Rhizobial Extracellular Signaling Molecules and Their Functions in Symbiotic Interactions with Legumes

Walter Giordano

Introduction

Rhizobia are soil bacteria that fix nitrogen after becoming established inside root nodules of legume plants. Rhizobia require a plant host; they are not capable of fixing nitrogen independently. Many legume species respond to inoculation with rhizobia by developing root nodules. The development of a root nodule in which rhizobia convert atmospheric nitrogen into ammonia requires the exchange of specific signaling molecules between the host plant and its microsymbionts. Flavonoids released by plant roots or seeds act as chemoattractants to rhizobia, and certain flavonoids have been shown to induce transcription of rhizobial (*nod*) genes. The products of these genes synthesize Nod factor, a lipochitooligosaccharide molecule whose effects include root hair deformation, root cortical cell division, and nodule morphogenesis (Downie and Walker 1999).

"Quorum sensing" (QS) is a mechanism whereby bacteria sense population density and regulate gene expression, leading to activation of specific phenotypes in the population. This process depends on the accumulation in the environment of signaling molecules termed autoinducers. Many Gram-negative bacteria use N-acyl homoserine lactones (AHLs) as signaling molecules. Some have been reported to use other fatty acid derivatives such as 3-hydroxypalmitic acid methyl ester and cis-unsaturated fatty acids. In contrast, many Gram-positive bacteria use amino acids or modified peptides as signaling molecules. Both Gram-positive and Gram-negative bacteria use isomers of methyl-2,3,3,4-tetrahydroxytetrahydrofuran (AI-2 autoinducer) as signals. Signaling molecules belonging to other structural classes (indole and its derivatives, quinolones, (S)-3-hydroxytridecan-4-one, and cyclic dipeptides) have also been described (Ryan and Dow 2008; Li and Nair 2012).

AHL molecules from different species are chemically distinct, although their basic structures are similar. The molecules consist of a homoserine lactone (HSL) ring, covalently linked via an amide bond to an acyl side chain (ranging from 4 to 18 carbons) which may be saturated or unsaturated. The carbon at the 3 position of the *N*-linked acyl chain may contain a hydrogen-, oxo-, or hydroxyl substitution. This variability, in combination with the ability of most bacteria to produce more than one type of AHL, provides a mechanism for specificity in QS communication and for the ability of bacteria to distinguish their own AHLs from those produced by other species. The identification of AHL-based QS systems in a variety of bacteria and the understanding of how these systems work have been facilitated by the use of AHL bioreporters. There are many such reporter systems. The two most widely used are those in

W. Giordano (✉)
Departamento de Biología Molecular, Universidad Nacional de Río Cuarto, Ruta 36 Km 601, X5804BYA Río Cuarto, Córdoba, Argentina
e-mail: wgiordano@exa.unrc.edu.ar

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Chromobacterium violaceum and *Agrobacterium tumefaciens*, which are often applied for visualization of AHLs separated by thin-layer chromatography (Sanchez-Contreras et al. 2007).

The role of QS in rhizobia-legume symbioses has been studied extensively in many species and has been documented in symbiotic nodulation (Yang et al. 2009), bacterial growth and nodule formation (Cao et al. 2009), symbiotic nitrogen fixation (Daniels et al. 2002), and successful symbiosis establishment (Marketon et al. 2002). On the other hand, many rhizobia with mutations of QS genes are able to establish effective symbioses with their legume hosts, indicating that the role of QS mechanisms is primarily to optimize bacteria/host interactions. Our study of peanut-nodulating rhizobia strains suggested that, depending on the bacterial strain, QS mechanisms may either (i) induce dispersion of cellular aggregates and thereby allow individual bacteria to colonize the peanut root or (ii) promote autoaggregation and thereby improve overall bacterial survival in soil (Nievas et al. 2012).

