Applied Soil Ecology 94 (2015) 49–58

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Review

A review: Quorum sensing in *Bradyrhizobium*

Pablo C. Bogino, Fiorela L. Nievas, Walter Giordano *

Departamento de Biología Molecular, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina

ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form 14 April 2015
Accepted 24 April 2015
Available online xxx

Keywords:
QS mechanisms
Chemical signal
Symbiosis

ABSTRACT

Quorum sensing (QS) systems are an important form of cellular communication in bacteria. QS systems are based on the synthesis and secretion of a chemical signal (autoinducer) that accumulates as a function of population growth until reaching a threshold concentration that permits coordinated expression of certain genes that regulate bacterial physiology and behavior. A wide variety of soil bacteria (rhizobia) capable of establishing symbiotic associations with plants produces small chemical signaling molecules to communicate among themselves for physiological adaptation to environmental changes. Most species of rhizobia associated with legume plants have QS systems that regulate their behavior in a variety of soil microhabitats, including the establishment of symbiosis with the host plant. Species of the large, complex genus *Bradyrhizobium* are ecologically and agriculturally important, but present knowledge is limited and fragmentary regarding their QS communication systems, types of autoinducer produced, and biological processes regulated by QS. Therefore, the objective was to review findings to date on QS mechanisms in *Bradyrhizobium*, and the role of these mechanisms in symbiosis development and bacterial survival strategies. Bacteria of genus *Bradyrhizobium* produce a variety of QS signaling molecules, some of which are not found in any other bacterial genus. Of particular interest are the synthesis of bradyoxetin by *Bradyrhizobium* japonicum and its role in symbiosis regulation, and the synthesis of various branched homoserine lactones (HSLs) by other *Bradyrhizobium* species. In peanut-nodulating strains, these HSLs are associated with the processes of biofilm formation, motility, and autoaggregation. A proposed model is presented of QS mechanisms in *Bradyrhizobium* strains and the physiological processes regulated. The findings reviewed here provide a basis for future studies of QS communication systems in rhizobia and of regulatory mechanisms in bacterial behavior and ecophysiology.

Contents

1. Introduction

The high level of organization achieved by many bacterial species is reflected by their ability to synthesize molecules that play important roles in cell signaling mechanisms or regulate the expression of specific genes in response to changes in population

* Corresponding author at: Departamento de Biología Molecular, Universidad Nacional de Río Cuarto, Ruta 36, Km 601, X5804BYA Río Cuarto, Córdoba, Argentina. Tel.: +54 358 4676114; fax: +54 358 4676232.
E-mail address: wgiordano@exa.unrc.edu.ar (W. Giordano).

http://dx.doi.org/10.1016/j.apsoil.2015.04.016
0929-1393/

density. Such processes, termed quorum sensing (QS) (Fuqua and Greenberg, 2002), allow bacteria to coordinate their behavior in response to dynamic changes in the environment (Dong et al., 2008; Bernard et al., 2007). Through QS, bacterial populations are capable of regulating a variety of physiological processes, including bioluminescence, motility, symbiosis, plasmid transfer, antibiotic production, virulence factors, and biofilm formation (González and Marketon, 2003; Sanchez-Contreras et al., 2007; Pierson and Pierson, 2007). The common characteristic is that each of these processes are performed only if the bacterial cell population density is sufficiently high to ensure the success of the communication (Winzer et al., 2002; Atkinson et al., 2007).

QS mechanisms are common in soil bacteria that form associations with plants, and help regulate various aspects of the mechanisms whereby the bacteria–plant interaction is established (Bernard et al., 2007; Parsek and Greenberg, 2005; Whitehead et al., 2001; Dong et al., 2007; White and Winans, 2007). Communication within bacterial populations or communities that are in direct contact with the roots of plants is crucial in determining the physiological status of rhizosphere communities. Thus, QS mechanisms are important for bacterial survival, diversity maintenance, and interactions with plants.

Most rhizobial species have been found to produce one or more molecules associated with a QS system that regulates some aspect of the rhizobia–legume symbiosis (González and Marketon, 2003; Zheng et al., 2006; Sanchez-Contreras et al., 2007). QS mechanisms are involved in numerous symbiosis-related rhizobial functions, including exopolysaccharide (EPS) production, motility, nitrogen fixation, and nodulation (Rinaudi and Giordano, 2010). Despite the clear connection between QS and symbiosis, various rhizobial species with QS gene mutations are capable of establishing effective symbioses with their legume hosts, suggesting that the primary role of the QS system is to promote full development of symbiosis through enhancement of rhizobia–legume interactions. Better understanding of linkages between QS and symbiosis is therefore a valuable tool for improvement of rhizobia–legume interactions at the agroproductive level. Some studies indicate that QS regulatory systems mediated by *N*-acyl-homoserine lactones (acyl-HSLs) are present in rhizobia and regulate various aspects of symbiotic interactions (Sanchez-Contreras et al., 2007). Such acyl-HSLs may serve as signaling molecules between rhizobia and legume hosts. In some cases, the host produces acyl-HSL-mimicking compounds that activate or disrupt rhizobial communication and thereby affect the symbiosis (Sanchez-Contreras et al., 2007; Gao et al., 2003). Acyl-HSL production by the plant-associated genera *Bradyrhizobium*, *Sinorhizobium*, *Rhizobium*, and *Mesorhizobium* is of particular interest in terms of its effect on rhizobia–legume symbiotic interactions.

Various species of *Bradyrhizobium* have great agroecological importance; however, our knowledge of QS communication mechanisms in this group remains fragmentary and ambiguous. One problem in this regard is the complexity of the genus and the mechanisms whereby different species establish symbiosis with different legume hosts. In the soybean symbiont *B. japonicum*, QS mechanisms have been shown to be involved in symbiosis regulation through synthesis of bradyoxetin. Other novel molecules such as acyl branched-HSLs and aryl-HSLs have been isolated from *B. japonicum* and other *Bradyrhizobium* species, but their biological functions have not been studied. Production of acyl-HSLs (well-known signaling molecules) has been reported for peanut-nodulating *Bradyrhizobium* strains and shown to be associated with bacterial survival mechanisms such as biofilm formation, motility, and autoaggregation. QS mechanisms clearly play important agroecological roles in *Bradyrhizobium* species, but the connections between these mechanisms and the physiology of the bacteria are not well understood. In this review article, we summarize current knowledge of QS mechanisms in *Bradyrhizobium*, the biological processes affected by these mechanisms, and the production of novel QS signals. Theoretical models are presented that take into account the available data and provide a basis for future studies.

2. QS and symbiosis

Rhizobia–legume symbiosis is a very specific interaction between the bacteria and the plant that leads to the formation of nitrogen-fixing nodules in plant roots. The development of a successful symbiotic program involves several steps whereby the two partners exchange chemical signals (plant flavonoids/bacterial Nod factors) and various genes are differentially expressed. These processes result in activation of a nodulation program in the plant and coordination of bacterial invasion of the root with initial division of root cortical cells (Oldroyd and Downie, 2004). The symbiotic association is established when differentiated rhizobia inside the nodules (bacteroids) reduce atmospheric nitrogen to ammonium ions that the plant can utilize, and these compounds are exchanged for energy sources from the plant (Gage, 2004; Jones et al., 2007). Biological nitrogen fixation is a crucial process in agricultural food production and long-term productivity of crops under sustainable and environmentally sound programs. Improved understanding of the factors that control this process will help enhance the effectiveness of symbiotic development as a strategy in sustainable agriculture. The simple two-signal model (flavonoids/Nod factors) can be extended to more complex signaling systems that involve both plant and bacterial compounds to direct the course of root colonization (infection) (Cooper, 2007). A variety of physiological mechanisms are controlled by QS in various rhizobial species; these include surface polysaccharide production, growth inhibition, adaptation to stationary phase, nodulation efficiency, symbiosome development, and nitrogen fixation (Sanchez-Contreras et al., 2007). HSL molecules produced by rhizobia that communicate through QS can therefore be regarded as signals involved in the symbiotic programs of the rhizobia.

Bacterial invasion of the plant root in rhizobia–legume symbiosis may occur in two different ways. Plants provide to rhizobia a specific mechanism whereby the bacteria reach the nodule primordium. In most cases, the mechanism involves root-hair curling and the development of “infection threads” (Gage and Margolin, 2000). Another infection mechanism, termed “crack entry”, observed in subtropical legumes such as *Arachis* and *Aeschynomene* sp., involves entry of bacteria into the root through epidermal injuries caused by the emergence of lateral roots (Boogerd and van Rossum, 1997). After entry, the rhizobia colonize intercellular spaces in root subepidermal tissue as small populations termed “infection pockets”. The invasion process then continues through intercellular bacterial dissemination and entry from the infection pocket to cells of the nodule primordium for direct capture (Boogerd and van Rossum, 1997). Depending on the invasion mechanism, groups of bacteria accumulated in either root-hair curling or infection pockets are the last external populations prior to entry into plant tissues. Such groups may act as signaling centers from which the bacteria produce the amounts of chemical signals (*e.g.*, Nod factors, EPSs, HSLs) necessary to initiate plant responses (Goormachtig et al., 2004). The involvement of QS mechanisms at this stage of the invasion program presumably leads to physiological processes that determine subsequent infection and development of the rhizobia–legume symbiosis.

