Plant Pathol. J. 15(2) : 79-86 (1999)

The Plant Pathology Journal

Symposium
Molecular Plant-Microbe Interactions
November 27, 1998, Taejon, Korea

Quorum-Sensing Signals in Gram-Negative Plant-Associated Bacteria

Ingyu Hwang

Plant Protectants Research Unit, Korea Research Institute of Bioscience and Biotechnology, Yusung P.O. Box 115, Taejon 305-600, Korea

(Received March 23, 1999)

Bacteria perceive their growth environment, gather information, interpret this information, and execute appropriate responses to go on in an orderly-regulated way. Plant-associate bacteria also possess sensory machineries to become adapted to new environments by processing input signals. The best-known example of processing external signals in Gram-negative bacteria is the so-called two-component system that consists of two proteins, a sensor and a response regulator. The two-component system uses phosphorylation as a means of transferring information (Parkinson and Kofoid, 1992). It has been known that plant-associated bacteria give signals to host plants and that small molecules originated from their hosts can be used as signals for a certain specific biological phenomenon. This signaling system plays important roles in plant-bacteria interactions. The two most well characterized cases are *Agrobacterium*-crown gall interactions and *Rhizobium* (*Bradyrhizobium*)-legume interactions.

More recently, it has become clear that bacteria within their population communicate each other and receive information from other bacteria. These examples include sporulation and fruit-body formation by *Myxococcus xanthus*, antibiotic production by *Streptomyces* species, and conjugation in *Enterococcus faecalis* (Dunny and Leonard, 1997). The paradigm of this inter-bacterial signaling system is autoinduction (or quorum sensing) of bioluminescence in the symbiotic marine bacterium *Photobacterium fischeri*. Autoinduction was first described in marine bacteria *Vibrio harveyi* and *P. fischeri* in the early 1970s (Nealson, 1977). *P. fischeri* produces a small diffusible compound called the autoinducer, *N*-(3-oxohexanoyl)-L-homoserine lactone (OHHL) (Fig. 1), which accumulates in the medium during growth. In recent years, a broad range of Gram-negative bacteria including plant-associated bacteria has been reported to produce autoinducers. Many other biological phenomena including pathogenicity, extracellular enzyme biosynthesis, antibiotic biosynthesis, conjugation, exopolysaccharide biosynthesis, swarming, bacteriocin production, nodulation, rhamnolipid biosurfactant biosynthesis, regulation of *rpoS* expression, cell aggregation, and cell division are found to be regulated by autoinduction (Table 1) (Swift et al., 1996).

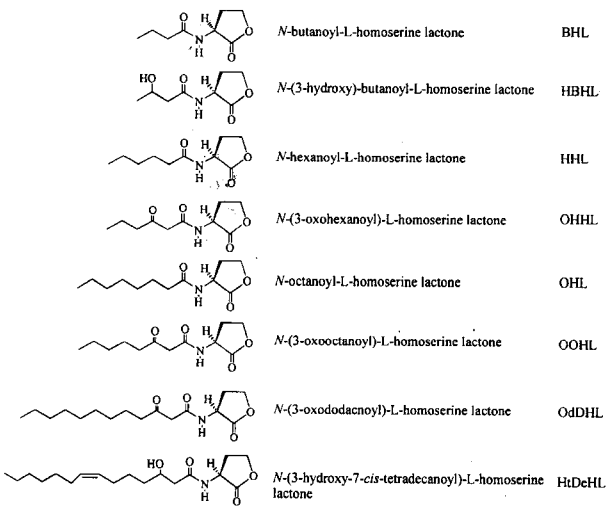

Fig. 1. The structures of autoinducer molecules.