AHL molecules may also provide a means for rhizobia to communicate with the legume host. Certain higher plants, including legumes, can synthesize AHL-mimicking compounds that may activate or disrupt rhizobial communication and thus affect rhizobial/host symbiosis (Gao et al. 2003). Conversely, the legume *Medicago truncatula* is able to perceive rhizobial AHL signals, with resulting changes in gene expression in the plant (Mathesius et al. 2003). A recent study indicates that research models such as *Arabidopsis thaliana* perceive AHLs in diverse manners (Zarkani et al. 2013).

Rhizobia are a highly diverse group of nitrogen-fixing symbiotic bacteria belonging to the α- and β-subclasses of Proteobacteria. Rhizobial genera include *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Methylobacterium* (α-rhizobia), *Burkholderia*, and *Cupriavidus* (β-rhizobia). Recent studies have revealed other rhizobial genera, some of which have arisen through lateral transfer of symbiotic genes. At present, 13 genera and 98 species of rhizobia (α- and β-proteobacteria) are known (www.rhizobia.co.nz/taxonomy/rhizobia). An integrated view of QS systems in rhizobia is presented in this chapter. Available data on QS signaling molecules in rhizobia are summarized in Table 1. The following sections present data for the four best-studied genera: *Sinorhizobium*, *Mesorhizobium*, *Rhizobium*, and *Bradyrhizobium.*

Sinorhizobium

Sinorhizobium (*Ensifer*) *meliloti* is best known for its ability to establish nitrogen-fixing symbioses with plant hosts in the genera *Medicago*, *Melilotus*, and *Trigonella*. The signaling and regulatory events in these symbioses have been well studied. Less is known regarding *S. meliloti* behaviors outside the host that affect the symbioses, e.g., bacterial QS signaling in the rhizosphere (Gao et al. 2012).

The Sin QS system of *S. meliloti* involves an AHL synthase, SinI, and at least two LuxR-type regulators, SinR and ExpR. SinR appears to be independent of AHLs for its control of *sinI* expression, whereas ExpR is almost completely dependent on AHLs. The *S. meliloti* genes *sinI*, *sinR*, and *expR* are all essential for QS regulation in this system, and their expression is dependent on not only the presence of AHLs but also the level of AHLs supplied in growth culture of strains incapable of producing AHLs (McIntosch et al. 2009). The *sinI* gene encodes an AHL synthase that catalyzes synthesis of several long-chain AHLs, including oxo-C14-HL, oxo-C16:1-HL, and C16:1-HL (Teplitski et al. 2003). Upstream of *sinI* is *sinR*, which encodes a transcription regulator that controls activity of the *sinI* promoter. The *sinR* promoter responds to environmental cues such as nutrient limitation by increasing *sinR* transcription (McIntosch et al. 2009). The SinR protein is necessary for activation of the *sinI* promoter, most likely through a SinR binding site, and increased SinR production therefore results in increased *sinI* expression.

The *expR* gene of the Sin system is disrupted by an insertion element in *S. meliloti* strains Rm1021 and Rm2011 (Pellock et al. 2002),