QS mechanisms have been identified and defined in many rhizobial species of agroecological importance, including *Sinorhizobium meliloti*, *Rhizobium leguminosarum*, *Rhizobium* etli, *Mesorhizobium* spp., and *Bradyrhizobium* spp. These rhizobial QS

Table 1
QS systems in members of the *Rhizobiaceae* family.

Bacterium	Legume host	QS system	Signaling molecule	Biological function regulated	Reference
Sinorhizobium meliloti					
Rm1021	*Medicago sativa*	SinR/SinI	C_{12}-HSL; 3-O-C_{14}-HSL 3-O-C_{16}-HSL 3-O-$C_{16:1}$-HSL C_{18}-HSL	Swarming motility, EPS production	Marketon and Gonzalez (2002), Marketon et al. (2003),Teplitski et al. (2003), Gao et al. (2005)
		ExpR	$C_{16:1}$-HSL	Swarming motility, EPS production, biofilm formation	Pellock et al. (2002), Gao et al. (2005), Rinaudi and González (2009)
		mel	C_8-HSL, short chain HSLs	Unknown	Marketon et al. (2002)
Rm41	*Medicago trunactula*	TraR/TraI	3-O-C_8-HSL	Plasmid transfer	He et al. (2003)
RU10/406	*Medicago sativa*	VisR/ VisN	Unknown effector	Motility, chemotaxis	Sourjik et al. (2000)
Rhizobium sp. NGR234	*Vigna unguiculata*	TraR/TraI	3-O-C_8-HSL	Plasmid transfer	He et al. (2003)
Rhizobium leguminosarum bv. *viciae*	*Vicia sativa*	CinR/CinI	3-OH-$C_{14:1}$-HSL	Growth inhibition, swarming motility	Lithgow et al. (2000)
		RhiR/RhiI	C_6-HSL; C_7-HSL C_8-HSL	Nodulation efficiency	Rodelas et al. (1999)
		TraR/TraI, BisR	C_8-HSL 3-O-C_8-HSL	Plasmid transfer	Danino et al. (2003)
		RaiR/RaiI	3-OH-C_8-HSL C_8-HSL	Unknown	Wisniewski-Dye et al. (2002)
		ExpR, CinS		Regulation of CinR/CinI–RaiR/RaiI systems	Edwards et al. (2009)
Rhizobium etli					
CNPAF512	*Phaseolus vulgaris*	CinR/CinI	3-OH-(slc)-HSL	Nitrogen fixation, symbiosome development, growth inhibition, swarming motility	Daniels et al. (2002, 2004),)
		RaiR/RaiI	Short chain HSL	Nitrogen fixation, growth inhibition	Daniels et al. (2002)
CFN42		TraR/TraI	3-O-C_8-HSL 3-OH-C_8-HSL	Plasmid transfer	Tun-Garrido et al. 2003
Agrobacterium tumefaciens		TraR/TraI	3-O-C_8-HSL	Plasmid transfer	Piper et al. (1993); Fuqua et al. (1994); Hwang et al. (1995)
		TraR2/ TraI2	3-O-C_8HSL	Unknown	Wang et al. (2014)
Mesorhizobium					
M. tianshanense	*Glycyrrhiza uralensis*, various legume plant species	MrtR/ MrtI	C_8-HSL 3-O-C_8-HSL 3-O-C_{12}-HSL	Growth rate, nodulation	Zheng et al. (2006), Cao et al. (2009)
M. loti	*Lotus corniculata*, various legume plant species	TraR/traI	3-O-C_6-HSL	Plasmid transfer	Ramsay et al. (2009)
Bradyrhizobium					
B. japonicum USDA110	*Glycine max*	Unknown	Bradyoxetin	*nod* gene regulation	Loh et al. (2001, 2002b),)
B. japonicum USDA110	*Glycine max*	BjaR/BjaI	Isovaleryl-HSL	Unknown	Lindemann et al. (2011)
B. japonicum native strains *B. elkanii* native strains	*Glycine max*	Unknown	Acyl-HSLs	Unknown	Pongslip et al. (2005)
B. sp. native strains.	*Arachis hypogaea*	Unknown	3-O-C_{10}-HSL 3-O-C_{12}-HSL 3-O-C_{14}-HSL	Biofilm formation, autoaggregation, swimming motility	Nievas et al. (2012b)
B. sp. ORS278 *B.* sp. BTai1	*Aeschynomene* genus	BraR/BraI	Cinnamoyl-HSL	Unknown	Ahlgren et al. (2011)

systems are involved in regulation of many important symbiosis-related phenotypes. Characteristics of QS systems for members of the family *Rhizobiaceae* are summarized in Table 1. No complete QS system has been identified in the genome of *Azorhizobium caulinodans*, the symbiont of *Sesbania rostrata*, although LuxR-type response regulators are present (Lee et al., 2008). One possibility is that *A. caulinodans* responds to HSL signals (mimicking compounds) originating from other microorganisms or plants in its microenvironment.

It has been difficult to identify QS elements such as signaling molecules and proteins homologous to LuxR–LuxI in certain plant-associated bacteria, particularly *Bradyrhizobium* species. Genes homologous to those in LuxR–LuxI QS systems have been found in the deciphered genomes of a few *Bradyrhizobium* species (Table 2).

Bradyrhizobium is a complex genus whose members form symbioses with many different legume species, including soybean, cowpea, and peanut. Numerous strains and even species of *Bradyrhizobium* have not yet been named (Willems et al., 2001; Vinuesa et al., 2005; Zhang et al., 2007), and little or no information is available on QS communication mechanisms in such species.

Studies to date on QS mechanisms in *Bradyrhizobium* have focused primarily on the species *B. japonicum* and the role of QS in its symbiotic relationship with soybean (*Glycine max*). The roles of various QS signaling molecules in other biological processes in *B. japonicum* are unclear. No information is available regarding QS systems in peanut (*Arachis*)-nodulating *Bradyrhizobium* species (Brelles-Marino and Bedmar, 2001; Pongslip et al., 2005).

3. QS in *B. japonicum*

The nodulation genes *nod*, *nol*, and *noe* in rhizobia are essential for nodule formation in legume roots. These genes encode a series of proteins that form a diffusible lipochitooligosaccharide (Nod factor) that acts as a signaling molecule for nodulation (Spaink, 2000).

nod gene expression in the soybean symbiont *B. japonicum* is a complex mechanism regulated by a cell density-dependent QS mechanism (Loh et al., 2001, 2002a) involving the expression of several regulatory pathways, including nodD1, nodVW, nwsAB, and nolA–nodD2 (Fig. 1A). Expression of regulatory *nod* genes is induced as a response to either plant isoflavonoids or changes in population density (quorum mechanism) (Jitacksorn and Sadowsky, 2008). *nod* genes are expressed in response to production of the isoflavonoid genistein by the soybean plant. The induction of genistein is mediated by NodD1, an LysR family regulatory protein found in all rhizobia (Göttfert et al., 1992).

The proteins NolA and NodD2 are part of a regulatory feedback mechanism that suppress *nod* gene expression in response to increased levels of Nod factors, particularly chitin tetrameric byproducts or intermediates. The increase in tetrameric Nod signals in response to soybean isoflavonoids as a result of *nodYABC* operon expression also induces *NolA* gene expression. The NolA regulator activates expression of the NodD2 regulator that suppresses the nodYABC operon (Loh and Stacey, 2001) (Fig. 1A). NolA and NodD2 also modulate *nod* gene expression in a cell density-dependent manner (Loh et al., 2001). *nod* gene induction was maximal in low-density cultures and much lower in high-density cultures. In agreement with this finding, soybean nodulation was reduced when plants were inoculated with high-density *B. japonicum* cultures (Jitacksorn and Sadowsky, 2008). NolA and NodD2 mediate this QS-regulated phenotype, which is expressed at high cell density when the ability to induce *nod* genes is reduced (Fig. 1B). An *nolA* gene mutant did not display such dependence on cell density; *nodYABC* genes were not suppressed even at high cell densities.

The establishment of symbiosis in *B. japonicum* is further regulated by two-component systems. The two-component system NodVW activates expression of *nod* genes by isoflavonoids and is essential for nodulation in cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), and siratro (*Macroptilium atropurpureum*), but not in soybean (Göttfert et al., 1990; Loh et al., 1997). The host specificity of *B. japonicum* is apparently due to the fact that NodD1-mediated Nod factor production is sufficient for soybean nodulation; in contrast, coordinated activity of NodD1 and NodVW is required for nodulation of cowpea, mung bean, and siratro (Loh et al., 2002a).

