inoculants. Biol Agric Hortic 29:145–158. https://doi.org/10.1080/01448765.2013.790303

Raaijmakers JM, Vlami M, de Souza JT (2002) Antibiotic production by bacterial biocontrol agents. Anton van Leeuwen 81:537–547. https://doi.org/10.1023/a:1020501420831

Ramos T, Bellaj ME, Idrissi-Tourane AE, Daayf F, Hadrami IE (1997) Les Phénolamides des Rachis de Palmes, Composants de la Réaction de Défense du Palmier Dattier vis-à-vis de *Fusarium oxysporum* f.sp. *albedinis*, Agent Causal du Bayoud. J Phytopathol 145:487–493

Rezzonico F, Zala M, Keel C, Duffy B, Moënne-Loccoz Y, Défago G (2007) Is the ability of biocontrol fluorescent pseudomonads to produce the antifungal metabolite 2,4-diacetylphloroglucinol really synonymous with higher plant protection? New Phytol 173:861–872

Ridge RW (1988) Investigation of the cytoskeleton of freeze substituted root hairs. *6ot*. Mag Tokyo 101:427–441

Rioux CR, Jordan DC, Rattray JBM (1986) Iron requirement of *Rhizobium leguminosarum* and secretion of anthranilic acid during growth on an iron-deficient medium. Arch Biochem 248:175–182

Robertson JG, Lyttleton P, Bullivant S, Grayston GF (1978) Membranes in lupin root nodules. I. The role of Golgi bodies in the biogenesis of Infection threads and peribacteroid membranes. J Cell Sci 30:129–149

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Ronchi AL, Grassano A, Balatti AP (1997) Perlite as a carrier for legume inoculants. Agrochimica 41:186–195

Roth LE, Stacey G (1989) Bacterium release into host cells of nitrogen-fixing soybean nodules: the symbiosome membrane comes from three sources. Eur J Cell Biol 49(1):13–23

Roughley RJ, Vincent J (1967) Growth and survival of *Rhizobium* spp. in peat culture. J Appl Bacteriol 30:362–376

Roy N, Bhattacharyya P, Chakrabartty PK (1994) Iron acquisition during growth in an iron deficient medium by *Rhizobium* sp. isolated from *Cicer arietinum*. Microbiology 140:2811–2820

Rugheim AM, Abdelgani ME (2009) Effects of microbial and chemical fertilization on yield and seed quality of faba bean. 9th conference of the African crop science society: science and technology supporting food security in Africa. Cape Town, South Africa 28 September–1 October 2009

Ruiz Duenas FJ, Martinez MJ (1996) Enzymatic activities of *Trametes versicolor* and *Pleurotus eryngii*, implicated in biocontrol of *Fusarium oxysporum* f. sp. *lycopersici*. Curr Microbiol 32:151–155

Ruiz B, Chávez A, Forero A, García-Huante Y, Romero A, Sánchez M, Rocha D, Sánchez B, Rodríguez-Sanoja R, Sánchez S, Langley E (2010) Production of microbial secondary metabolites: regulation by the carbon source. Crit Rev Microbiol 36(2):146–167

Sagolshemcha R, Devi YN, Singh WR (2017) Plant growth promoting effect and biocontrol potential of *Rhizobium* spp. against *Macrophomina phaseolina*. Int J Curr Microbiol App Sci 6(6):2695–2701

Sáncheza AC, Gutiérrezc RT, Santanab RC, Urrutiab AR, Fauvarta M, Michielsa J, Vanderleydena J (2014) Effects of co-inoculation of native Rhizobium and Pseudomonas strains on growth parameters and yield of two contrasting *Phaseolus vulgaris* L. genotypes under Cuban soil conditions. Eur J Soil Biol 62:105–112

Seneviratne G (2003) Development of eco-friendly, beneficial microbial biofilms. Curr Sci 85:1395–1396

Seneviratne G, Zavahir JS, Bandara WMMS, Weerasekara MLMAW (2008) Fungal-bacterial biofilms: their development for novel biotechnological applications. World J Microbiol Biotechnol 24:739–743. https://doi.org/10.1007/s11274-007-9539-8

Shaban W, El-Bramawy M (2011) Impact of dual inoculation with *Rhizobium* and *Trichoderma* on damping off, root rot diseases and plant growth parameters of some legumes field crop under greenhouse conditions. Intl Res J Agric Sci Soil Sci 1:98–108

Siddiqui ZA (2006) PGPR: prospective biocontrol agents of plant pathogens. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 111–142. https://doi.org/10.1007/1-4020-4152-7_4

Siddiqui IA, Shaukat SS (2002) Mixtures of plant disease suppressive bacteria enhance biological control of multiple tomato pathogens. Biol Fert Soils 36:260–268

Siddiqui IA, Ehteshamul-Haque S, Zaki MJ, Abdul G (2000) Effect of urea on the efficacy of *Bradyrhizobium* sp. and *Trichoderma harzianum* in the control of root infecting fungi in mungbean and sunflower. Sarhad J Agric 16:403–406

Siddiqui IA, Shaukat SS, Hussain-Sheikh I, Khan A (2006) Role of cyanide production by *Pseudomonas fluorescens* CHA0 in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. World J Microbiol Biotechnol 22(6):641–650