Table 1 Summary of rhizobial QS signaling molecules

Rhizobial species	Signaling molecules	Phenotypes	References
S. meliloti			
Rm1021	3-O-C14-HSL, C16:1-HSL, 3-O-C16:1-HSL, 3-O-C16-HSL, C18-HSL, C12-HSL	EPSs production, swarming	Marketon et al. (2002), Teplitski et al. (2003), Gao et al. (2005), Hoang et al. (2008), Gao et al. (2012)
Rm41	3-O-C8-HSL	Plasmid transfer	Marketon and Gonzalez (2002)
Mesorhizobium			
M. loti R7A	Unknown acyl-HSLs	Symbiosis island transfer	Ramsay et al. (2009)
M. loti NZP2213	3-O-C6-HSL, C8-HSL, C10-HSL, C12-HSL	Nodulation efficiency	Yang et al. (2009)
M. tianshanense	Unknown acyl-HSLs	Legume nodulation	Cao et al. (2009)
M. huakuii	Unknown acyl-HSLs	Biofilm formation, nodulation	Wang et al. (2004), Gao et al. (2006)
Rhizobium			
R. Etli CNPAF512	3-OH-slc-HSL, short-chain acyl-HSLs	Nitrogen fixation, symbiosome development, growth inhibition	Rosemeyer et al. (1998), Daniels et al. (2002)
R. leguminosarum A34	3-OH-C14:1-HSL	Growth inhibition, polysaccharide degradation	Lithgow et al. (2000), Edwards et al. (2009)
	C6-HSL, C7-HSL, C8-HSL	Nodulation efficiency	Cubo et al. (1992); Rodelas et al. (1999)
	3-O-C8-HSL, C8-HSL	Plasmid transfer	Wilkinson et al. (2002), Danino et al. (2003)
Rhizobium sp.			
NGR234	3-O-C8-HSL	Plasmid transfer	He et al. (2003)
Bradyrhizobium			
B. japonicum USDA110	Bradyoxetin	*Nod* gene control	Loh and Stacey (2001), Loh et al. (2002)
	Isovaleryl-HSLs	*BjaI* gene control	Lindemann et al. (2011)
B. japonicum USDA110/290 and *B. elkanii*	Acyl-HSLs like autoinducers	Not reported	Pongsilp et al. (2005)
Bradyrhizobium sp.	Acyl-HSLs like autoinducers	Autoaggregation, biofilm formation, motility	Nievas et al. (2012)

which have been most intensively studied. Under nonstarvation conditions in the laboratory, these strains produce detectable quantities of the symbiotically important exopolysaccharide (EPS) succinoglycan (also known as EPS I) but does not produce galactoglucan (EPS II). The presence of a functional *expR* open reading frame (ORF) on a plasmid or in the genome is sufficient to promote the production of symbiotically active EPS II, e.g., in strain Rm8530, which has an intact *expR*, is termed *expR*+, and has a mucoid phenotype. Restoration of the *expR* gene in strains Rm1021 and Rm2011 confers bacterial QS capability and a notable increase in production of EPS I and EPS II (Pellock et al. 2002). The DNA binding activity of ExpR depends upon the presence of AHLs. The ExpR-AHL complex regulates many promoters (the precise number is not known) throughout the genome. Binding has been demonstrated for the promoters of genes controlling EPS II production (*wgeA* and *wgaA*), genes related

to EPS I production (*exoI* and *exsH*), genes controlling flagellum production (*visNR*), and the Sin system genes *sinR* and *sinI* (McIntosch et al. 2009). Because of the importance of EPSs in bacterial attachment, rhizobial cell surface components in combination with bacterial functional signals are essential for this process (Bogino et al. 2013). Accordingly, strains Rm1021 and Rm2011 did not form organized biofilms (Rinaudi and Giordano 2010) and displayed poorly autoaggregative phenotypes (Sorroche et al. 2010).

The amount of AHLs in the environment presumably determines the predominance of positive vs. negative feedback mechanisms and eventually leads to an equilibrium state between the mechanisms at high population densities (McIntosh et al. 2009). Recent studies confirmed the presence of previously detected ExpR-DNA binding sites in *S. meliloti* and identified several additional sites, some of which regulate genes not previously known to be members of the ExpR/AHL regulon. The activities of ExpR/AHL-dependent promoters were titrated against AHL levels, with varying effects on AHL sensitivity. The findings suggest a type of temporal expression program whereby the activity of each promoter is subject to a specific range of AHL concentrations. Genes responsible for EPS production are activated at lower AHL concentrations than those required for repression of genes that control flagellum production. Several features of ExpR-regulated promoters determine their response to AHLs. The location of the ExpR-binding site relative to the relevant transcription start site within each promoter region determines whether ExpR/AHL activates or represses promoter activity. The strength of the response depends on the AHL concentration. This differential sensitivity to AHLs provides a bacterial colony with a transcription control program that is both dynamic and precise (Charoenpanich et al. 2013).