NwsAB is another two-component system for *nod* gene regulation in *B. japonicum*. NwsB (encoded by *nwsB*) is a regulatory protein required for full expression of *nod* genes in the presence of genistein. NwsB activity complements the effect of NodW, and cross-talk may occur between the NodVW and NwsAB systems (Grob et al., 1993). At high cell population density, NwsB is required to regulate the expression of genes *nolA* and *nodD2*, which suppress *nod* gene expression. A *nwsB* mutant was able to synthesize the quorum signal even though it was incapable of responding to this signal because *nolA* and *nodD2* expression was not induced (Loh et al., 2001). These findings suggest that NwsB determines the ability of soybean isoflavonoids to induce *nod* gene expression in *B. japonicum*, through *nod* gene activation at low cell density and *nod* gene suppression at high cell density (Loh et al., 2002a; Loh and Stacey, 2001) (Fig. 1A, B).

As in other QS systems, such dependence on population density is mediated through production of an extracellular signal (cell density factor; CDF) that accumulates as a function of culture density (Loh et al., 2001). Isolation and purification of CDF from *B. japonicum* USDA110 (recently renamed *B. diazoefficiens*; Delamuta et al., 2013) revealed that it is a novel autoinducer molecule different from other QS signals. CDF is composed of two aromatic rings linked by an imino group; each ring contains an amino oxetane group in position p. This molecule, termed bradyoxetin, has the proposed structure 2-{4-[[4-(3-aminooxetan-2-yl)phenyl]-(imino)methyl]phenyl}oxetan-3-ylamine (Loh et al., 2002b). Bradyoxetin acts as an NolA inducer leading to *nod* gene suppression.

Bradyoxetin production is downregulated by Fe^{3+}, and is maximal under iron starvation conditions, reflecting an apparent link between nutrient limitation and QS, as described for other bacteria (Bollinger et al., 2001). Bradyoxetin has been suggested to function as a siderophore in addition to its autoinducer activity. Its molecular structure is very similar to that of the siderophore mugineic acid (Drechsel and Jung, 1998). However, bradyoxetin is not a high-affinity siderophore in free-living bacteria. One

Table 2
Number of homologous *luxR–luxI* genes in various *Bradyrhizobium* genomes. http://genome.microbedb.jp/rhizobase/.

Genome	*luxR* gene			*luxI* gene
	Putative *N*-acyl-HSL transcriptional regulator	Transcriptional regulatory protein	Two-component transcriptional regulator	
B. japonicum USDA 110	1 (blr1062)	15	–	1 (blr1063) putative autoinducer synthase
B. japonicum USDA 6	1 (BJ6T_10880)	9	4	1 (BJ6T_10890) putative autoinducer synthase
B. sp. *BTAi1*	1 (BBta_7112)	10	8	1 (BBta_7113) putative autoinducer (acyl-HSL) synthase
B. sp. ORS278	1 (BRADO0942)	6	7	1 (BRADO0941) putative autoinducer (acyl-HSL) synthase

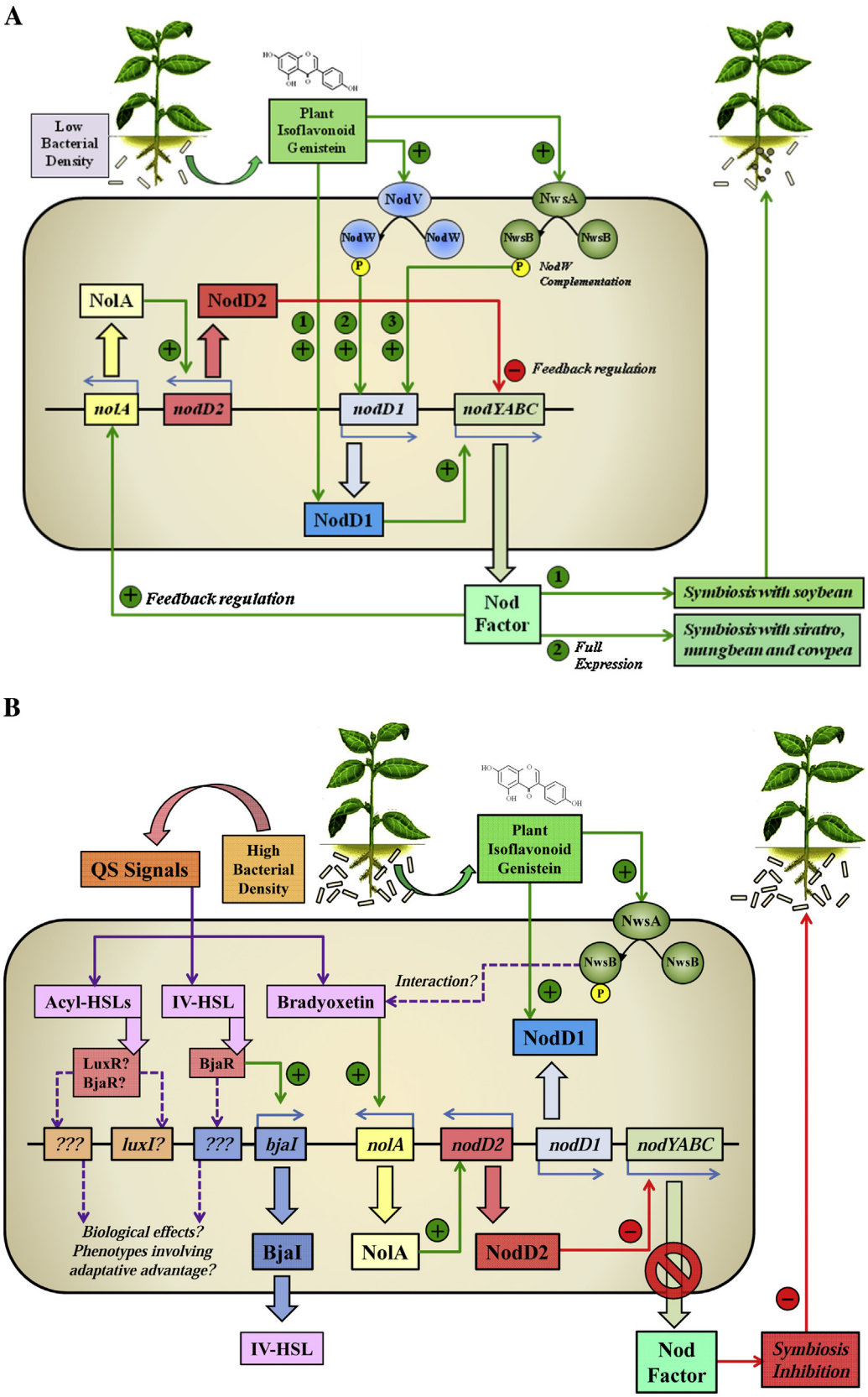

Fig. 1. Proposed models of relationships among bacterial population density, QS, symbiosis, and other biological processes in *Bradyrhizobium japonicum*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Green arrows: gene expression or process development. Red arrows: gene suppression or process inhibition. Dashed purple arrows: putative gene expression or suppression, or putative process development.
(A) Control of symbiosis at low bacterial density. Plant isoflavonoids are crucial for induction of *nod* gene expression (*nodYABC* operon) and Nod factor synthesis through activation of regulatory protein NodD1 and the two-component systems NodVW and NwsAB. NodD1-mediated Nod factor production is sufficient for establishment of