Singh PK, Singh M, Vyas D (2010) Biocontrol of fusarium wilt of chickpea using arbuscular mycorrhizal fungi and *Rhizobium leguminosorum* biovar. Caryologia 63:349–353

Singh HB, Sarma BK, Keswani C (eds) (2016) Agriculturally important microorganisms: commercialization and regulatory requirements in Asia. Springer, Singapore

Singh HB, Sarma BK, Keswani C (eds) (2017) Advances in PGPR. CABI, London

Smith MJ, Shoolery JN, Schwyn B, Holden I, Neilands JB (1985) Rhizobactin, a structurally novel siderophore from *Rhizobium meliloti*. J Am Chem Soc 107:1739–1743. https://doi.org/10.1021/ja00292a047

Smitha M, Singh R (2014) Biocontrol of phytopathogenic fungi using mycolytic enzymes produced by rhizospheric bacteria of *Cicer arietinum*. Indian J Agric Biochem 27:215–218

Spaink H (1992) Rhizobial lipopolysaccharides: answers and questions. Plant Mol Biol 20:977–986

Sparow SD, Ham GE (1983) Survival of *Rhizobium phaseoli* in six carrier materials. Agron J 75:181–184

Sridevi M, Mallaiah K (2008) Factors effecting chitinase activity of *Rhizobium* sp. from Sesbania sesban. Biologia 63:307–312

Stephens JHG, Rask HM (2000) Inoculant production and formulation. Field Crops Res 65:249–258

Stokkermans TJW, Peters NK (1994) *Bradyrhizobium elkanii* lipooligosaccharide signal induce complete nodule structures on *Glycine soja* Siebold et Zucc. Planta 193:413–420

Sutton WD, Pankhurst CE, Craig AS (1981) The rhizobium bacteroid state. In: Bourne GH, Danielli JF (eds) International review of cytology, supplement 13. Academic, New York, pp 149–177

Szekeres A, Kredics L, Antal Z, Kevei F, Manczinger L (2004) Isolation and characterization of protease overproducing mutants of *Trichoderma harzianum*. FEMS Microbiol Lett 233:215–222

Temprano F, Albareda M, Camacho M, Daza A, Santamaría C, Rodríguez-Navarro ND (2002) Survival of several *Rhizobium*/*Bradyrhizobium* strains on different inoculant formulations and inoculated seeds. Int Microbiol 5:81–86. https://doi.org/10.1007/s10123-002-

Triplett EW, Barta TM (1987) Trifolitoxin production and nodulation are necessary for the expression of superior nodulation competitiveness by *Rhizobium leguminosarum* bv. *trifolii* strain T24 on clover. Plant Physiol 85:335–342

Triveni S, Prasanna R, Shukla L, Saxena AK (2013) Evaluating the biochemical traits of novel *Trichoderma*-based biofilms for use as plant growth-promoting inoculants. Ann Microbiol 63:1147–1156. https://doi.org/10.1007/s13213-012-0573-x

Van Kammen A (1984) Suggested nomenclature for plant genes involved in nodulation and symbiosis. Plant Mol Biol Rep 2:43–45

van Rhijn P, Vanderleyden J (1995) The Rhizobium–plant symbiosis. Microb Rev 59(1):124–142

VandenBosch KA, Bradley DJ, Knox JP, Perotto S, Butcher GW, Brewin NJ (1989) Common components of the infection thread matrix and the inter cellular space identified by immune cytochemical analysis of pea nodules and uninfected roots. EMBO J 8(2):335–341

Verdine GL (1996) The combinatorial chemistry of nature. Nature 384:11–13

Verma DPS, Hong Z (1996) Biogenesis of the peribacteriod membrane in root nodules. Trends Microbiol 4(9):364–368

Verma D, Long S (1983) Molecular biology of Rhizobium plant symbiosis. In: Jeon K (ed) Intracellular symbiosis. Academic, New York, pp 211–245

Vincent J (1974) Root nodule symbioses with *Rhizobium*. In: Quispel A (ed) Biology of nitrogen fixation. North Holland Press, Amsterdam, pp 265–341

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–750

Weidemann C, Tenhaken R, Höhl U, Barz W (1991) Medicarpin and maackiain 3-O-glucoside-6′-O-malonate conjugates are constitutive compounds in chickpea (*Cicer arietinum* L.) cell cultures. Plant Cell Rep 10:371–374. https://doi.org/10.1007/bf00193162

Weigand F, Köster J, Weltzien H, Barz W (1986) Accumulation of phytoalexins and isoflavone glucosides in a resistant and a susceptible cultivar of *Cicer arietinum* during infection with *Ascochyta rabiei*. J Phytopathol 115:214–221

Yu GY, Sinclair JB, Hartman GL, Bertagnolli BL (2002) Production of iturin a by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. Soil Biol Biochem 34:955–963. https://doi.org/10.1016/S0038-0717(02)00027-5

Zahran HH (1999) Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63(4):968–989

Zakhia F, Jeder H, Domergue O, Willems A, Cleyet-Marel JC, Gillis M, Dreyfus B, de Lajudie P (2004) Characterisation of wild legume nodulating bacteria (LNB) in the infra-arid zone of Tunisia. Syst Appl Microbiol 27(3):380–395