Rm1021 and Rm41 (the parent strain of AK631) are two independently isolated *S. meliloti* strains that are commonly used for studying various aspects of symbiosis with alfalfa (*M. sativa*). Both of these strains display autoinducer activity but have different AHL patterns. TraR, a QS transcriptional activator found in the family Rhizobiaceae, is regulated negatively by the antiactivator TraM via formation of a TraR-TraM heterocomplex (Zheng et al. 2012). The *traR*/*traM* locus homologous to the *tra* system of *A. tumefaciens* (Lang and Faure 2014) is part of a QS system unique to AK631 strain and is involved in regulating conjugal plasmid transfer in the presence of a second QS system, *sinR*/*sinI*, that is present in both AK631 and Rm1021 (Marketon and González 2002). The *traR*/*traM* QS regulators may also be involved in other functions (e.g., host range specificity) uniquely in AK631. The *sinRI* locus, common to Rm1021 and AK631, may regulate components of symbiosis or the free-living state in these strains (Marketon and González 2002).

Disruption of *sinI* expression eliminates these AHLs, and *sinR* disruption results in basal AHL levels. The same *sinI* and *sinR* mutations lead to reduced numbers of pink nodules in nodulation assays and a slight delay in appearance of these nodules, indicating a role of QS in symbiosis. The *sinI* and *sinR* mutants are still capable of producing several short-chain AHLs, one of which was identified as octanoyl homoserine lactone. Marketon et al. (2002) proposed that these short-chain AHLs belong to another QS system in Rm1021, which is termed *mel* system, for "*S. meliloti*."

Mesorhizobium

Mesorhizobium is a moderately fast-growing genus that fixes nitrogen in symbiotic association with legumes. *M. loti* is able to form determinant-type globular nodules and perform nitrogen fixation in several *Lotus* species. The genome sequence of *M. loti* contains ten predicted LuxR homologs and six predicted AHL synthases (Kaneko et al. 2000).

A number of bacterial processes are regulated by exchanges of chemical signals that permit a bacterial community (monospecies and multispecies biofilms) to coordinate its responses to novel environmental challenges or opportunities (Burmølle et al. 2014). Such responses include pathogenesis, symbiosis, antibiotic

production, motility, genetic competence, and biofilm formation. Diffusible signals have been suggested to help a bacterial community take a census of its population size; this is a QS phenomenon. The abovementioned chemical signals consist of a set of diffusible AHLs. The key regulatory components of these signaling systems are LuxI-type proteins (which act as AHL synthases) and LuxR-type proteins (which act as AHL receptors and AHL-dependent transcription factors). The TraI and TraR proteins of *A. tumefaciens* are members of this family. The symbiosis island ICE*Ml*Sym of *M. loti* R7A is an integrative and conjugative element (ICE) that carries genes required for nitrogen-fixing symbiosis with *Lotus* species. ICE*Ml*Sym encodes homologs (TraR, TraI1, TraI2) of proteins that regulate plasmid transfer by QS in *A. tumefaciens* (Ramsay et al. 2009). Horizontal transfer is activated by TraR and AHLs. In wild-type cultures, the ICE is excised at low frequency. Ramsay et al. (2013) demonstrated recently that QseM, a widely conserved ICE-encoded protein, is an antiactivator of TraR.

Various isolates of *M. tianshanense* that form nodules on various types of licorice plants produce several different AHL molecules. Root hair attachment and nodulation in this species are controlled by *mrtI/R* QS genes (Cao et al. 2009).

M. huakuii is best known as a symbiont of *Acacia* and *Astragalus*, but was recently shown to form nodules in *Thermopsis* spp. (Ampomah and Huss-Danell 2011). Many AHLs were detected in this species, some at extremely low concentrations (Zhu et al. 2003). To investigate QS regulation in *M. huakuii*, the *Agrobacterium* QS regulator TraR was heterologously expressed in the bacterium. AHL production in supernatant was lower in the resulting strains than in wild type, but intracellular AHL levels were similar, suggesting that AHLs in *M. huakuii* can be bound to intracellular TraR proteins and thus become unavailable for the bacterium's own QS systems. TraR-overexpressing *M. huakuii* formed thinner biofilms than did wild type, suggesting a role of QS in biofilm formation (Wang et al. 2004).