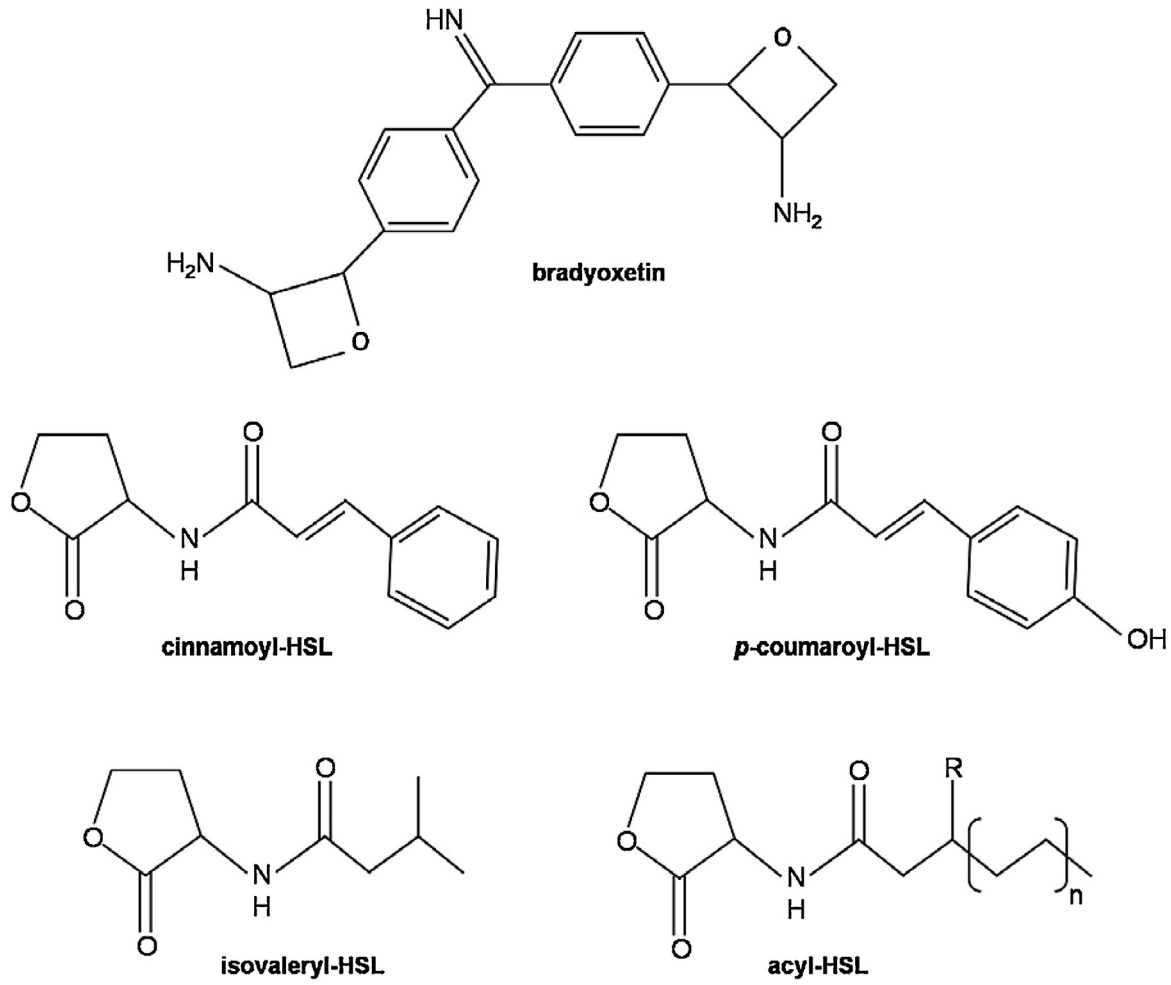

Fig. 2. Structures of QS signaling molecules produced by *Bradyrhizobium*.
The structure of bradyoxetin is quite different from those of four homoserine lactones (HSLs). For acyl-HSL, R represents a variable substituent (hydrogen, hydroxyl, or oxo).

possibility is that bradyoxetin is a nodule-specific siderophore and becomes active under iron-limiting conditions in the symbiosome, suppressing *nod* gene expression in the plant (Loh et al., 2002b). Iron is an essential element sequestered by the plant for nodule development, nodulation regulation, and proper functioning of nitrogen assimilation enzymes (nitrogenase, nitrate reductase, nitrite reductase) and cytochromes (Johnston et al., 2001). In this regard, iron is presumably a limiting nutrient for symbiotic bacteria; *i.e.*, low iron level in combination with high bacterial population density in the small symbiosome compartment leads to increased bradyoxetin content, consequent *nolA* and *nodD2* gene expression, and suppression of *nod* genes, thereby avoiding the energetic cost of synthesizing Nod factor molecules.

The presence of two oxetane rings in the bradyoxetin molecule may confer antibiotic properties, in view of the structural similarity to oxetin, an antibiotic molecule produced by the actinomycete *Streptomyces* sp. OM2317 (Omura et al., 1984). MerR family proteins such as NolA are activated in the presence of toxic compounds; along this line, synthesis of bradyoxetin as an antibiotic may enhance protection against stress and confer a significant competitive advantage over other rhizobia during the nodulation process (Thorne and Williams, 1999).

The production of acyl-HSL-like signaling molecules has been demonstrated in native strains of soybean-nodulating *Bradyrhizobium* strains (Pongslip et al., 2005). Of 142 strains analyzed, only 22% were capable of producing acyl-HSL molecules when *Agrobacterium tumefaciens* NT1 (pZLR4) (Cha et al., 1998) was used as a biosensor to detect autoinducer production. All strains positive for autoinducer activity belonged to the species *B. japonicum* or *Bradyrhizobium elkanii*. Neither LuxR–LuxI type QS systems nor biological functions regulated by the acyl-HSL molecules have been identified in these native strains.

B. japonicum strain USDA110 has genes homologous to *luxI–luxR*, termed *bjaI–bjaR* (Kaneko et al., 2002). Lindemann et al. (2011) reported the synthesis in *B. japonicum* USDA110 of a novel signaling molecule catalyzed by BjaI synthase. The novel molecule consisted of a branched chain acyl group attached to HSL, identified as isovaleryl HSL (IV-HSL). IV-HSL was produced and

symbiosis with soybean (1). Coordinated activities of the proteins NwsAB–NodVW–NodD1 are required for full expression of *nod* genes leading to nodulation of cowpea, mung bean, and siratro (2). Synthesis of Nod factor is regulated through a feedback mechanism: increased levels of Nod factor activate expression of *nolA-nodD2* genes, leading to synthesis of NodD2 regulator which inhibits expression of the *nodYABC* operon. (B) Control of symbiosis at high bacterial density, and proposed mechanism of QS development. *B. japonicum* is able to produce various QS signals. Regulation of symbiosis establishment is associated with the QS molecule bradyoxetin. Bradyoxetin inhibits symbiosis through activation of the NolA–NodD2 regulatory system and suppression of *nod* gene. This mechanism also requires participation of NwsB protein, suggesting a possible interaction with bradyoxetin. The QS signaling molecule IV-HSL upregulates its own synthesis through binding to BjaR regulator and activation of *bjaI* gene. Production of acyl-HSL has been observed for native soybean-nodulating *Bradyrhizobium* strains. The biological processes regulated by IV-HSL and acyl-HSL remain to be clarified.

displayed activity at concentrations (~5 nM) much lower than those of acyl-HSLs (0.1–10 μM) produced by most bacteria (Thiel et al., 2009). The $BjaR_1$ regulator, a LuxR homolog, showed high affinity for IV-HSL. In analogy to other acyl-HSL QS systems, *bjaI* expression was upregulated in response to IV-HSL addition. *B. japonicum* USDA110 is able to respond to acyl-HSLs but not to synthesize them. The high sensitivity of the $BjaR_1$ regulator to IV-HSL and the low specificity to acyl-HSLs may reflect a strategy of *B. japonicum* to both avoid detection of IV-HSL by other bacterial strains and detect QS signals from other microorganisms, in order to acquire a competitive advantage in the rhizospheric microniche (Lindemann et al., 2011). This QS system has been well described for *B. japonicum*, but the biological functions of IV-HSL remain unclear (Fig. 1B). Expression of a new LuxR family member (encoded by *blr1880*) was highly upregulated in bacteroids of *B. japonicum*, suggesting a link between symbiosis development and QS (Pessi et al., 2007).

The observed production of bradyoxetin and IV-HSL by *B. japonicum* and of acyl-HSLs by native *Bradyrhizobium* strains indicate that nodulation and other biological processes are modulated by different autoinducers and different global regulator families to coordinate the cellular physiology of soybean-nodulating *Bradyrhizobium* (Fig. 1B).

4. QS in strains of *Bradyrhizobium* sp.

In contrast to well-studied models of rhizobia–legume interaction such as *S. meliloti*—alfalfa, *R. leguminosarum*—bean, and *B. japonicum*—soybean, the symbiotic *Bradyrhizobium* sp.-peanut (*Arachis hypogaea* L.) interaction is less well understood. The latter interaction has great agroecological importance, particularly in countries where peanut is a major crop (*e.g.*, China, India, USA, Argentina). There is a clear positional effect during inoculation on the symbiotic ability of *Bradyrhizobium* strains. A series of studies has shown that application of the inoculant bacterial strain directly to the soil ("in-furrow" inoculation) prior to sowing increases the efficiency of biological nitrogen fixation and competitiveness for nodulation in comparison with "on-seed" inoculation (Bogino et al., 2006, 2008, 2011). The mechanisms whereby position affects competitiveness for nodule occupancy vary depending on the strain and probably include chemotaxis, adhesion, and motility. These same mechanisms are also involved in biofilm formation and are presumably subject to QS regulation (Parsek and Greenberg, 2005; Rinaudi and Giordano, 2010). Our limited knowledge of QS mechanisms in *Bradyrhizobium* strains is summarized in the following subsections.

