Chapter 4
N-Acyl Homoserine Lactone Quorum Sensing in Gram-Negative Rhizobacteria

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4.1 Introduction

In the last 15 years microbiologists have become aware that in most bacteria a major level of regulation exists which involves intercellular communication via the production and response to signal molecules. The concentration of the signal molecules increases alongside the bacterial population density and when it reaches a critical level, when a sufficient number of cells are present, bacteria respond and modulate gene expression. This cell-density-dependent modulation of gene expression has been termed quorum sensing (QS) (Fuqua et al. 1994). This allows bacteria to modify their behavior and act as multicellular entities; it is believed that in natural ecosystems bacteria are always aiming at establishing communities rather than choosing to exist as solitary cells. The reason being that intercellular communication provides significant advantages to a group of bacteria such as improving access to environmental niches, enhancing its defense capabilities against other microorganisms or eukaryotic host-defense mechanisms, and facilitating the adaptation to changing environmental conditions.

Bacterial QS signaling compounds at present can be broadly divided in two main classes, one being produced by Gram-positive bacteria and the other by Gram-negative bacteria. Gram-positive bacteria produce short, usually modified peptides processed from precursors which are then exported out of the cell and are then sensed by the bacterium through a signal transduction cascade (Bassler 2002; Sturme et al. 2002). A typical Gram-negative QS system, on the other hand (Fig. 4.1), involves the production of an acylated homoserine lactone (AHL) which was first described in the marine bioluminescent bacterium *Vibrio fischeri* in which QS regulates light production (Ruby 1996). Other types of less common signaling molecules have also been identified (Barber et al. 1997; Flavier et al. 1997a), including a furanosyl borate diester which appears to be employed by bacteria for interspecies communication (Chen et al. 2002).

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Several AHL QS systems have been described for Gram-negative plant-associated bacteria, including *Pseudomonas putida, P. chlororaphis/P. aureofaciens, P. syringae, Burkholderia cepacia, B. glumae, Erwinia carotovora, E. chrysanthemi, E. stewartii, Ralstonia solanacearum, Agrobacterium tumefaciens, Rhizobium etli, R. leguminosarum*, and *Sinorhizobium meliloti*. Among them, QS is involved in the regulation of antibiotic biosynthesis, extracellular enzymes, antifungal production, plasmid conjugation, biofilm formation, virulence factors, and rhizosphere gene

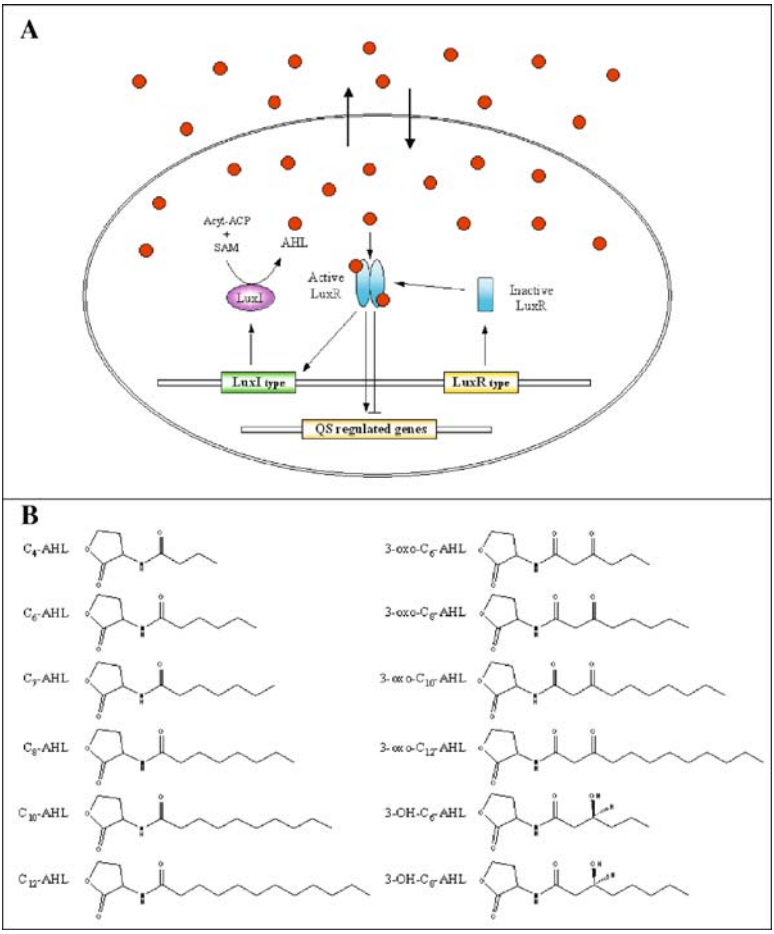

Fig. 4.1 **a** A typical *N*-acyl homoserine lactone (AHL) dependent quorum sensing (QS) system in Gram-negative bacteria. The LuxI-type proteins are the main class of enzymes capable of synthesizing AHLs and they use the cellular metabolites *S*-adenosyl-methionine (SAM) and acetylated acyl carrier proteins (ACP) to form AHLs. At high cell density, the AHL signal accumulates and interacts directly with the LuxR-type protein, inducing a conformational change (usually allowing multimerization) altering the affinity for specific DNA sequences (known as *lux* boxes) at target gene promoters changing gene expression (see text for all details). **b** Some common AHL signal molecules

expression (Loh et al. 2002; Pierson et al. 1998b; von Bodman et al. 2003). The scope of this review is to outline only the current knowledge on the AHL QS systems of rhizosphere bacteria, discussing recent advances in the role of gene regulation by QS and potential functions in bacteria–bacteria and plant–bacteria interactions. More general excellent reviews on QS in bacteria have recently been published (Fuqua et al. 2001; Miller and Bassler 2001; Whitehead et al. 2001).