The Paradigm of Quorum Sensing: Regulation of Bioluminescence in *P. fischeri*

P. fischeri is a light-organ symbiont of certain species of bony fish and squid. When they live as symbionts where they can grow to high cell densities, they generate bioluminescence. However, when they live as free-living organisms, the production of bioluminescence is turned off since it is a highly energy-consuming process. Two divergent *lux*

*Corresponding author.
Phone) +82-42-860-4552, Fax) +82-42-861-2675
E-mail) ingyu@kribb4680.kribb.re.kr

Table 1. Many Gram-negative bacteria produce various *N*-acyl homoserine lactone signal molecules

Bacterium	Phenotype	Signal molecule[a]	Signal generator	Response regulator
Aeromonas hydrophila	Extracellular protease	BHL, HHL	AhyI	AhyR
Aeromonas salmonicida	Extracellular protease	BHL, HHL	AsaI	AsaR
Agrobacterium tumefaciens	Conjugation	OOHL	TraI	TraR
Chromobacterium violaceum	Antibiotics, Exoenzymes, Cyanide, Violacein	HHL	CviI	CviR
Enterobacter agglomerans	Unknown	OHHL	EagI	
Erwinia carotovora subsp. *carotovora*	Carbapenem, Exoenzymes, Virulence	OHHL	CarI, ExpI	CarR, ExpR
Escherichia coli	Cell division			SdiA
Nitrosomonas europaea	Emergence from lag phase	OHHL		
Obesumbacterium proteus	Unknown	OHHL	OprI	OprR
Pantoea stewartii subsp. *stewartii*	Exopolysaccharide, Virulence	OHHL	EsaI	EsaR
Photobacterium fischeri	Bioluminescence	OHHL, OHL	LuxI AinS	LuxR AinR
Pseudomonas aeruginosa	Alkaline protease, Elastase, Exotoxin A Chitinase, Cyanide, Rhamnolipid, Lectins, Haemolysin, RpoS	OdDHL, BHL	LasI RhlI	LasR RhlR
Pseudomonas aureofaciens	Phenazine antibiotics	HHL	PhzI	PhzR
Pseudomonas syringae pv. *tabaci*	Unknown		PsyI	PsyR
Ralstonia solanacearum	Virulence, Exopolysaccharide	HHL, OHL	SolI	SolR
Rhizobium leguminosarum	Nodulation, Bacteriocin *small*	HtDeHL	RhiI	RhiR
Rhodobacter sphaeroides	Community escape	7,8-*cis*-tDHL	CerI	CerR
Serratia liquefaciens	Swarming, Phospholipase	BHL	SwrI	
Vibrio anguillarum		ODHL	VanI	VanR
Vibrio harveyi	Bioluminescence, Polyhydroxybutyrate metabolism	HBHL	LuxLM	LuxN
Xanthomonas campestris	Exoenzymes			
Xenorhabdus nematophilus	Virulence, Bacterial lipase	HBHL		
Yersinia enterocolitica	Unknown	HHL, OHHL	YenI	YenR
Yersinia pseudotuberculosis	Unknown	OHHL, HHL	YepI	YepR
Yersinia ruckeri	Unknown		YukI	YukR

[a]BHL = *N*-butanoyl-L-homoserine lactone; OHL = *N*-octanoyl-L-homoserine lactone; HHL = *N*-hexanoyl-L-homoserine lactone; OHHL = *N*-(3-oxohexanoyl)-L-homoserine lactone; OOHL = *N*-(3-oxooctanoyl)-L-homoserine lactone; ODHL = *N*-(3-oxodecanoyl)-L-homoserine lactone; OdDHL = *N*-(3-oxododecanoyl)-L-homoserine lactone; HBHL = *N*-(3-hydroxy)-butanoyl-L-homoserine lactone; HtDeHL = *N*-(3R-hydroxy-7-*cis*-tetradecanoyl)-L-homoserine lactone; 7,8-*cis*-tDHL = 7,8-*cis*-*N*-(tetradecanoyl)-L-homoserine lactone.