4.1. Autoinducers produced by Bradyrhizobium sp.

The major signaling molecules in Gram-negative bacteria are acyl-HSLs (Atkinson and Williams, 2009). Over 100 species of *Proteobacteria* use acyl-HSLs as diffusible molecules that act as QS signals (Fuqua and Greenberg, 2002). However, novel HSL signaling molecules have been detected and identified as aryl-HSLs and IV-HSLs (Lindemann et al., 2011; Ahlgren et al., 2011) only recently in *Bradyrhizobium* strains phylogenetically related to peanut-nodulating strains. Structures of various signaling molecules produced by *Bradyrhizobium* strains are shown in Fig. 2. Schaefer et al. (2008) identified a new HSL derivative, *N*-(*p*-coumaroyl)-HSL (pC-HSL, an aryl-HSL) in the photosynthetic bacterium *Rhodopseudomonas palustris*. Other variants with the chemical structures cinnamoyl-HSL (another aryl-HSL) and IV-HSL (a branched-chain HSL) have been found in *Bradyrhizobium* strains (Lindemann et al., 2011; Ahlgren et al., 2011).

Only a few studies have addressed QS mechanisms in peanut-nodulating *Bradyrhizobium* strains. One QS system was described in *Bradyrhizobium* BTAi1, an unusual strain that is photosynthetic and capable of forming nitrogen-fixing nodules on the stems of *Aeschynomene* sp. (Ahlgren et al., 2011; Molouba et al., 1999). This symbiosis is established through a "crack entry" mechanism similar to that in peanut-nodulating rhizobia. The QS system in *B.* BTAi1 responds to cinnamoyl-HSL, which is produced by BraI in nM-order concentrations (comparable to those of IV-HSL in *B. japonicum* USDA110) and detected by the BraR regulator. The *braI* gene is positively autoregulated by cinnamoyl-HSL. The high affinity of BjaR for IV-HSL and of BraR for cinnamoyl-HSL, and the relative specificity of these two receptors for other HSLs, may reflect a strategy used by *Bradyrhizobium* strains to respond to signals from other bacteria and avoid detecting their own signals in various microecosystems (soil, rhizosphere, plant). The genes and chemical structures of QS signals in these *Bradyrhizobium* strains have been elucidated (Lindemann et al., 2011; Ahlgren et al., 2011), but the effects of the signals on rhizobial symbiosis or other physiological processes are unknown. These findings provide a useful baseline for further studies of QS mechanisms in *Bradyrhizobium* strains, particularly peanut-nodulating strains. Strains isolated from peanut grown in various sites in southern Córdoba province, Argentina (Bogino et al., 2006, 2010; Nievas et al., 2012a) were found to produce acyl-HSL-like signaling molecules, particularly long-chain (C_6–C_{16}) molecules. This was the first report of QS molecule production by peanut-nodulating *Bradyrhizobium* strains (Nievas et al., 2012b).

In studies of a collection of >50 native peanut-nodulating rhizobial strains from various agrogeographic locations in central/south Córdoba province in Argentina, 34% of the evaluated strains were capable of synthesizing long-chain acyl-HSL-like molecules. Through quantification of β-galactosidase activity induction in *A. tumefaciens* NTL4 (pZLR4) (Cha et al., 1998), the rhizobial strains were classified according to high, moderate, or low production of acyl-HSL-like molecules (Nievas et al., 2012b). These findings were consistent with those of Pongslip et al. (2005), who observed production of long-chain acyl-HSL-like molecules in a geographically and genetically diverse collection of soybean-nodulating bacteria. TLC and HPLC MS/MS analyses of peanut strain extracts revealed various types of signaling molecules corresponding to C_6 acyl-HSL and C_{10}, C_{12}, and C_{14} acyl-HSL having a 3-keto substituent ($3OC_{10}$, $3OC_{12}$, and $3OC_{14}$) (Nievas et al., 2012b). These findings are consistent with many previous reports of high diversity of acyl-HSLs produced by rhizobia (Sanchez-Contreras et al., 2007; Brelles-Marino and Bedmar, 2001; Atkinson and Williams, 2009). For example, *R. leguminosarum* bv. *viciae* synthesizes mainly $3OC_8$ acyl-HSL and C_6 acyl-HSL (Danino et al., 2003), and *S. meliloti* Rm 1021 produces at least seven distinct acyl-HSLs, notably $3OC_{14}$ acyl-HSL (Marketon and González, 2002). There appears to be overlap in the production and recognition of acyl-HSLs by various strains, suggesting common QS mechanisms, or cross-talk, among rhizobia.

4.2. Biological processes regulated by QS in Bradyrhizobium sp.

In preliminary studies of peanut-nodulating *Bradyrhizobium* strains, addition of various types and concentrations of acyl-HSLs to cultured bacteria affected physiological processes related to survival, *i.e.*, biofilm formation, motility, and autoaggregation (Nievas et al., 2012b).

Biofilms are bacterial communities attached to a surface in which cells are embedded in a self-produced matrix of extracellular polymeric compounds (Branda et al., 2005). Bacteria growing in natural habitats typically undergo transitions involving repeated cycles of differentiation from free-living planktonic forms to complex communities organized on and attached to biotic or abiotic surfaces (biofilms) (Webb et al., 2003). The occurrence of these cycles is governed by complex regulatory systems that

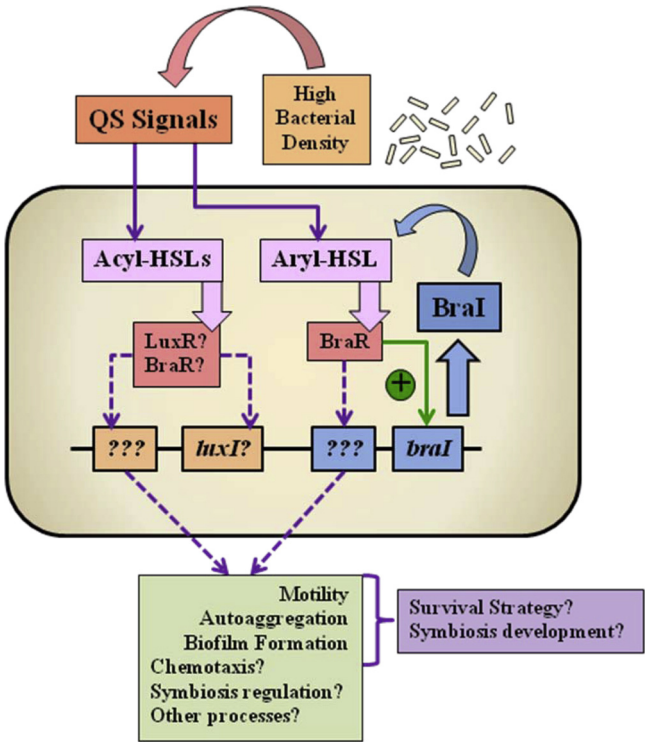

Fig. 3. Proposed model of QS mechanisms in *Bradyrhizobium* strains and the physiological processes regulated. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Green arrows: gene expression or process development. Dashed purple arrows: putative gene expression or suppression, or putative process development. Production of aryl-HSL by *Bradyrhizobium* ORS278 upregulates its own synthesis through binding to BraR regulator and activation of *braI* gene. Production of acyl-HSL in peanut-nodulating *Bradyrhizobium* strains is associated with physiological processes related to bacterial survival.

respond continuously to metabolic and environmental changes. QS regulation is a central component in regulation of major aspects of biofilm development, including adhesion, motility, maturation, and dispersion (Dong et al., 2008; Parsek and Greenberg, 2005; Daniels et al., 2004; Stoodley et al., 2002).

Recent studies show that acyl-HSLs participate in physiological processes that control physical cell-cell interactions in peanut-nodulating *Bradyrhizobium* strains. Such interactions are essential for bacterial survival in the environment and for establishment of a symbiotic association with the legume host. Exogenous addition of acyl-HSLs ($3OC_{10}$, $3OC_{12}$, $3OC_{14}$) was found to alter the processes of biofilm formation, autoaggregation, and bacterial motility (swimming) (Nievas et al., 2012b). Bacteria displayed increased biofilm formation ability in the presence of $3OC_{12}$ acyl-HSL, and to a lesser degree $3OC_{10}$ or $3OC_{14}$.

The mechanism whereby QS regulates biofilm formation by peanut-nodulating *Bradyrhizobium* strains is largely unknown. Such regulation may occur at various biological levels through effects on the production or structures of surface components such as lipopolysaccharide (LPS), capsular polysaccharides (CPSs), flagella, pili, and extracellular compounds (*e.g.*, EPSs) essential for initial adhesion of bacteria to surfaces and subsequent biofilm development (Koutsoudis et al., 2006; Rinaudi and González, 2009; De Araujo et al., 2010; Rinaudi and Giordano, 2010). Biofilm formation ability is crucial for survival of bacteria in the rhizospheric environment and for their colonization/invasion of plant roots. The biological role of QS systems mediated by acyl-HSLs in peanut-nodulating *Bradyrhizobium* strains remains to be elucidated, but recent findings suggest that these signaling molecules promote biofilm formation (Nievas et al., 2012b).