4.2 AHL-Mediated QS Regulation

The typical model system for AHL QS regulation is rather simple, being most commonly mediated by two proteins belonging to the LuxI/LuxR protein families (Fig. 4.1). These families originate from the LuxI–AHL synthase and LuxR–AHL-response regulator, which is the first AHL QS system discovered involved in regulating light production in *V. fischeri* (Ruby 1996). LuxI-type proteins are the main class of enzymes capable of synthesizing AHLs and they use the cellular metabolites *S*-adenosyl-methionine and acetylated acyl carrier proteins to form AHLs. Many different Gram-negative bacteria have been reported to produce AHLs via LuxI-type proteins, differing only in the length of the acyl-chain moiety and substitution at position C3, which can be either unmodified or carries an oxo or hydroxyl group (Fig. 4.1b). Most AHLs are believed to diffuse across the cell wall; however, long-chain AHLs also utilize efflux pumps for translocation (Kohler et al. 2001; Pearson et al. 1999). The AHLs then interact directly at quorum concentration with the cognate LuxR-type protein and this protein–AHL complex can then bind at specific gene promoter sequences called *lux* boxes affecting expression of QS target genes. In most cases, the LuxR–AHL complex positively regulates the *luxI* family gene, creating a positive induction loop resulting in significant signal amplification. LuxR-type proteins display preferential binding for the AHL produced by the cognate LuxI-family protein, guaranteeing a good degree of selectivity; however, LuxR-family proteins can also to some extent respond to AHLs of different length and substitution of the acyl-chain moiety, raising important implications for the role of AHLs in interspecies communication. In some cases, for example, in *P. aeruginosa, P. aureofaciens*, and *Rhizobium* spp., bacteria possess multiple LuxI/LuxR systems producing multiple AHLs which can be hierarchically organized (see later). Recent reviews have appeared which cover in depth information on AHL structure and synthesis, LuxI- and LuxR-type proteins, LuxR–AHL interactions, and AHL QS regulons (Bassler 2002; Fuqua et al. 2001; Miller and Bassler 2001; Whitehead et al. 2001).

4.3 AHL QS and the Rhizosphere

The rhizosphere is the environment which surrounds and is influenced by the root system and has an important impact on the health and yield of crops. Plants release many compounds in the rhizosphere and microbial communities establish themselves

creating a microenvironment of plant–microbe associations (Bais et al. 2004). Some of these interactions are beneficial to both plants and microbes, involving nutrient exchange, and are encouraged, for example, in the case of nitrogen-fixing bacteria or plant-growth-promoting rhizobacteria (PGPR). On the other hand, the rhizosphere can be an environment of growth, establishment, and attack of disease-causing microorganisms, resulting in crop damage and loss. Plants therefore have evolved strategies to defend themselves from pathogens, one of which is to favor the colonization of the rhizosphere by PGPR which will then exclude deleterious pathogenic microorganisms from this environment. The generally accepted mechanisms of biocontrol of phytopathogens by PGPR are competition for a substrate, production of inhibitory substances, and induction of systematic resistance (Compant et al. 2005; Haas and Defago 2005).

In the last 10 years it has become apparent that a diversity of *Proteobacteria* isolated from the rhizosphere use AHL signal molecules for QS-dependent gene expression. Among these are strains belonging to the species or genera of *P. aureofaciens/P. chlororaphis, P. putida, P. syringae, Burkholderia, Serratia, Erwinia, Ralstonia*, and *Rhizobium* and related genera involved in legume symbiosis. These bacteria employ AHL QS, for example, to regulate the production of biologically active secondary metabolites, enzymes, or exopolysaccharide which can improve the biological control activities of PGPR or are virulence determinants for plant pathogens. In then following we describe and discuss the current knowledge of AHL QS control in these beneficial or pathogenic rhizosphere-associated bacteria.

4.4 AHL QS in *Pseudomonas*

Pseudomonads can colonize several environmental niches and *P. aeruginosa* is also an important and dangerous human opportunistic pathogen as it can infect and chronically colonize the lungs of humans suffering from cystic fibrosis. In fact in the scientific community, pseudomonads are studied (1) for their role as human and plant pathogens, (2) for their remarkable catabolic potential, metabolism, and physiological versatility, and (3) for rhizosphere colonization and potential biological control agents. AHL QS has been most extensively studied in *P. aeruginosa*, making it one of the most studied systems in bacteria (Juhas et al. 2005; Smith and Iglewski 2003). Two systems, LasI/LasR and RhlI/RhlR, are present in *P. aeruginosa*; in the LasI/LasR system, LasI synthesizes *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (3-oxo-C_{12}-AHL), which interacts with LasR and regulates target promoters. In the RhlI/RhlR system, RhlI directs the synthesis of *N*-butanoyl-L-homoserine lactone (C_4-AHL), which interacts with the cognate regulator RhlR and regulates target gene promoters. The two systems are under positive feedback control and are intimately connected forming a regulatory cascade; LasR/3-oxo-C_{12}-AHL positively regulates the *lasI* AHL synthase, creating a positive induction loop, and also activates *rhlR* expression initiating the RhlI/RhlR systems (Latifi et al. 1996; Seed et al. 1995). The LasI/LasR and RhlI/RhlR regulons have been extensively studied