operons are responsible for the production of bioluminescence (Fig. 2). The expression of the *lux* operon of *P. fischeri* requires the transcriptional activator LuxR. LuxR, in turn, requires OHHL as a coinducer. The gene, *luxI*, located at the 5' end of the *lux* operon, is responsible for the synthesis of OHHL. During growth, *P. fischeri* produces OHHL that diffuses out of the cells into the culture supernatants. When total OHHL reaches a certain overall concentration as a function of cellular growth, the autoinducer is believed to bind to LuxR, converting it to a functional activator (Fuqua et al., 1994). The active form of LuxR binds to called *lux* box upstream of the *lux* operon and activates transcription of the *lux* genes. Thus, the expression of the *lux* genes is dependent upon the cells reaching a critical population density. Biochemical evidences for OHHL binding to LuxR and OHHL and LuxR complex binding to the *lux* box are limited due to insolubility of intact LuxR. Only genetic evidences exist for OHHL binding to LuxR, however a truncated form of LuxR lacking the N-terminal region has been purified and used to prove its binding to the *lux* box (Stevens et al., 1994). An intragenic suppressor mutant of LuxR activating the *lux* gene expression independent of OHHL has been isolated and found to have a mutation in the C-terminal region (Poellinger et al., 1995).

Interestingly, a second autoinducer, *N*-octanoyl-L-homoserine lactone (OHL) (Fig. 1), exists in *P. fischeri*. *ainS* is responsible for OHL biosynthesis, and OHL activates the *lux* operon in *E. coli* (Gilson et al., 1995). The C-terminal end of AinS shows homology to a LuxM gene of *V. harveyi*, which is required for the synthesis of a *V. harveyi* bioluminescence autoinducer, suggesting the occurrence of convergent evolution in the synthesis of autoinducer signal molecules (Gilson et al., 1995).

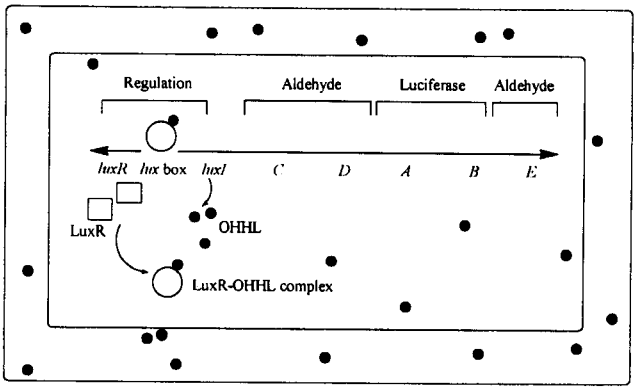

Fig. 2. Organizations and functions of LuxR and OHHL of *Photobacterium fischeri*. Arrows indicate the direction of transcription of the *lux* operon. Rectangles indicate LuxR protein and open circles with black dots denote active forms of LuxR. Black dots indicate OHHL. Small and big rectangles indicate a bacterial cell and a light organ, respectively.

Production of *N*-acyl Homoserine Lactone Signals Is Common Among Gram-negative Plant-Associated Bacteria

Regulation of exoenzyme biosynthesis and antibiotic production in *Erwinia carotovora*. In early 1990s, it was unveiled that the regulation of sets of bacterial genes by autoinduction is not limited to marine bacteria. Bainton et al. (1992) first reported that a terrestrial bacterium produces an acyl-homoserine lactone. Two research groups worked on *E. carotovora* demonstrated that avirulent mutants which have mutations in one of the regulatory genes show a pleiotropic defect in the growth phase-dependent transcriptional activation of exoenzyme gene expression (Jones et al., 1993; Pirhonen et al., 1993). Phenotypes of these mutants were complemented by adding exogenous OHHL purified from *P. fischeri*. This extracellular complementation supported that *E. carotovora* subsp. *carotovora* also produces the same signal molecule. It was turned out that OHHL produced from *E. carotovora* subsp. *carotovora* regulates cell-density-dependent carbapenem antibiotic production and expression of cell-wall-degrading exoenzymes involved in pathogenicity (Jones et al., 1993; Pirhonen et al., 1993). Two sets of LuxI and LuxR homologs, CarI and CarR and ExpI and ExpR, are found in *E. carotovora*. However, disruption of *expR* has no significant effects on OHHL biosynthesis, pathogenicity, and exoenzyme production whereas *carI* interruption led to a downregulation of exoenzyme biosynthesis and subsequent loss of pathogenicity (Jones et al., 1993; Pirhonen et al., 1993). Thus, CarI and CarR in *E. carotovora* apparently act for both carbapenem antibiotic production and exoenzyme biosynthesis.