QS systems are involved in control of bacterial motility (Soutourina and Bertin, 2003; Hoang et al., 2008; Conrad, 2012). Motility of peanut-nodulating *Bradyrhizobium* strains (similarly to biofilm formation ability) was enhanced in the presence of acyl-HSLs (Nievas et al., 2012b). These bacteria must reach infectable root sites to establish a symbiotic association with the host and obtain essential nutrients. Bacterial motility is a crucial process for survival in varied edaphic niches, for reaching infectable root sites, and for dispersion in nutritionally limited environments (Hoang et al., 2008). Adequate motility increases the likelihood that rhizobia will survive as free-living cells in soil and/or establish themselves in biofilm communities when conditions prevent symbiosis with a legume host. Motility is also important in colonization of surfaces, the first step in biofilm formation (O'Toole and Kolter, 1998; Stanley and Lazazzera, 2004). The role of a QS system in biofilm formation by peanut-nodulating *Bradyrhizobium* strains may be indirect; *i.e.*, QS signals primarily promote motility, and movement secondarily enhances adhesion of bacteria to new surfaces.

Results of a study by Nievas et al. (2012b) suggest that QS signaling molecules can either inhibit or promote the autoaggregation process in peanut-nodulating *Bradyrhizobium* strains. Depending on the bacterial strain, QS mechanisms may either induce dispersion of cellular aggregates (allowing individual bacteria to colonize new microniches) or promote autoaggregation (improving overall bacterial survival) in soil.

A proposed model based on present knowledge of QS mechanisms in *Bradyrhizobium* strains is shown in Fig. 3. Recent findings that peanut-nodulating strains produce various acyl-HSL signaling molecules (Nievas et al., 2012b) and that certain *Bradyrhizobium* strains synthesize other signaling molecules with novel chemical structures (Loh et al., 2002b; Lindemann et al., 2011; Ahlgren et al., 2011) give promise for discovery of more new signaling molecules in future studies. *Bradyrhizobium* strains have been shown to synthesize a wide variety of QS molecules, and presumably express genes for synthesis of a wide range of HSLs or multiple *luxI* genes specific for each type of QS signal. The determinants that govern QS-regulated biological processes in *Bradyrhizobium* sp. in general, and peanut-nodulating strains in particular, will be identified and characterized in future studies.

5. Conclusions

Bradyrhizobium sp. clearly have more than one QS system of communication. The presently limited knowledge of the genetic mechanisms and physiological processes regulated by QS in this bacterial genus should inspire more extensive studies along these lines. The development of differing research strategies for different *Bradyrhizobium* strains will help clarify the QS mechanisms involved in bacteria–bacteria and bacteria–legume communication and signaling processes. Molecular genetic studies of the systems responsible for producing signaling molecules are essential for understanding the mechanisms whereby the bradyrhizobia communicate with each other and interact symbiotically with the host plant. Mechanistic studies of the molecular and biochemical processes regulated by QS will help clarify the key physiological events governed by QS communication and the role of such communication in bacterial survival in various environments and development of symbiotic relationships. It is also important to identify the active compounds in host legumes that affect bacterial QS processes, and the role of these compounds in development of symbiosis and in bacterial colonization of the rhizosphere.

Improved understanding of these interaction processes can be extended to other regulatory mechanisms in bacterial

ecophysiology, and to techniques for improving agricultural yields of legume crops symbiotically associated with *Bradyrhizobium* sp.

Acknowledgements

Preparation of this review article and the authors' studies described herein were supported by grants from the Secretaría de Ciencia y Técnica (UNRC), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), and Consejo Nacional de Investigaciones Científicas y Técnicas of the República Argentina (CONICET). FLN was supported by a fellowship from the CONICET. PCB and WG are career members of CONICET. The authors are grateful to Dr. S. Anderson for English editing of the manuscript.

References

Ahlgren, N.A., Harwood, C.S., Schaefer, A.L., Giraud, E., Greenberg, P., 2011. Aryl-homoserine lactone quorum sensing in stem-nodulating photosynthetic bradyrhizobia. Proc. Natl. Acad. Sci. U. S. A. 108, 7183–7188.

Atkinson, S., Camara, M., Williams, P., 2007. *N*-Acylhomoserine lactones, quorum sensing and biofilm development in Gram-negative bacteria. In: Kjellberg, S., Givskow, M. (Eds.), The Biofilm Mode of Life. Mechanisms and Adaptations. Horizon Bioscience, Wymondham, UK, pp. 95–122.

Atkinson, S., Williams, P., 2009. Quorum sensing and social networking in the microbial world. J. R. Soc. Interface 6, 959–978.

Bernard, A.M., Bowden, S.D., Burr, T., Coulthrust, S.J., Monson, R.E., Salmond, G.P.C., 2007. Quorum sensing, virulence and secondary metabolite production in plant soft-rotting bacteria. Philos. Trans. R. Soc. B 362, 1165–1183.

Bogino, P., Banchio, E., Rinaudi, L., Cerioni, G., Bonfiglio, C., Giordano, W., 2006. Peanut (*Arachis hypogaea*) response to inoculation with *Bradyrhizobium* sp. in soils of Argentina. Ann. Appl. Biol. 148, 207–212.

Bogino, P., Banchio, E., Bonfiglio, C., Giordano, W., 2008. Competitiveness of a *Bradyrhizobium* sp: strain in soils containing indigenous rhizobia. Curr. Microbiol. 56, 66–72.

Bogino, P., Banchio, E., Giordano, W., 2010. Molecular diversity of peanut-nodulating rhizobia in soils of Argentina. J. Basic Microbiol. 50, 274–279.

Bogino, P., Nievas, F., Banchio, E., Giordano, W., 2011. Increased competitiveness and efficiency of biological nitrogen fixation in peanut via in-furrow inoculation of rhizobia. Eur. J. Soil Biol. 47, 187–193.

Bollinger, N., Hassett, D.J., Iglewski, B.H., Costerton, J.W., McDermott, T.R., 2001. Gene expression in *Pseudomonas aeruginosa*: evidence of iron override effects on quorum sensing and biofilm-specific gene regulation. J. Bacteriol. 183, 1990–1996.

Boogerd, F.C., van Rossum, D., 1997. Nodulation of groundnut by *Bradyrhizobium*: a simple infection process by crack entry. FEMS Microbiol. Rev. 21, 5–27.

Branda, S.S., Vik, S., Friedman, L., Kolter, R., 2005. Biofilms: the matrix revisited. Trends Microbiol. 13, 20–26.

Brelles-Marino, G., Bedmar, E.J., 2001. Detection, purification and characterization of quorum-sensing signal molecules in plant-associated bacteria. J. Biotechnol. 91, 197–209.

Cao, H., Yang, M., Zheng, H., Zhang, J., Zhong, Z., Zhu, J., 2009. Complex quorum-sensing regulatory systems regulate bacterial growth and symbiotic nodulation in *Mesorhizobium tianshanense*. Arch. Microbiol. 191, 283–289.

Cha, C.E., Gao, O., Chen, Y.C., Shaw, P.D., Farrand, S.K., 1998. Production of acyl-hosmoserine lactone quorum-sensing signals by Gram-negative plant-associated bacteria. Mol. Plant Microbe Interact. 11, 1119–1129.

Conrad, J.C., 2012. Physics of bacterial near-surface motility using flagella and type IV pili: implications for biofilm formation. Res. Microbiol. 163, 619–629.

Cooper, J.E., 2007. Early interactions between legumes and rhizobia: disclosing complexity in a molecular dialogue. J. Appl. Microbiol. 103, 1355–1365.

Daniels, R., De Vos, D.E., Desair, J., Raedschelders, G., Luyten, E., Rosemeyer, V., 2002. The *cin* quorum sensing locus of *Rhizobium etli* CNPAF512 affects growth and symbiotic nitrogen fixation. J. Biol. Chem. 277, 462–468.

Daniels, R., Vanderleyden, J., Michiels, J., 2004. Quorum sensing and swarming migration in bacteria. FEMS Microbiol. Rev. 28, 261–289.

Danino, V.E., Wilkinson, A., Edwards, A., Downie, J.A., 2003. Recipient-induced transfer of the symbiotic plasmid pRL1JI in *Rhizobium leguminosarum* bv. *viciae* is regulated by quorum sensing relay. Mol. Microbiol. 50, 511–525.

De Araujo, C., Balestrino, D., Roth, L., Charbonnel, N., Forestier, C., 2010. Quorum sensing affects biofilm formation through lipopolysaccharide synthesis in *Klebsiella pneumoniae*. Res. Microbiol. 161, 595–603.

Delamuta, J.R., Ribeiro, R.A., Ormeño-Orrillo, E., Melo, I.S., Martínez-Romero, E., Hungria, M., 2013. Polyphasic evidence supporting the reclassification of *Bradyrhizobium japonicum* group Ia strains as *Bradyrhizobium diazoefficiens* sp. nov. Int. J. Syst. Evol. Microbiol. 63, 3342–3351.

Dong, Y.H., Wang, L.H., Zhang, L.H., 2007. Quorum-quenching microbial infections: mechanisms and implications. Philos. Trans. R. Soc. B 362, 1201–1211.