and have been found to regulate the production of multiple virulence factors, including elastase, alkaline protease, exotoxin A, rhamnolipids, pyocyanin, lectins, superoxide dismutases, and biofilm formation (Smith and Iglewski 2003). The effects of the two AHL QS systems have been tested in various models of *P. aeruginosa* infection, including several mouse models and alternative infection models of *Caenorhabditis elegans, Arabidopsis thaliana*, and *Dictyostelium discoideum*, all of which have shown that AHL QS MUTANTS showed reduction in virulence (reviewed recently in Juhas et al. 2005; Smith and Iglewski 2003). Genetic and microrray studies on regulons of *P. aeruginosa* have shown that the expression of over 300 genes is affected by AHL QS; thus, it is a major global regulatory response/regulation system (Hentzer et al. 2003; Schuster et al. 2003; Wagner et al. 2003; Whiteley et al. 1999). It is not intended to review and discuss in detail AHL QS in *P. aeruginosa* since studies have been focused on its pathogenicity to humans and because it is not regarded as a predominant rhizosphere bacterial species as is the case for *P. putida, P. fluorescens*, and *P. chlororaphis/P. aureofaciens*.

One of the first reports of AHL QS in *Pseudomonas* was the PhzI/PhzR of the wheat plant growth promoting rhizosphere colonizing *P. aureofaciens* (synonym of *P. chlororaphis*) strain 30-84 producing and responding to *N*-hexanoyl-L-homoserine lactone (C_6-AHL) (Pierson et al. 1994, 1995; Wood et al. 1997). PhzR/C_6-AHL regulates transcription, through binding to *lux*-box-like sequences in the promoter region, of the phenazine operon *phzXYFABCD* (Wood et al. 1997). The production of phenazine antibiotics in the wheat rhizosphere by *P. aureofaciens* strain 30-84 is important for its biocontrol properties by antagonizing the fungus *Gaeumannomyces graminins* var. *tritici*, which is the causal agent of take-all disease of wheat. A second AHL QS system is present in *P. aureofaciens* strain 30-84 which has been designated CsaI/CsaR (Zhang and Pierson 2001). The two systems are not organized in a hierarchical way (as is the case for the Las and Rhl systems of *P. aeruginosa*) and appear to function independently. CsaI/CsaR is not involved in the regulation of phenazine production, whereas it regulates exoprotease production in a synergistic way together with the PhzI/PhzR system; the precise molecular mechanism by which this occurs is still unknown (Zhang and Pierson 2001). In addition, CsaI/CsaR is involved in the regulation of cell-surface properties and is important for rhizosphere colonization. Interestingly, the most notable reduction in rhizosphere colonization in this strain was observed when both the PhzI/PhzR and CsaI/CsaR systems were inactivated (Zhang and Pierson 2001). The role of AHL QS in phenazine regulation has also been investigated in the plant-beneficial rhizobacterium *P. chlororaphis* strain PCL1391 (Chin et al. 2001, 2005). Similarly to *P. aureofaciens* 30-84, also in strain PCL1391, phenazine-1-carboxamide is regulated by a PhzI/PhzR QS system which produces and responds to C_6-AHL (Chin et al. 2001, 2005). The two PhzI/PhzR systems are highly identical and initial studies have shown that both *phzI/phzR* systems are themselves under considerable regulation (Chancey et al. 1999; Chin et al. 2005). *P. chlororaphis* PCL1391 has been shown to produce other types of AHL molecules in addition to C_6-AHL; however, the genetic determinants as well as the possible roles in gene regulation are currently unknown (Chin et al. 2001, 2005). Recently a PhzI/PhzR system has also been reported in

rhizosphere *P. fluorescens* 2-79 in which it is also involved in the regulation of the antifungal secondary metabolite phenzine-1-carboxamide (Khan et al. 2005). Unlike the PhzI/PhzR systems of *P. chlororaphis/P. aurefaciens* 30-84 and PCL1391, the Phz/PhzR of *P. fluorescens* 2-79 produces and responds to *N*-(3-oxo-hexanoyl)-L-homoserine lactone (3-oxo-C_6-AHL) despite it being almost 90% identical to the other two PhzI/PhzR systems. Genetic and molecular studies have shown that in *P. fluorescens* PhzI/PhzR regulates the transcription of the phenazine biosynthesis operon in response to 3-oxo-C_6-AHL and hence to cell density (Khan et al. 2005).

AHL QS has been studied in two strains of plant-beneficial *P. putida* rhizobacteria (Bertani and Venturi 2004; Steidle et al. 2002). *P. putida* strain IsoF produces and responds to a 3-oxo-C_{12}-AHL via the PpuI/PpuR AHL QS system. This system has been shown to be important for biofilm formation; an important trait for colonization when growing on surfaces. Similarly, in *P. putida* strain WCS358 a PpuI/PpuR system identical to that of strain IsoF has been identified and characterized; however, no phenotypes have yet been identified which are regulated by this system. Interestingly, the PpuI/PpuR system is highly identical to the LasI/LasR system of *P. aeruginosa*, both systems responding to the same AHL molecule and being regulated in a similar way (Bertani and Venturi 2004).

The plant pathogen *P. syringae* has been reported to be able to synthesize AHLs (Dumenyo et al. 1998; Elasri et al. 2001). *P. syringae* pv. *syringae*, the causal agent of brown spot of bean, has an AHL QS system designated AhlI/AhlR which produces and responds to 3-oxo-C_6-AHL and was shown to be important for cell aggregation and epiphytic fitness for *in planta* growth and disease (Quinones et al. 2004). Interestingly, an extensive study analyzing the AHL production ability of 137 soil-borne and plant-associated *Pseudomonas* sp. bacterial strains revealed that AHL production was more common among plant-associated bacteria than among free-living soil-borne ones (Elasri et al. 2001). This study involved strains belonging to *P. syringae, P. chlororaphis, P. fluorescens*, and *P. putida*. It was observed that none of the last three *Pseudomonas* sp. isolated from the soil produced AHLs, whereas it was a very common feature if they were isolated from the rhizosphere. This raises the question of the possible important role of AHL QS in plant–bacteria interaction.