Rhizobium

The genus *Rhizobium* is currently known to include 30 rhizobial species and 11 nonrhizobial species (www.rhizobia.co.nz/taxonomy/rhizobia). *R. etli* establishes a nitrogen-fixing symbiosis by infecting the roots of its leguminous host *Phaseolus vulgaris* (common bean). *R. etli* has multiple AHL synthase genes. The *cinR* and *cinI* genes are required for normal symbiotic nitrogen fixation and swarming and for normal expression levels of *raiI*, which encodes another AHL synthase. The expression of *raiI* in *R. etli* is regulated by RaiR (Rosemeyer et al. 1998; Daniels et al. 2002). QS genes similar to the *cinI/R* genes have been identified in *R. etli* and *M. tianshanense* (*cinI/R* and *mrtI/R*, respectively). Despite high sequence similarities, the roles of *cin* in *R. etli* and *mrt* in *M. tianshanense* are different from their roles in *R. leguminosarum*. In *R. etli,* a *cinI* mutation increased lag phase, slowed growth, and led to abnormal symbiosome development and nitrogen fixation (Daniels et al. 2002). It is possible that the observed symbiotic phenotypes resulted from growth problems in the mutants. The *cin* locus in *R. etli* is required for normal swarming (Daniels et al. 2004), and induction of *cinI* and *cinR* leads to enhanced expression of RaiI-made AHLs. RaiR is involved in the restriction of nodule number. Rosemeyer et al. (1998) found that in vitro mutation of *raiI* led to increases in nodule number and nitrogenase activity, although no significant increase in nitrogen fixation was observed *in planta*.

R. leguminosarum has three biovars: bv. *viciae* (which nodulates peas, vetch, and lentils), bv. *trifolii* (which nodulates clover), and bv. *phaseoli* (which nodulates *Phaseolus*). bv. *viciae* has received the most research attention. Four LuxI-type AHL synthase genes have been identified to date in isolates of bv. *viciae* (Wisniewski-Dye and Downie 2002).

R. leguminosarum A34 contains *cin*, *rai*, *rhi*, and *tra* QS genes. *cinI* and *cinR* are located on the chromosome and are on top of a regulatory cascade that induces production of

RaiI-, RhiI-, and TraI-made AHLs (Lithgow et al. 2000; Wisniewski-Dye and Downie 2002). CinI-made 3-hydroxy-C14:1-HSL was originally termed "small bacteriocin" because it was found to inhibit growth of *Rhizobium* strains carrying the symbiotic plasmid pRL1JI (Schripsema et al. 1996). This compound was also involved in adaptation to stationary phase, because cultures entering stationary phase at high population density showed no loss of viability over long periods, whereas cultures entering stationary phase at low population density did. The addition of 3-hydroxy-C14:1-HSL to cultures at low population density reversed such loss of viability (Thorne and Williams 1999). Mutation of *cinI* or *cinR* did not cause growth difficulties under laboratory conditions, and pea nodulation was normal (Lithgow et al. 2000).

The *traI* and *traR* genes on the symbiotic plasmid pRL1JI are homologous to those in *A. tumefaciens*. Expression of *traR* and *traI* is induced by CinI-made AHLs and results in recipient-induced plasmid transfer. The crucial factor in this process is the presence of BisR, a LuxR-type regulator encoded on pRL1JI that can act as either an inducer or repressor (Danino et al. 2003). In strains carrying pRL1JI (donor strains), BisR represses *cinI* expression, thus preventing synthesis of CinI-made 3-hydroxy-C14:1-HSLs (Wilkinson et al. 2002). In strains that do not carry pRL1JI (recipient strains), such repression does not occur, and CinI therefore produces 3-hydroxy-C14:1-HSLs. When a recipient strain and donor strain come into close proximity, BisR in the donor strain senses the 3-hydroxy-C14:1-HSLs produced by the recipient strain, and the activated BisR then induces *traR* expression (Wilkinson et al. 2002). TraR is activated by TraI-made AHLs and induces expression of plasmid transfer genes, thus initiating conjugation of the symbiotic plasmid to the recipient strain (Danino et al. 2003). The bivalent mode of action of BisR (activator and repressor) is thus responsible for a regulatory mechanism that allows a recipient strain to induce plasmid transfer in the presence of a possible donor strain. This mechanism leads to very high conjugation frequencies and prevents the waste of energy that would occur if unnecessary plasmid transfer took place; i.e., plasmid transfer is initiated only in the presence of recipient strains that do not carry a BisR-containing plasmid (Danino et al. 2003).