A negative regulation by LuxR homolog for production of extracellular polysaccharide in *Pantoea stewartii* subsp. *stewartii*. In *E. carotovora*, overexpression of CarR represses exoenzyme production and can be relieved by adding exogenous OHHL (McGowan et al., 1995). This was a first description that LuxR homologs may act as a repressor. A similar phenomenon was observed in *P. stewartii* subsp. *stewartii* (this bacterium formerly was named *Erwinia stewartii*), which causes Stewartii's wilt on sweet corn and leaf blight on maize. Extracellular polysaccharide (EPS) stewartan governs pathogenicity of this bacterium and its production is controlled by quorum sensing (Beck von Bodman and Farrand, 1995). EsaI is responsible for the quorum sensing signal, OHHL, and EsaR is a cognate gene regulator like LuxI and LuxR (Beck von Bodman and Farrand, 1995). However, unlike *luxR* an *esaR* null mutation renders high level production of OHHL in a cell-density-independent manner, indicating that the regulatory system of EPS production in *P. stewartii* subsp. *stewartii* uses EsaR to repress EPS biosynthesis at low cell densities (Beck von Bodman et al., 1998). In contrast to most other described autoinduction systems, EsaR operates as a negative regulator of EPS biosynthesis rather than a gene activator. Quorum-sensing mechanisms play a role in delaying the production of EPS in *P. stewartii* subsp. *stewartii* at low cell densities so that it does not interfere with early disease development (Beck von Bodman et al., 1998).

Regulation of Ti plasmid conjugation by opines: A path to cell-to-cell communication. *Agrobacterium tumefaciens* strains carrying Ti plasmid cause crown gall tumors on many plants and have two independent DNA transfer systems. A T-DNA transfer from bacterial cells to plant nucleus is mediated by Vir proteins. Agrobacteria use plant phenolic compounds as signal molecules to initiate this process, and expression of *vir* genes is induced by two-components systems consisting of the autokinase VirA and the response regulator VirG (Citovsky et al., 1992). While the T-DNA transfer is a DNA transfer system from bacterial cells to plant cells, whole Ti plasmid can be transferred from donor agrobacteria to receiver cells. This conjugal transfer is induced primarily by plant metabolites called opines. Conjugal transfer of octopine or nopaline type Ti plasmid is induced by so-called conjugal opines, octopine or agrocinopines, respectively. However, expression of *tra* and *trb* genes is actually regulated by quorum sensing systems (Hwang et al., 1994; Li et al., 1999; Piper et al., 1993). Expression of genes in the *tra* and *trb* regions requires the transcriptional activator, TraR. TraR is a LuxR homolog and like LuxR, requires autoinducer, *N*-(3-oxooctanoyl)-L-homoserine lactone (OOHL) (Fig. 1) (Piper et al., 1993; Zhang et al.. 1993). TraI is responsible for OOHL biosynthesis and uses *S*-adenosylmethionine and 3-oxooctanoyl-

acyl carrier protein to make the homoserine lactone moiety and 3-oxooctanoyl moiety of OOHL, respectively (Moré et al., 1996). In octopine-type strains, the synthesis of TraR is activated by the octopine response regulator, OccR (Fuqua et al., 1994). In nopaline-type strains, expression of *traR* is repressed by the agrocinopine catabolism repressor, AccR (Beck von Bodman et al., 1992). Agrocinopines relieve the repressor activity of AccR, resulting in high level expression of *traR* (Fig. 3). This indicates that quorum-sensing is subordinate to the opine regulon and that hierarchical gene regulatory systems arise from fortuitous gene association in which *traR* has become associated with an operon controlled by the opine-responsive transcriptional regulator (Fig. 3) (Piper et al., 1999).