4.5 AHL QS in *Burkholderia*

Just like in the *Pseudomonas* genus, *Burkholderia* species can populate very different niches, including plants, soil, water, and rhizosphere; they may have both pathogenic and symbiotic interactions with plants and are also pathogenic to humans (Coenye and Vandamme 2003). The species *B. cepacia* was originally described by Burkholder (1950) in 1950 as the causative agent of bacterial rot of onions causing a disease called sour skin. In the last 10 years several taxonomic studies resulted in

the classification of the *Burkholderia* genus, also creating a group of nine closely species, designated as the *B. cepacia* complex (BCC), isolated from both clinical and environmental sources (Coenye and Vandamme 2003). BCC strains have emerged as opportunistic pathogens in patients with cystic fibrosis causing serious chronic infections. Some species of the BCC are potential biocontrol agents as they can efficiently colonize the root rhizosphere of several important crops and antagonize growth of microbial plant pathogens (Coenye and Vandamme 2003; O'Sullivan and Mahenthiralingam 2005).

Several studies involving cell–cell communication in *Burkholderia* have been performed mainly involving species of the BCC where the AHL QS systems are very well conserved. All reported QS systems of *Burkholderia* consist of the AHL synthase CepI, which mainly directs the synthesis of *N*-octanoyl-L-homoserine lactone (C_8-AHL), which then interacts with LuxR-family member CepR, leading to induction or repression of gene expression (Venturi et al. 2004a). CepI/CepR systems are highly conserved and thus very homologous within the *Burkholderia* genus (Aguilar et al. 2003a, b; Gotschlich et al. 2001; Lutter et al. 2001; Yao et al. 2002). A second AHL QS system (called BviI/BviR), in addition to the CepI/CepR system, has been reported in some strains of *B. vietnamiensis* involving *N*-decanoyl-L-homoserine lactone (C_{10}-AHL) (Conway and Greenberg 2002; Venturi et al. 2004a); strains belonging to this species have been the subject of bioremediation studies. The role of CepI/CepR and BviI/BviR and whether the two systems interact in *B. vietnamiensis* are currently unknown. Another system, called CciI/CciR, has been reported in a highly transmissible opportunistic human pathogen of *B. cenocepacia* (Mahenthiralingam et al. 2005). CciI/CciR is part of a 31-kb pathogenicity island and recently it has been demonstrated that the CepI/CepR and the CciI/CciR systems are interacting with each other (Malott et al. 2005).

The AHL QS system of *Burkholderia*, just like in *P. aeruginosa*, contributes to the virulence as determined using various infection models including plants, nematodes, and murines (Bernier et al. 2003; Huber et al. 2004; Sokol et al. 2003). In the environmental isolate *B. cepacia* ATCC 25419^T, the CepI/CepR system is associated with onion pathogenicity; *cepI* and *cepR* mutants are less virulent in onion rot since attenuated tissue maceration was observed (Aguilar et al. 2003a). This reduction in maceration is mainly due to the lower levels of extracellular polygalacturonase activity since in *cepI/cepR* knockout mutants display 40% of enzyme activity when compared with the parent strain. A systematic study on the CepI/CepR regulon of a *B. cenocepacia* strain has revealed that just like in *P. aeruginosa*, also in *Burkholderia*, QS is a global regulatory system modulating gene expression of approximately 6% of the loci present in the genome (Aguilar et al. 2003b; Riedel et al. 2003).

AHL QS plays an important role in virulence in the soil- and seed-borne rice grain rot (also known as panicle blight) pathogen *B. glumae* (Kim et al. 2004). *B. glumae* contains a system called TofI/TofR which has high identity (75%) to the CepI/CepR system, also producing and responding to C_8-AHL (Kim et al. 2004). The TofI/TofR system of *B. glumae* has been implicated in the regulation of toxoflavin, an essential toxin for the rice pathogenicity of this organism (Kim et al. 2004). Toxoflavin production occurs best in the late-exponential phase and

employs QS through TofR/C_8-AHL, ensuring high expression of the toxoflavine biosynthesis genes at high cell densities. It is currently unknown if this occurs directly through activation by TofR/C_8-AHL of the toxoflavin genetic loci or whether the TOF system regulates another regulator called ToxJ which then activates the toxoflavin operons (Kim et al. 2004).