Lithgow et al. (2000) showed that CinR induces production of RhiI-made AHLs, which are present on the symbiotic plasmid. The *rhiI* and *rhiR* genes were first identified in *R. leguminosarum* based on the high expression level of RhiA protein, which was not produced by strains lacking the *nod-nif* gene region. RhiR regulates expression of the *rhiABC* genes in response to RhiI-made C6-, C7-, and C8-HSLs. *rhiA* encodes a protein of unknown function that is highly expressed in the rhizosphere (Cubo et al. 1992). Mutation of *rhiA* or *rhiR* caused a decrease in nodule number in strains that were already nodulation compromised (Cubo et al. 1992). Mutations in the *cin* and *rhi* QS systems also affect biofilm formation, rhizosphere growth, and symbiotic interactions (Russo et al. 2006; Edwards et al. 2009). The *cinS* gene, which is co-transcribed with the AHL synthase gene *cinI*, is required for full induction of *rhiR* and *raiR*, whose products (in combination with their partner AHL synthases) regulate other genes in a QS-regulated hierarchy (Frederix et al. 2011). Expression of the *rhi* genes is inhibited by the presence of flavonoids; this is a *nod*-dependent effect mediated via *rhiR* expression. RhiA is present in all strains of bv. *viciae* but is absent in bv. *trifolii* and *phaseoli*, suggesting that this protein may function to optimize interactions between bv. *viciae* and its hosts (peas, vetch lentils).

Rhizobium sp. strain NGR234 is a unique α-proteobacterium that forms nitrogen-fixing nodules with a greater variety of legumes than any other microsymbiont. Transfer of the symbiotic plasmid of this strain apparently has the potential to be under QS regulation because the *traI* and *traR* genes are on the plasmid pNGR234a. TraI synthesizes 3-oxo-C8-HSL, and two other AHLs have been detected in a *traI* mutant, indicating that the corresponding synthase(s) is encoded elsewhere in the genome (He et al. 2003). NGR234 carries at least six loci linked to the quenching of QS signals as well as one gene (*ngrI*) that may encode a novel type of autoinducer I molecule (Schmeisser et al. 2009).

Bradyrhizobium

This genus comprises a diverse group of soil microorganisms having the ability to establish associations with legume (e.g., soybean, peanut) and nonlegume plants (e.g., *Parasponia*). Detectable amounts of AHLs were found in 20 % of *B. japonicum* and *B. elkanii* strains examined with an AHL detection bioassay (Pongsilp et al. 2005). In *B. japonicum* strain USDA110, nodulation genes are expressed in a population density-dependent manner under regulation by bradyoxetin, a factor in the growth medium. Bradyoxetin was shown to be an iron chelator and is therefore not a typical QS regulation signaling molecule (Loh and Stacey 2001; Loh et al. 2002). It acts as a NolA inducer leading to *nod* gene suppression, and NwsB activity is modulated in a cell density-dependent manner. The observed production of AHLs in *B. japonicum* indicates that nodulation and other biological processes are modulated by different autoinducers and different global regulator families to coordinate cellular physiology (Westenberg 2002; Pongsilp et al. 2005; Lindemann et al. 2011).