Although TraI and TraR are key elements in regulation of conjugal transfer of Ti plasmid, there is another negative level of regulation by TraM (Hwang et al., 1995). TraM suppresses expression of *tra* and *trb* genes mediated by TraR and OOHL, mutations in *traM* in pTiC58 confers a transfer-constitutive phenotype, and strains carrying the Ti

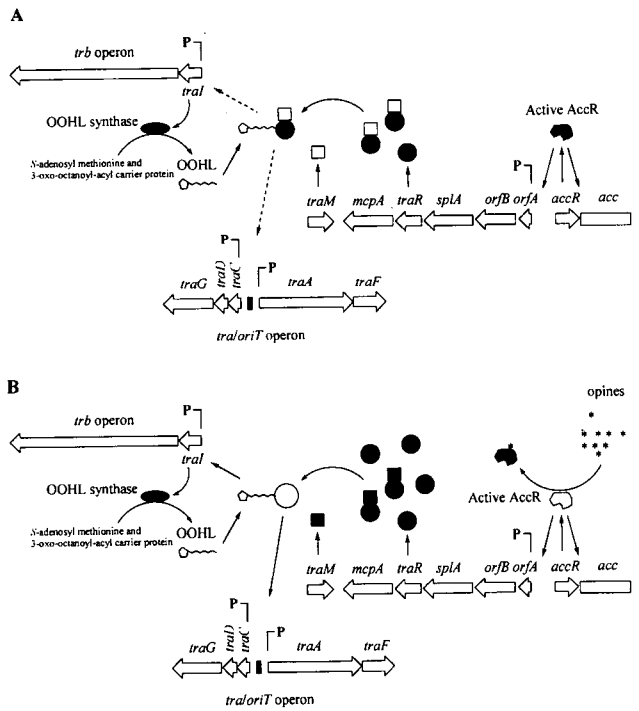

Fig. 3. Regulation of conjugal transfer of pTiC58 under uninduced conditions (A) and induced conditions (B). In the absence of agrocinopines, the active form of AccR represses expression of *traR* transcribing from the upstream promoter and of *acc* operon. TraM binds to TraR, resulting in an inactive form of TraR. This form of TraR fails to activate *tra* and *trb* gene expression mediated by OOHL. When opines are present, TraR is produced more through inactivation of AccR by agrocinopines. TraR under this condition exists more than TraM, and OOHL then binds to free TraR to activate *tra* and *trb* genes.

plasmids produce easily detectable amounts of OOHL (Hwang et al., 1995). TraM functions only when overexpressed with respect to TraR, and this suppression can be overcome by overexpressing TraR. However, suppression by TraM cannot be overcome by adding excess OOHL. Genetic and biochemical data suggest that TraM modulates autoinduction by interacting with TraR to inhibit premature conjugation (Hwang et al., unpublished). Thus, TraM appears to prevent the basal level of TraR present in uninduced cells from activating transcription of the *tra* genes (Fig. 3).

More recently, a second *traR*-like gene, *trlR*, was found in octopine-mannityl opine-type Ti plasmids pTi15955 and pTiR10 (Oger et al., 1998; Zhu and Winans, 1998). This gene is located in an operon coding for a mannopine transport system and is expressed as parts of the mannityl opine regulons. *trlR* has a frameshift mutation compared to *traR*, resulting in a truncated protein lacking the carboxy-terminal domain thought to be the DNA-binding region of TraR (Oger et al., 1998; Zhu and Winans, 1998). Expression of *trlR* is inducible by mannopine, and genetic analyses indicate that *trlR* is a dominant negative allele of *traR* and inhibits conjugation by forming inactive heteromultimers with TraR (Oger et al., 1998; Zhu and Winans, 1998). This explains why octopine is a conjugal opine for conjugal transfer of pTi15955 and pTiR10 and mannopine is not.