4.6 AHL QS in *Erwinia*

Erwinia spp. are Gram-negative bacterial necrotrophic plant pathogens. They are the causative agents of plant diseases such as soft rots, the potato disease blackleg (stem rot), and Stewart's wilt. AHL QS regulation of pathogenicity factors has been studied in *E. carotovora, E. stewartii* subsp. *stewartii* (Ess; synonym *P. stewartii*), and in *Erwinia chrysnathemi*. In *E. carotovora*, AHL QS systems have been reported in three subspecies: *E. carotovora* subsp. *carotovora* (Ecc), which is pathogenic to many different crops, *E. carotovora* subsp. *atroseptica* (Eca), whose genome has been sequenced and which attacks mainly potato (reviewed in Perombelon 2002), and *E. carotovora* spp. *betavasculorum*, which is pathogenic to sugar beet (Costa and Loper 1997). The major AHL signal molecule produced by *Erwinia* spp. is 3-oxo-C_6-AHL, which in Ecc and Eca is generated by CarI (Swift et al. 1993) and in *E. stewartii* by EsaI. *E. amylovora*, a bacterial pathogen that causes fire blight in plants, produces both 3-oxo-C_6-AHL and *N*-(3-hydroxyhexanoyl)-L-homoserine lactone (3-OH-C_6-AHL) (Venturi et al. 2004b) and the AHL synthase gene, *eamI*, and its putative activator gene, *eamR*, were recently identified and found to be involved in disease symptom development (Molina et al. 2005). *Erwinia* spp. produce an array of exoenzymes (Barras et al. 1994) and some subspecies produce the antibiotic 1-carbapen-2-em-3-carboxylic acid (carbapenem). The production of carbapenem is directly regulated by QS since CarR/AHL binds upstream of the CarA-H biosynthetic operon, resulting in transcriptional activation (Bainton et al. 1992; McGowan et al. 1995; Williams et al. 1992). Mutations in either *carI* or *carR* block carbapenem synthesis (McGowan et al. 1995). Self-resistance to carbapenem antibiotic is obtained by expressing CarF and CarG, which are expressed on a basal level in an AHL-independent manner, while their upregulated expression is CarR/AHL-dependent (McGowan et al. 2005). AHLs are also required to induce the production of plant cell wall degrading exoenzymes (PCWDEs; Jones et al. 1993). CarR is not the AHL receptor activator driving PCWDE production, as exoenzyme production is unaltered in Ecc harboring a disrupted *carR* gene, whereas *carI* mutations affect exoenzyme production. The LuxR-family regulator involved in this regulation is currently unidentified. As the regulators characterized in different *Erwinia* spp. share a higher degree of amino acid identity with each other than with other members of the LuxR-like proteins, Andersson et al. (2000) proposed that they may form a distinct subfamily.

The involvement of QS in plant infection by *Erwinia* spp. is manifested also in experiments with transgenic tobacco plants producing AHLs (Mae et al. 2001) or

transgenic potato plants expressing the lactonase enzyme AiiA (Dong et al. 2001), which show increased resistance to *Erwinia* infections. The "quenching" of the QS of *Erwinia* spp. can also be achieved by coinfecting *Erwinia* spp. with bacterial strains that are capable of degrading the AHL signals; recently *Bacillus thuringiensis*, the most widely used biocontrol agent for insect control, was found to effectively stop the otherwise rapid spread of *E. carotovora* cells in plant tissues by the production of AHL lactonases (Dong et al. 2004).

The search for additional QS-regulated genes that are related to pathogenicity of *Erwinia* spp. is ongoing, and has revealed such genes both in Eca and in Ecc. Seven novel genes that are either activated or repressed by the presence of AHLs were found in Ecc (Pemberton et al. 2005). One of these genes, Nip_{Ecc}, was found to be a member of the Nep-1-like (NPL) proteins family (Nep1 is an elicitor of plant necrosis from *Fusarium oxysporum*; Bailey 1995). Nip_{Ecc} was found to cause necrosis when infiltrated into *Nicotiana tabacum* leaves. The Eca homologue of Nip_{Ecc}, Nip_{Eca}, was found to be also involved in pathogenicity. As for exoenzyme production, also nip expression was not affected by *carR* mutations, and *eccR* mutation (*eccR* codes for EccR which is a second AHL LuxR-family response protein) led to a slight increase in nip transcription (Pemberton et al. 2005). A novel gene, *svx*, encoding for a virulence factor which is also regulated by QS was recently found in Eca, the mutant of the *svx* gene was found to have reduced virulence, and *carI* mutants did not produce Svx (Corbett et al. 2005).

E. chrysanthemi strain 3937 produces three different AHLs: 3-oxo-C_6-AHL, C_6-AHL and *N*-decanoyl-L-homoserine lactone (C_{10}-AHL). The genes for the QS signal generator (*expI*) and a response regulator (*expR*) were identified and shown to have high similarity to the *expI/expR* genes of *E. carotovora* (Nasser et al. 1998). ExpI is responsible for only two of the AHLs produced. Disruption of *expI* had no apparent effect on the growth-phase-dependent expression of *hrpN* and *pelE*, or on the virulence of *E. chrysanthemi* in witloof chicory leaves (Ham et al. 2004).

4.7 AHL QS in Rhizobia

Bacteria belonging to the genera *Rhizobium, Mesorhizobium, Sinorhizobium, Bradyrhizobium*, and *Azorhizobium* (collectively referred to as rhizobia) grow in the soil as free-living organisms and can also live as nitrogen-fixing symbionts inside root nodule cells of legume plants playing important roles in agriculture by inducing nitrogen-fixing nodules on the roots of legumes such as peas, beans, clover, and alfalfa (Gage 2004). Several species of rhizobia have been shown to produce AHLs playing important roles in plant–bacteria interactions (Gonzalez and Marketon 2003).

R. leguminosarum bv. *viciae* has a genome consisting of a circular chromosome and six plasmids and possesses several AHL QS systems designated Rhi, Cin, Tra, and Rai (reviewed in Gonzalez and Marketon 2003). The Rhi system is located on the symbiotic plasmid pRL1JI and is composed of *rhiI* and *rhiR* genes responsible for