Dong, Y.H., Zhang, X.F., An, S.W., Xu, J.L., Zhang, L.H., 2008. A novel two component system BqS–BqR modulates quorum sensing-dependent biofilm decay in *Pseudomonas aeruginosa*. Commun. Integr. Biol. 1, 88–96.

Drechsel, H., Jung, G., 1998. Peptide siderophores. J. Pept. Sci. 4, 147–181.

Edwards, A., Frederix, M., Wisniewski-Dyé, F., Jones, J., Zorreguieta, A., Downie, J.A., 2009. The cin and rai quorum-sensing regulatory systems in *Rhizobium leguminosarum* are coordinated by ExpR and CinS, a small regulatory protein coexpressed with CinI. J. Bacteriol. 191, 3059–3067.

Fuqua, W.C., Winans, S.C., Greenberg, E.P., 1994. Quorum sensing in bacteria: the LuxR–LuxI family of cell density-responsive transcriptional regulators. J. Bacteriol. 176, 269–275.

Fuqua, C., Greenberg, E.P., 2002. Listening in on bacteria: acyl-homoserine lactone signalling. Nat. Rev. Mol. Cell Biol. 3, 685–695.

Gage, D.J., 2004. Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. Microbiol. Mol. Biol. Rev. 68, 280–300.

Gage, D.J., Margolin, W., 2000. Hanging by a thread: invasion of legume plants by rhizobia. Curr. Opin. Microbiol. 3, 613–617.

Gao, M., Teplitski, M., Robinson, J.B., Bauer, W.D., 2003. Production of substances by *Medicago truncatula* that affect bacterial quorum sensing. Mol. Plant Microbe Interact. 16, 827–834.

Gao, M., Chen, H., Eberhard, A., Gronquist, M.R., Robinson, J.R., Rolfe, B.G., Bauer, W. D., 2005. *sinI* and *expR* dependent quorum sensing in *Sinorhizobium meliloti*. J. Bacteriol. 187, 7931–7944.

González, J.E., Marketon, M.M., 2003. Quorum sensing in nitrogen-fixing rhizobia. Microbiol. Mol. Biol. Rev. 67, 574–592.

Goormachtig, S., Capoen, W., Holsters, M., 2004. Rhizobium infection: lessons from the versatile nodulation behaviour of water tolerant-legumes. Trends Plant Sci. 9, 518–522.

Göttfert, M., Grob, P., Hennecke, H., 1990. Proposed regulatory pathway encoded by the *nodV* and *nodW* genes, determinants of host specificity in *Bradyrhizobium japonicum*. Proc. Natl. Acad. Sci. U. S. A. 87, 2680–2684.

Göttfert, M., Holzhäuser, D., Bäni, D., Hennecke, H., 1992. Structural and functional analysis of two different *nodD* genes in *Bradyrhizobium japonicum* USDA110. Mol. Plant Microbe Interact. 7, 364–369.

Grob, P., Michel, P., Hennecke, H., Göttfert, M., 1993. A novel response regulator is able to suppress the nodulation defect of a *Bradyrhizobium japonicum nodW* mutant. Mol. Gen. Genet. 241, 531–541.

Hwang, I., Cook, D.M., Farrand, S.K., 1995. A new regulatory element modulates homoserine lactone-mediated autoinduction of Ti plasmid conjugal transfer. J. Bacteriol. 177, 449–458.

He, X., Chang, W., Pierce, D.L., Seib, L.O., Wagner, J., Fuqua, C., 2003. Quorum sensing in *Rhizobium* sp. strain NGR234 regulates conjugal transfer (tra) gene expression and influences growth rate. J. Bacteriol. 185, 809–822.

Hoang, H.H., Gurich, N., González, J., 2008. Regulation of motility by ExpR/Sin quorum-sensing system in *Sinorhizobium meliloti*. J. Bacteriol. 190, 861–871.

Jitacksorn, S., Sadowsky, M.J., 2008. Nodulation gene regulation and quorum sensing control density-dependent suppression and restriction of nodulation in the *Bradyrhizobium japonicum*-soybean symbiosis. Appl. Environ. Microbiol. 74, 3749–3756.

Johnston, A.W., Yeoman, K.H., Wexler, M., 2001. Metals and the rhizobial–legume symbiosis-uptake, utilization and signalling. Adv. Microb. Physiol. 45, 13–56.

Jones, K.M., Kobayashi, H., Davies, B.W., Taga, M.E., Walker, G.C., 2007. How rhizobial symbionts invade plants: the *Sinorhizobium*–Medicago model. Nat. Rev. Microbiol. 5, 619–633.

Kaneko, T., Nakamura, Y., Sato, S., Minamisawa, K., Uchiumi, T., Sasamoto, S., Watanabe, A., Idesawa, K., Iriguchi, M., Kawashima, K., Kohara, M., Matsumoto, M., Shimpo, S., Tsuruoka, H., Wada, T., Yamada, M., Tabata, S., 2002. Complete genomic sequence of nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* USDA110. DNA Res. 9, 189–197.

Koutsoudis, M.D., Dimitrios, T., Minogue, T.D., von Bodman, S., 2006. Quorum sensing regulation governs bacterial adhesion biofilm development, and host colonization in *Pantoea stewartii* subspecies *stewartii*. Proc. Natl. Acad. Sci. U. S. A. 103, 5983–5988.

Lee, K.B., De Backer, P., Aono, T., Liu, C.T., Suzuki, S., Suzuki, T., Kaneko, T., Yamada, M., Tabata, S., Kupfer, D.M., Najar, F.Z., Wiley, G.B., Roe, B., Binnewies, T.T., Ussery, D. W., D'Haeze, W., Den Herder, J., Gevers, D., Vereecke, D., Holsters, M., Oyaizu, H., 2008. The genome of the versatile nitrogen fixer *Azorhizobium caulinodans* ORS571. BMC Genomics doi:http://dx.doi.org/10.1186/14710-2164-9-271.

Lindemann, A., Pessi, G., Schaefer, A.L., Mattmann, M., Christensen, Q.H., Kessler, A., Hennecke, H., Blackwell, H.E., Greenberg, E.P., Harwood, C.S., 2011. Isovaleryl-homoserine lactone, an unusual branched-chain quorum-sensing signal from the soybean symbiont *Bradyrhizobium japonicum*. Proc. Natl. Acad. Sci. U. S. A. 108, 16765–16770.

Lithgow, J.K., Wilkinson, A., Hardman, A., Rodelas, B., Wisniewski-Dye, F., Williams, P., Downie, J.A., 2000. The regulatory locus *cinRI* in *Rhizobium leguminosarum* controls a network of quorum-sensing loci. Mol. Microbiol. 37, 81–97.

Loh, J., Garcia, M., Stacey, G., 1997. NodV and NodW, a second flavonoid recognition system regulating *nod* gene expression in *Bradyrhizobium japonicum*. J. Bacteriol. 179, 3013–3020.

Loh, J.T., Yuen-Tsai, J.P.Y., Stacey, M.G., Lohar, D., Welborn, A., Stacey, G., 2001. Population density-dependent regulation of the *Bradyrhizobium japonicum* nodulation genes. Mol. Microbiol. 42 (1), 37–46.

Loh, J., Lohar, D.P., Andersen, B., Stacey, G., 2002a. A two-component regulator mediates population-density-dependent expression of the *Bradyrhizobium japonicum* nodulation genes. J. Bacteriol. 184 (6), 1759–1766.

Loh, J., Carlson, R.W., York, W.S., Stacey, G., 2002b. Bradyoxetin a unique chemical signal envolved in symbiotic gene regulation. Proc. Natl. Acad. Sci. U. S. A. 99, 14446–14451.