Hierarchical autoinduction in *Ralstonia solanacearum*. *R. solanacearum* causes vascular wilt in many plants including tomato, potato, and tobacco. Production of EPS of this bacterium occludes the vascular systems of plants and hence is a major pathogenicity factor (Schell, 1996). The expression of virulence determinants in *R. solanacearum* is controlled by a complex regulatory network in which PhcA, a LysR-type transcriptional regulator, plays a central role (Schell, 1996). The transcriptional activity of *phcA* is controlled by an autoregulatory system responding to 3-hydroxypalmitic acid methyl ester (Flavier et al., 1997a). On the other hand, *eps* genes are differentially expressed during exponential multiplication indicative of cell-density-dependent regulation (Flavier et al., 1997a). Apparently *R. solanacearum* produces *N*-hexanoyl homoserine lactone (HHL) and *N*-octanoyl homoserine lactone (OHL) (Fig. 1) (Flavier et al., 1997b). LuxI and LuxR homologs were found, and *solI* is responsible for HHL and OHL biosynthesis (Flavier et al., 1997b). Mutations in *solI* neither abolish pathogenicity nor affect the expression of virulence genes (Flavier et al., 1997b). However, since expression of *solI* and *solR* requires PhcA which responds to 3-hydroxypalmitic acid methyl ester, autoinduction systems in *R. solanacearum* are a part of a more complex autoregulatory hierarchy (Flavier et al., 1997b).

Novel signal molecules from *Xanthomonas campestris*

pv. *campestris*: Regulation of pathogenicity, EPS production, and pigment biosynthesis. *X. campestris* pv. *campestris* is a major pathogen of cruciferous plants and produces extracellular enzymes including proteases, pectinases, endoglucanase, and polysaccharides that are important for pathogenicity of this bacterium (Barber et al., 1997). A cosmid clone complementing mutants showing reduced symptom production carries at least seven regulatory genes, designated *rpfA-G*, controlling pathogenicity factors (Tang et al., 1991). Cells carrying mutations in *rpf* genes show reduced virulence but still have an ability to cause hypersensitive response on nonhost plants. The phenotype of *rpfF* can be restored by a low molecular weight diffusible substance (DSF). DSF is produced at the early stationary phase and declined subsequently and heat-stable, and its activity is not destroyed by alkaline treatment and acid hydrolysis (Barber et al., 1997). DSF apparently is not *N*-acyl homoserine lactone because it does not activate reporter genes of known screening systems designed to detect *N*-acyl homoserine lactone, but rather it may be a fatty-acid derivative (Barber et al., 1997). However, it is still possible that DSF may be a modified *N*-acyl homoserine lactone. DSF production is limited to certain strains of xanthomonads (Barber et al., 1997). Recently, Poplawsky et al. (1998) reported that biosynthesis of EPS, exoenzymes, and xanthomonadin in *X. campestris* pv. *campestris* is under the control of two intercellular regulatory signals. The chemical properties of these two signal molecules remain uncertain.

Cell-to-cell signaling in the nitrogen-fixing bacterium *Rhizobium leguminosarum* and *R. etli*. All three biovars of *R. leguminosarum* produce *small* bacteriocin that inhibits the growth of some strains of this bacterium. The growth inhibiting function is encoded by Sym plasmid pRL1JI and activated by an autoinducer molecule and its cognate transcriptional activator RhiR (Gray et al., 1996). This autoinducer molecule is *small* bacteriocin, *N*-(3*R*-hydroxy-7-*cis*-tetradecanoyl)-L-homoserine lactone (HtDeHL) which is required to activate the rhizosphere-expressed *rhiABC* operon (Schripsema et al., 1996).