producing and responding to C_6-AHL and C_8-AHL (Rodelas et al. 1999). RhiI/RhiR has been shown to regulate the expression of the *rhiABC* operon, the function of which is currently unknown but it is believed to be involved in the early stages of the symbiotic process. Moreover it was demonstrated that the plant signal compounds known as flavonoids inhibit the expression of both *rhiR* and the *rhiABC* operon (Cubo et al. 1992). The TraI/TraR system is present on the plasmid pRL1JI, it produces and responds to *N*-(3-oxo-octanoyl)-L-homoserine lactone (3-oxo-C_8-AHL), and is involved in the regulation of plasmid transfer (Danino et al. 2003). The RaiI/RaiR system mainly produces and responds to *N*-(3-hydroxyoctanoyl)-L-homoserine lactone (3-OH-C_8-AHL) and is intimately connected with the CinI/CinR system (Wisniewski-Dye et al. 2002; see later). The CinI/CinR system is present on the chromosome and is responsible for the production and response to *N*-(3-hydroxy-7-*cis*-tetradecenoyl)-L-homoserine lactone (3-OH-$C_{14:1}$-AHL) (Lithgow et al. 2000). This signaling molecule is rather unusual in that it inhibits the growth of several strains of *R. leguminosarum* and was previously known as a small bacteriocinin. The CinI/CinR system appears to be at the top of the AHL regulatory cascade since it influences several AHL QS systems, including the RhiI/RhiR and TraI/TraR systems, as well as being involved in the transfer of pRL1JI (Gonzalez and Marketon 2003; Lithgow et al. 2000).

R. etli strain CNPAF512 differs from *R. leguminosarum*, since it possesses only the RaiI/RaiR and the CinI/CinR AHL QS systems. Both systems are present on the chromosome in this strain and are important for growth inhibition and nitrogen fixation (Daniels et al. 2002). *R. etli* strain CFN42 contains one chromosome and six plasmids (p42a to p42f) and possesses the Tra and part of the Cin AHL QS systems, both being involved in the mobilization of the p42a symbiotic plasmid (Tun-Garrido et al. 2003).

S. meliloti is a free-living soil bacterium capable of establishing a symbiotic relationship with the alfalfa plant (*Medicago sativa)*. Several strains of *S. meliloti* have been reported to produce one or more AHLs, suggesting the presence of QS systems in this species (Cha et al. 1998; Gonzalez and Marketon 2003; Shaw et al. 1997). The well-characterized *S. meliloti* strain Rm1021 contains two different AHL QS systems on its chromosome: the Sin and the Mel systems (Marketon and Gonzalez 2002).The SinI/SinR system is responsible for the production of long-chain AHLs, ranging from *N*-dodecanoyl-L-homoserine lactone (C_{12}-AHL) to *N*-octadecanoyl-L-homoserine lactone (C_{18}-AHL); *sinI* and *sinR* mutants lead to a decrease in the number of pink nodules during nodulation assays, suggesting a role for QS in symbiosis (Marketon and Gonzalez 2002). In addition, SinI/SinR is necessary for the synthesis of EPSII, an exopolysaccharide important for the nodule invasion process (Marketon et al. 2003). The Mel system appears to be responsible for the production of short-chain AHLs, but the genetic loci as well as its function have not yet been identified (Marketon and Gonzalez 2002). A third AHL QS system has been identified in *S. meliloti* strain Rm41. This system, named TraI/TraR for its homology to the QS system in *A. tumefaciens* and *Rhizobium*, is present on a plasmid called pRme41a and has been shown to be controlling conjugal plasmid transfer (Marketon and Gonzalez 2002).

4.8 AHL QS in Other Gram-Negative Rhizobacteria

The production of exopolysaccharides and plant cell wall degrading enzymes by the phytopathogen *Ralstonia solanacearum* contributes significantly to its virulence and they are produced maximally at high cell densities. *R. solanacearum* contains an AHL QS system designated SolI/SolR which produces and responds to C_6-AHL and/or C_8-AHL (Flavier et al. 1997b). At present there is no evidence that SolI/SolR is directly involved in virulence gene expression; however SolI/SolR is part of the regulatory cascade as it is regulated by a "higher-level" autoinduction system responsive to 3-hydroxypalmitic acid methyl ester via the LysR family regulator PchA (Flavier et al. 1997b, 1998). In addition, *solI/solR* is also additionally regulated by the stationary phase RpoS sigma factor. The QS system is therefore regulated by two other global regulatory systems which are both required for the expression of virulence factors.

Members of the *Serratia* genus are able to colonize a wide variety of surfaces in water and soils and are opportunistic pathogens for plants, insects, fish, and humans (Grimont and Grimont 1978). In *S. liquefaciens* MG1 the AHL QS system SwrI/SwrR produces and responds to C_4-AHL and is involved in (1) the regulation of swarming motility through the direct control of the *swrA* gene which encodes a peptide synthetase responsible for the synthesis of the biosurfactant serraweetin 2 which reduces surface tension and allows swarming motility to occur (Lindum et al. 1998) and (2) mature biofilm formation through the regulation of two loci (called *bsmA* and *bsmB*) responsible for the formation of cell aggregates at a specific time point in biofilm development (Labbate et al. 2004). In *Serratia* sp. ATCC 39006 the AHL QS system SmaI/SmaR produces and responds to C_4-AHL and is involved in the regulation of the antibiotic prodigiodin, of the secondary metabolite carbapenem (a broad spectrum β-lactam antibiotic also produced by *Erwinia* sp.), and of the exoenzymes pectate lyase and cellulose (Fineran et al. 2005; Slater et al. 2003; Thomson et al. 2000). The biocontrol strain *S. plymutica* IC1270 has been reported to produce AHLs; however, the genetic loci have not yet been isolated (Ovadis et al. 2004).