Production of AHL signaling molecules has been demonstrated in native strains of soybean-nodulating *Bradyrhizobium* (Pongsilp et al. 2005). From several strains analyzed, only 22 % were capable of producing AHL molecules when *A. 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 *B. elkanii*. The *luxI-luxR* genes responsible for AHL synthesis and the biological functions regulated by AHLs were not identified.

The strain USDA110 of *B. japonicum* has genes homologous to *luxI-luxR*, termed *bjaI-bjaR*. No AHLs derived from BjaI have yet been detected. Lindemann et al. (2011) reported the synthesis of a novel signaling molecule catalyzed by BjaI synthase in USDA110. The molecule consists of a branched-chain fatty acyl-HSL, identified as isovaleryl-HSL (IV-HSL). The $BjaR_1$ regulator, a LuxR homolog, has high affinity for IV-HSL. In analogy to results in other AHL QS systems, *bjaI* expression was upregulated by IV-HSL addition. USDA110 is able to respond to AHLs but not to synthesize them. The high sensitivity and low specificity of the $BjaR_1$ regulator to AHLs may reflect a strategy of *B. japonicum* to both avoid detection of AHLs 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 AHLs in the system has not revealed.

Soil bacteria of the genus *Bradyrhizobium* form symbiotic relationships with peanut (*Arachis hypogaea* L.) root cells and fix atmospheric nitrogen (Bogino et al. 2006). In comparison 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 interaction is poorly understood. Novel AHL signaling molecules have been recently detected and identified in *Bradyrhizobium* strains that are phylogenetically related to peanut-nodulating strains (Ahlgren et al. 2011; Lindemann et al. 2011). We showed recently that addition of various types and concentrations of AHLs to cellular cultures of peanut-nodulating *Bradyrhizobium* sp. affects physiological processes related to bacterial survival, particularly autoaggregation, biofilm formation, and motility (Nievas et al. 2012). However, the symbiotic or other functional roles of these autoinducers in these strains remain essentially unknown.

Conclusions

The interactions between root-associated bacteria and plants encompass many levels. We have summarized here the effects of bacterial QS molecules on the symbiotic interactions between rhizobia and members of the Fabaceae family. QS is a mechanism whereby bacteria regulate their gene expression in a population density-dependent manner. Many aspects of

nodulation are also regulated by QS. The best-studied QS signaling systems involve AHLs as signaling molecules. Not surprisingly, the genetic determinants of AHL production and perception are usually integrated in complex regulatory networks and affect numerous aspects of bacterial lifestyles.

Rhizobia have a variety of QS regulatory systems that affect or regulate plasmid transfer, symbiotic interactions, surface polysaccharides, growth inhibition, and stationary-phase adaptation. Several rhizobial species studied to date have been to establish effective symbioses even when genes for AHL synthases and certain AHL receptors are mutated. Rhizobia may therefore not be as dependent as are many bacterial pathogens on QS for successful interactions with their hosts. Findings to date indicate that QS-regulated functions in the bacterium and host serve primarily to optimize various interactions between the partners. The manners in which host plants perceive AHLs appear to be diverse; AHL-producing bacteria therefore have great potential for agricultural applications.

QS signals have been detected in many species of legume-nodulating rhizobia. Besides the role of rhizobial QS signals in symbiotic interactions with legumes, these signals play important roles in microbial cross-communication. Under natural conditions, bacteria typically occur as a mixture of species and have therefore developed many ways to communicate with each other and to "listen in" on other conversations. It has been proposed that bacteria use AI-2 for interspecies communication and that the variable chemical nature of AHLs allows intraspecies communication. In this regard, certain LuxR-type regulators may interact with non-cognate AHL molecules, and such interactions may lead to unwanted activation or inhibition of QS.

It is likely that several additional genes regulated by the above-described systems remain to be identified. Although the regulatory mechanism of one of the most complex QS cascades has been well elucidated, many of the functions regulated by QS genes are poorly understood. Molecular genetic studies of the systems responsible for production of signaling molecules are essential for understanding the mechanisms whereby rhizobia communicate with each other and interact symbiotically with the host plant.

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