Rhizobium etli (formerly classified as *R. leguminosarum* bv. *phaseoli*) forms nitrogen-fixing nodules on the roots of the common bean and produces at least seven different autoinducer molecules. One of them is *small* bacteriocin, and two autoinducers are synthesized by a LuxI homolog, RaiI (Rosemeyer et al., 1998). A *raiI* mutant still releases three different autoinducers and a *raiR* mutant releases four different autoinducers (Rosemeyer et al., 1998). HtDeHL is involved in the restriction of nodule number, however nitrogen-fixing activity in terms of acetylene reduction per nodule is not affected (Rosemeyer et al., 1998).

Meaning of cell-density signals in biological control by *Pseudomonas aureofaciens* and *Ps. fluorescens*. *Ps. aureofaciens* and *Ps. fluorescens* produce phenazine antibiotics and have been used as biocontrol agents to protect wheat from take-all disease caused by *Gaeumannomyces graminis* var. *tritici*. *Ps. aureofaciens* strain 30-84 produces three phenazine antibiotics, phenazine-1-carboxylic acid, 2-hydroxy-phenazine-1-carboxylic acid, and 2-hydroxy-phenazine (Pierson and Thomashow, 1992). These antibiotics also play important roles in microbial competition and rhizosphere survival. Mutants of *Ps. aureofaciens* unable to produce phenazines lost the ability to inhibit the growth of fungal pathogen, and by analyzing these mutants the phenazine biosynthetic region (*phz* genes) was localized in the 9.2-kb *Eco*RI fragment (Pierson and Thomashow, 1992). This region contains a LuxI and LuxR homolog, PhzI and PhzR, and addition of exogenous culture filtrates resulted in *phz* gene expression at low cell densities indicative of the presence of cell-density signals (Pierson et al., 1994). Like many others, a typical sign of the presence of cell-density signals in *Ps. aureofaciens* is the fact that phenazines are produced only during late-exponential and stationary growth phase. However, chemical natures of this autoinducer have not been characterized.

Unknown roles of *N*-acyl homoserine lactone in many plant-associated bacteria. When many isolates (106 isolates) of plant-associated bacteria were screened for production of *N*-acyl homoserine lactone using four different indicator systems, most of *Agrobacterium* and *Rhizobium* isolates, about 50% of *Erwinia* and *Pseudomonas* isolates, and some *Xanthomonas* isolates gave positive reactions (Cha et al., 1998). They produce various kinds of *N*-acyl homoserine lactone as judged by TLC analysis, and some of isolates produce multiple autoinducers. Among various *N*-acyl homoserine lactones, OHHL and OOHL are very common molecules, and the pseudomonads and erwinia produce OHHL most. Interestingly, the production of *N*-acyl homoserine lactone is dependent upon strains within the same species and pathovars. This phenomenon is somewhat unexpected because plant-associated bacteria classified as a same group of species or pathovars should have very similar or same ecological fitness and an equal ability to adapt to new environments. Although many plant-associated bacteria produce quorum sensing signals, the phenotypes depending upon a cell density are known only for a few. As mentioned above, regulation of conjugal transfer of Ti plasmid, regulation of exoenzyme production and secretion of *E. carotovora*, and regulation of phenazine biosynthesis by *Ps. aureofaciens* are among the known traits regulated by quorum sensing signals. Biological traits regulated by quorum sensing in many other plant-associated bacteria remain to be characterized. Especially, roles of these signals in ecological fitness of plant-associated bacte-

ria and possible cross-communications among bacteria are interesting subjects to study.