4.9 Interspecies Signaling via AHLs Among Bacteria in the Rhizosphere

Most studies involving production and response to AHL of plant-associated bacteria have been performed in the laboratory and might not reflect what occurs in vivo in close proximity to the plant or *in planta*. Scientists need to focus more attention on the *in situ* production and ability to respond to AHL signal molecules in order to understand when bacteria are coordinating their gene expression in response to cell density or if they are subjected to interference by other bacteria or by the plant. Using green fluorescent protein based AHL sensor plasmids

(which are able to respond to the presence of AHLs by producing the easily detectable green fluorescent protein) Steidle et al. (2001) have demonstrated that *P. putida* and *S. liquefaciens* can perceive AHL signals in the rhizosphere of tomato plants when coinoculated with an AHL-producing strain in axenically grown tomato plants. *P. putida* can also perceive AHL signals produced by the indigenous rhizosphere community as it responds to AHLs when inoculated in nonsterile soil (Steidle et al. 2001). This latter result clearly demonstrates that AHL molecules are produced at quorum concentrations in the rhizosphere and that they can be utilized/perceived by the bacterial consortium, implicating interspecies communication. Interspecies communication via AHLs has also been demonstrated between *Burkholderia* and *Pseudomonas* including in the biofilm mode of growth (Lewenza et al. 2002; McKenney et al. 1995; Riedel et al. 2001). Similarly, cross-talk via AHLs has also been demonstrated in the rhizosphere of wheat as a naturally coexisting nonisogenic bacterial population exchange AHL signal with phenazine-producing *P. aureofaciens* strain 30-84 (Pierson et al. 1998a).

Interspecies communication can also be significantly affected by microorganisms which have the capability of degrading the AHLs. Over the past 5 years scientists have reported that a diversity of soil microbes are capable of biodegrading AHLs by cleaving either the amide or the lactone bonds. These enzyme activities could have potent negative effects on AHL signal accumulation as has been demonstrated in pure culture laboratory studies, in soil microcosms, and in transgenic plants expressing bacterial proteins (reviewed in Dong and Zhang 2005).

4.10 AHL Interference, Coordination, and Response by the Plant

A question which is now beginning to be addressed by the scientific community is how eukaryotic hosts are responding to and/or defending themselves against bacterial AHL signal molecules. Plants have been shown to produce chemical compounds that can interfere with QS systems in bacteria by acting as agonists or antagonists of AHL signaling pathways. The chemical structure of these AHL mimics is currently not known and they are referred to as mimics because of their functional interference with bacterial AHLs. Pea seedling exudates inhibited AHL QS in *Chromobacterium violaceum*, whereas they induced the system in *S. liquefaciens* and in LuxR-, LasR-, and AhyR-based engineered *Escherichia coli* AHL sensor systems (Teplitski et al. 2000). In addition, extracts from rice, soybean, tomato, *Medicago truncatula*, and the green alga *Chlamydomonas reinhardtii* all contain AHL mimic molecules (Bauer and Mathesius 2004; Gao et al. 2003; Teplitski et al. 2000, 2004). The implications of a plant interfering with the AHL QS system can be the following: (1) if a plant pathogenic bacterium employs QS in order to prevent activation of virulence gene expression at low cell densities, the interference by the plant to prematurely express these

genes would result in the bacterium revealing its presence at a time during the infection at which the plant can effectively prevent its establishment, and (2) on the other hand, in symbiotic plant–bacteria interactions direct signaling via AHLs may permit the coordination of gene expression, resulting in a beneficial interaction for both partners. Interestingly, tobacco plants genetically modified to produce AHLs could induce AHL QS target gene expression in bacteria and consequently restore biocontrol activity of a *P. aureofaciens* AHL-deficient mutant. These transgenic plants could also reestablish pathogenicity to an AHL-defective *E. carotovora* mutant (Fray et al. 1999). Similarly, plants genetically modified to produce a bacterial lactonase enzyme able to degrade AHL molecules displayed significant resistance to disease caused by *E. carotovora* (Dong et al. 2001). AHLs have also been shown to be able to modulate gene expression in plant cells as shown in a proteomic study. Three-day-old roots of *M. truncatula* were exposed to 3-oxo-C_{12}-AHL or *N*-(3-oxo-9-*cis*-hexadecanoyl)-L-homoserine lactone (3-oxo-$C_{16:1}$-AHL) and protein expression was examined by two-dimensional gel electrophoresis and the abundance of 150 proteins showed altered levels depending on the identity, time of exposure, and concentration of the AHL (Mathesius et al. 2003). Our understanding of how plants interfere, coordinate, or respond to bacterial AHL signal molecules is at an early stage and these are important questions to address in the future.

4.11 Conclusions

Several AHL QS systems belonging to rhizobacteria have been reported and studied (summarized in Table 4.1); most systems have been isolated in the course of deciphering the regulation of particular target genes. From studies in other bacteria, it is very likely that also in rhizobacteria AHL QS is a global regulatory network controlling the expression of several hundred genes, thus changing the gene expression profile of bacteria. It will be important to determine if bacterial species which have evolved to particularly adapt to colonize the rhizosphere display unique features with respect to AHL QS. In order to establish this, more *in situ* studies are required especially to determine if AHL QS is influenced by the plant or the microbial consortium present in the rhizosphere. Initial investigations have shown that the plant responds to AHLs, synthesizes AHL analogues and that in the rhizosphere interspecies communication via AHL takes place. These are very important observations indicating that AHL QS could be a way to communicate in the bacterial community and across kingdoms. These results will encourage the scientific community to dedicate more attention to *in situ* studies; understanding the role of these systems will most probably have an impact on more appropriate and effective bioinoculants as well as designing efficient strategies for combating bacterial plant pathogens.