Loh, J., Stacey, G., 2001. Feedback regulation of the *Bradyrhizobium japonicum* nodulation genes. Mol. Microbiol. 41, 1357–1364.
Marketon, M.M., Glenn, S.A., Eberhard, A., González, J.E., 2003. Quorum sensing controls exopolysaccharide production in *Sinorhizobium meliloti*. J. Bacteriol. 185, 325–331.
Marketon, M.M., González, J.E., 2002. Identification of two quorum-sensing systems in *Sinorhizobium meliloti*. J. Bacteriol. 184, 3466–3475.
Marketon, M.M., Gronquist, M.N., Eberhard, A., González, J.E., 2002. Characterization of *Sinorhizobium meliloti sinR/sinI* locus and production of novel *N*-acyl homoserine lactones. J. Bacteriol. 184, 5686–5695.
Molouba, F., Lorquin, J., Willems, A., Hoste, B., Giraud, E., Dreyfus, B., Gillis, M., de Lajudie, P., Masson-Boivin, C., 1999. Photosynthetic bradyrhizobia from *Aeschynomene* spp. are specific to stem-nodulated species and form a separate 16S ribosomal DNA restriction fragment length polymorphism group. Appl. Environ. Microbiol. 65, 3084–3094.
Nievas, F., Bogino, P., Nocelli, N., Giordano, W., 2012a. Genotypic analysis of isolated peanut-nodulating rhizobial strains reveals differences among populations obtained from soils with different cropping histories. Appl. Soil Ecol. 53, 74–82.
Nievas, F., Bogino, P., Sorroche, F., Giordano, W., 2012b. Detection, characterization, and biological effect of quorum-sensing signaling molecules in peanut-nodulating Bradyrhizobia. Sensors 12, 2851–2873.
Oldroyd, G.E.D., Downie, J.A., 2004. Calcium, kinases and nodulation signalling in legumes. Nat. Rev. Mol. Cell Biol. 5, 566–576.
Omura, S., Murata, M., Imamura, N., Iwai, Y., Tanaka, H., Furusaki, A., Matsumoto, T., 1984. Oxetin, a new antimetabolite from an actinomycete. Fermentation, isolation, structure and biological activity. J. Antibiot. 37, 1324–1332.
O'Toole, G.A., Kolter, R., 1998. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. 30, 295–304.
Parsek, M.R., Greenberg, E.P., 2005. Sociomicrobiology: the connections between quorum sensing and biofilms. Trends Microbiol. 13, 27–33.
Pessi, G., Ahrens, C.H., Rehrauer, H., Lindemann, A., Hauser, F., Fischer, H.M., Hennecke, H., 2007. Genome-wide transcript analysis of *Bradyrhizobium japonicum* bacteroids in soybean root nodules. Mol. Plant Microbe Interact. 20 (11), 1353–1363.
Pellock, B.J., Teplitski, M., Boinay, R.P., Bauer, W.D., Walker, G.C., 2002. A LuxR homolog controls production of symbiotically active extracellular polysaccharide II by *Sinorhizobium meliloti*. J. Bacteriol. 184, 5067–5076.
Pierson, L.S., Pierson, E.A., 2007. Roles of diffusible signals in communication among plant-associated bacteria. Phytopathology 97, 227–232.
Piper, K.R., Beck von Bodman, S., Farrand, S.K., 1993. Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid transfer by autoinduction. Nature 362, 448–450.
Pongslip, N., Triplett, E.W., Sadowsky, M.J., 2005. Detection of homoserine lactone like quorum sensing molecules in *Bradyrhizobium* strains. Curr. Microbiol. 51, 250–254.
Ramsay, J.P., Sullivan, J.T., Jambari, N., Ortori, C.A., Heeb, S., Williams, P., Barrett, D.A., Lamont, I.L., Ronson, C.W., 2009. A LuxRI-family regulatory system controls excision and transfer of the *Mesorhizobium loti* strain R7A symbiosis island by activating expression of two conserved hypothetical genes. Mol. Microbiol. 73, 1141–1155.
Rinaudi, L.V., Giordano, W., 2010. An integrated view of biofilm formation in rhizobia. FEMS Microbiol. Lett. 304, 1–11.
Rinaudi, L.V., González, J.E., 2009. The low-molecular-weight fraction of exopolisaccharide II from *Sinorhizobium meliloti* is a crucial determinant of biofilm formation. J. Bacteriol. 191, 7216–7224.
Rodelas, B., Lithgow, J.K., Wisniewski-Dye, F., Hardman, A., Wilkinson, A., Economou, A., Williams, P., Downie, J.A., 1999. Analysis of quorum-sensing-dependent control of rhizosphere-expressed (*rhi*) genes in *Rhizobium leguminosarum* bv. *viciae*. J. Bacteriol. 181, 3816–3823.
Sanchez-Contreras, M., Wolfgang, B.D., Mengsheng, G., Robinson, J.B., Downie, A., 2007. Quorum sensing regulation in rhizobia and its role in symbiotic interactions with legumes. Philos. Trans. R. Soc. B 362, 1149–1163.
Schaefer, A.L., Greenberg, E.P., Oliver, C.M., Oda, Y., Huang, J.J., Bittan-Banin, G., Peres, C.M., Schmidt, S., Juhaszova, K., Sufrin, J.R., Harwood, C.S., 2008. A new class of homoserine lactone quorum-sensing signals. Nature 454, 595–599.
Sourjik, V., Muschler, P., Scharf, B., Schmitt, R., 2000. VisN and VisR are global regulators of chemotaxis, flagellar, and motility genes in *Sinorhizobium* (*Rhizobium*) *meliloti*. J. Bacteriol. 182, 782–788.
Soutourina, O.A., Bertin, P.N., 2003. Regulation cascade of flagellar expression in Gram-negative bacteria. FEMS Microbiol. Rev. 27, 505–523.
Spaink, H.P., 2000. Root nodulation and infection factors produced by rhizobial bacteria. Ann. Rev. Microbiol. 54, 257–288.
Stanley, N.R., Lazazzera, B.A., 2004. Environmental signals and regulatory pathways that influence biofilm formation. Mol. Microbiol. 52, 917–924.
Stoodley, P., Sauer, K., Davies, D.G., Coleman, F., 2002. Biofilms as complex differentiated communities. Ann. Rev. Microbiol. 56, 187–209.
Teplitski, M., Eberhard, A., Gronquist, M.R., Gao, M., Robinson, J.B., Bauer, W.D., 2003. Chemical identification of *N*-acyl homoserine lactone quorum-sensing signals produced by *Sinorhizobium meliloti* strains in defined medium. Arch. Microbiol. 180, 494–497.
Thiel, V., Kunze, B., Verma, P., Wagner-Dobler, I., Schulz, S., 2009. New structural variants of homoserine lactones in bacteria. Chem. BioChem. 10, 1861–1868.
Thorne, S.H., Williams, H.D., 1999. Cell density-dependent starvation survival of *Rhizobium leguminosarum* bv. *phaseoli*: identification of the role of an *N*-acyl homoserine lactone in adaptation to stationary-phase survival. J. Bacteriol. 181, 981–990.
Tun-Garrido, C., Bustos, P., Gonzalez, V., Brom, S., 2003. Conjugative transfer of p42a from *Rhizobium etli* CFN42, which is required for mobilization of the symbiotic plasmid, is regulated by quorum sensing. J. Bacteriol. 185, 1681–1692.
Vinuesa, P., León-Barrios, M., Silva, C., Willems, A., Jarabo-Lorenzo, A., Pérez-Galdona, R., Werner, D., Martínez-Romero, E., 2005. *Bradyrhizobium canariense* sp. nov., an acid-tolerant endosymbiont that nodulates endemic genistoid legumes (*Papilionoideae: Genisteae*) from the Canary Islands, along with *Bradyrhizobium japonicum* bv. *genistearum*, *Bradyrhizobium* genospecies alpha and *Bradyrhizobium* genospecies beta. Int. J. Syst. Evol. Microbiol. 55, 569–575.
Wang, C., Yan, C., Fuqua, C., Zhang, L.H., 2014. Identification and characterization of a second quorum-sensing system in *Agrobacterium tumefaciens* A6. J. Bacteriol. 196, 1403–1411.
Webb, J.S., Givskov, M., Kjelleberg, S., 2003. Bacterial biofilms: prokaryotic adventures in multicellularity. Curr. Opin. Microbiol. 6, 578–585.
White, E.W., Winans, S.C., 2007. Cell–cell communication in the plant pathogen *Agrobacterium tumefaciens*. Philos. Trans. R. Soc. B 362, 1135–1148.
Whitehead, N.A., Barnard, A.M.L., Slater, H., Simpson, N.J.L., Salmond, G.P.C., 2001. Quorum sensing in Gram-negative bacteria. FEMS Microbiol. Rev. 25, 365–404.
Willems, A., Doignon-Bourcier, F., Goris, J., Coopman, R., de Lajudie, P., De Vos, P., Gillis, M., 2001. DNA–DNA hybridization study of *Bradyrhizobium* strains. Int. J. Syst. Microbiol. 51, 1315–1322.
Winzer, K., Hardie, K.R., Williams, P., 2002. Bacterial cell-to-cell communication: sorry, can't talk now gone to lunch. Curr. Opin. Microbiol. 5, 216–222.
Wisniewski-Dye, F., Jones, J., Chhabra, S.R., Downie, J.A., 2002. *raiIR* Genes are part of a quorum-sensing network controlled by *cinI* and *cinR* in *Rhizobium leguminosarum*. J. Bacteriol. 184, 1597–1606.
Zhang, W.T., Yang, J.K., Yuan, T.Y., Zhou, J.C., 2007. Genetic diversity and phylogeny of indigenous rhizobia from cowpea (*Vigna unguiculata* (L.) Walp.). Biol. Fertil. Soils 44, 201–210.
Zheng, H., Zhong, Z., Lai, X., Wen-Xin, C., Shunpeng, L., Zhu, J., 2006. A LuxR/LuxI-type quorum-sensing system in a plant bacterium, *Mesorhizobium tianshanense*, controls symbiotic nodulation. J. Bacteriol. 188, 1943–1949.