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Table 4.1 *N*-Acyl homoserine lactone (*AHL*) quorum sensing (*QS*) systems of rhizobacteria

Species	AHL-QS	AHL molecule	Regulated phenotype	References
Pseudomonas aeruginosa	*lasI, lasR* *rhlI, rhlR*	3-oxo-C_{12}-AHL C_4-AHL	Elastase, alkaline protease, exotoxin A, rhamnolipids, pyocyanin, lectins, superoxide dismutases, biofilm formation	Juhas et al. (2005) Smith and Iglewski (2003)
Pseudomonas aureofaciens 30-84	*phzI, phzR* *csaI, csaR*	C_6-AHL Unknown	Phenazine antibiotics exoprotease, cell surface components, rhizosphere colonization	Pierson et al. (1995) Wood et al. (1997)
Pseudomonas chlororaphiss PCL1391	*phzI, phzR*	C_6-AHL Others Unknown	Phenazine antibiotics	Chin et al. (2001) Chin et al. (2005)
Pseudomonas fluorescens 2-79	*phzI, phzR*	3-oxo-C_6-AHL	Phenazine-1-carboxamide regulation	Khan et al. (2005)
Pseudomonas putida IsoF	*PpuI, PpuR*	3-oxo-C_{12}-AHL	Biofilm development	Steidle et al. (2002)
Pseudomonas putida WCS358	*PpuI, PpuR*	3-oxo-C_{12}-AHL	Unknown	Bertani and Venturi (2004)
Pseudomonas syringiae	*ahlI, ahlR*	3-oxo-C_6-AHL	Cell aggregation, epiphytic fitness	Dumenyo et al. (1998), Elasri et al. (2001)
Burkholderia cenocepacia	*cciI, cciR*	Unknown	Unknown	Malott et al. (2005)
Burkholderia cepacia	*cepI, cepR*	C_8-AHL	Unknown	Aguilar et al. (2003a)
Burkholderia glumae	*tofI, tofR*	C_8-AHL	Toxoflavin regulation	Kim et al. (2004)
Burkholderia vietmamiensis	*bviI, bviR*	C_{10}-AHL	Unknown	Conway and Greenberg (2002), Venturi et al. (2004a)
Erwinia amylovora Ea02	*eamI, eamR*	3-oxo-C_6-AHL 3-oH-C_6-AHL	Virulence Extracellular polysaccharide production, hydrogen peroxide tolerance	Venturi et al. (2004b) Molina et al. (2005)
Erwinia carotovora subsp. *betavasculorum* Ecb168	*ecbI, ecbR*	Unknown	Antibiotic production, extracellular protease	Costa and Loper (1997)

(continued)

Table 4.1 (continued)

Species	AHL-QS	AHL molecule	Regulated phenotype	References
Erwinia carotovora subsp. *atrseptica* SCRI1043	*carI, carR*	3-oxo-C_6-AHL 3-oxo-C_8-AHL, C_8-AHL, C_{10}-AHL	Carbapenem production Exoenzyme synthesis	Smadja et al. (2004)
Erwinia carotovora subsp. *carotovora*	*carI, carR* *expI, expR* *hslI, hslR*	3-oxo-C_6-AHL	Carbapenem production Exoenzyme synthesis Hrp secretion system	Bainton et al. (1992) Jones et al. (1993) McGowan et al. (1995), Swift et al. (1993)
Erwinia chrysanthemi 3937	*expI, expR*	3-oxo-C_6-AHL, C_6-AHL, C_{10}-AHL	Unknown	Nasser et al. (1998)
Erwinia stewartii subsp. *stewartii* SS104	*esaI, esaR*	3-oxo-C_6-AHL, 3-oxo-C_8-AHL	Exopolysaccharide production	Beck von Bodman and Farrand (1995), Beck von Bodman et al. (1998)
Rhizobium etli CNPAF512	*cinI, cinR* *raiI, raiR*	OH-long-chain AHLs Short-chain AHLs	Nitrogen fixation, growth inhibition, symbiosome development Nitrogen fixation, growth inhibition	Daniels et al. (2002)
Rhizobium etli CNPAF512	*traI, traR* Unknown	3-oxo-C_8-AHL 3-OH-C_8-AHL	Conjugal plasmid transfer Unknown	Tun-Garrido et al. (2003)
Rhizobium leguminosarum bv. *viciae*	*rhiI, rhiR* *cinI, cinR* *traI, traR* *raiI, raiR*	C_6-AHL, C_7-AHL, C_8-AHL 3-OH-$C_{14:1}$-AHL 3-oxo-C_8-AHL 3-OH-C_8-AHL	Influences nodulation Mediates growth inhibition Regulation of plasmid transfer Unknown	Rodelas et al. (1999) Lithgow et al. (2000) Danino et al. (2003) Wisniewski-Dye et al. (2002)
Sinorhizobium meliloti Rm1021	*sinI, sinR* *melI, melR*	Long-chain AHL Short-chain AHLs	Exopolysaccharide EPSII synthesis Unknown	Marketon et al. (2003) Marketon and Gonzalez (2002)
Sinorhizobium meliloti Rm41	*traI, traR* *sinI, sinR*	3-oxo-C_8-AHL, 3-oxo-$C_{16:1}$-AHL, $C_{16:1}$-AHL, C_{16}-AHL 3-oxo-C_{14}-AHL	Conjugal plasmid transfer Exopolysaccharide EPSII synthesis	Marketon and Gonzalez (2002) Marketon and Gonzalez (2002)

(continued)

Table 4.1 (continued)

Species	AHL-QS	AHL molecule	Regulated phenotype	References
Ralstonia solanacearum	*solI, solR*	3-Hydroxypalmitic acid methyl ester	Virulence gene expression	(Flavier et al. (1998)
Serratia liquefaciens MG1	*swrI, swrR*	C_4-AHL	Regulation of swarming motility Mature biofilm formation	Lindum et al. (1998) Labbate et al. (2004)
Serratia ATCC 39006	*smaI, smaR*	C_4-AHL	Regulation of antibiotic prodigiodin, exoenzymes pectate lyase, cellulose	Fineran et al. (2005)

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