Other Quorum Sensing Systems

Two levels of quorum sensing regulation in *Ps. aeruginosa*. *Ps. aeruginosa* is an opportunistic human pathogen causing cystic fibrosis, and multiple factors such as alginate, toxins, haemolysins, and proteases are important for pathogenesis. Production of these virulence factors depends upon growth environment and particularly cell densities. *Ps. aeruginosa* produces two *N*-acyl homoserine lactone, *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL) and *N*-butyryl homoserine lactone (BHL) (Fig. 1) (Passador et al. 1993; Pearson et al. 1994). *lasI* and *rhlI* are OdDHL and BHL synthase, respectively (Pearson, 1994; Ochsner and Reiser, 1995). OdDHL and its cognate transcriptional activator LasR activate expression of *lasB*, *lasA*, *apr*, and *toxA* genes (Pearson, 1997). BHL and RhlR are responsible for rhamnolipid, elastase, haemolysin, alkaline protease, cyanide, and lectin production (Ochsner and Reiser, 1995). They also enhance expression of *rpoS* in *E. coli* (Latifi et al., 1996). OdDHL apparently blocks BHL from binding to RhlR, thus inhibiting *rhlA* expression (Pesci et al., 1997). This shows a first description of biological interactions of two quorum sensing signal molecules in one bacterium to coordinate expression of different genes. Recently, it was found that a *lasI* mutant failed to form biofilms which are important for pathogenicity of *Ps. aeruginosa*, and that flat and undifferentiated biofilms were sensitive to the biocide sodium dodecyl sulfate (Davies et al., 1998). Adding exogenous OdDHL to the mutant recovers a biofilm formation, indicating that it requires cell-to-cell communication. This finding suggests a new possible target to control cystic fibrosis.

Examples of other signaling systems by *N*-acyl homoserine lactone. After autoinduction was first described for the production of bioluminescence in *P. fischeri*, many other similar but in some cases somewhat different regulatory circuits have been reported. Recently, a various bacterial phenotypes are known to be regulated by quorum sensing systems, and numbers of Gram-negative bacteria producing *N*-acyl homoserine lactone are growing (Table 1) (Swift et al., 1996). Different bacteria produce various autoinducers and these molecules can be easily detected by thin-layer chromatography and other chemical analyses (Shaw et al., 1997).

References

Bainton, N. J., Stead, P., Chhabra, S. R., Bycroft, B. W., Salmond, G. P. C., Stewart, G. S. A. B. and Williams, P. 1992. *N*-(3-oxohexanoyl)-L-homoserine lactone regulates carbapenem antibiotic production in *Erwinia carotovora*. *Biochem. J.* 288:997-1004.

Barber, C. E., Tang, J. L., Feng, J. X., Pan, M. Q., Wilson, T. J. G., Slater, H., Dow, J. M., Williams, P. and Daniels, M. J. 1997. A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is required by a small diffusible signal molecule. *Mol. Microbiol.* 24:555-566.

Beck von Bodman, S., Hayman, G. T. and Farrand, S. K. 1992. Opine catabolism and conjugal transfer of the nopaline Ti plasmid pTiC58 are coordinately regulated by a single repressor. *Proc. Natl. Acad. Sci. USA* 89:643-647.

Beck von Bodman, S. and Farrand, S. K. 1995. Capsular polysaccharide biosynthesis and pathogenicity in *Erwinia stewartii* require induction by an *N*-acyl homoserine lactone autoinducer. *J. Bacteriol.* 177:5000-5008.

Beck von Bodman, S., Majerczak, D. R. and Coplin, D. L. 1998. A negative regulator mediates quorum-sensing control of exopolysaccharide production in *Pantoea stewartii* subsp. *stewartii*. *Proc. Natl. Acad. Sci. USA* 95:7687-7692.

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

Citovsky, V., McLean, B. G., Greene, E., Howard, E., Kudau, G., Thorstenson, Y., Zupan, J. and Zambryski, P. 1992. *Agrobacterium*-plant cell interaction: induction of *vir* genes and T-DNA transfer. In: *Molecular Signals in Plant-Microbe Communications*, ed. by D. P. S. Verma, pp.169-199. CRC Press, Boca Raton.

Davies, D. G., Parsek, M. R., Pearson, J. P., Iglewski, B. H., Costerton, J. W. and Greenberg, E. P. 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 280:295-298.

Dunny, G. M. and Leonard, B. A. B. 1997. Cell-cell communication in Gram-positive bacteria. *Annu. Rev. Microbiol.* 51:527-564.

Flavier, A. B., Ganova-Raeva, L. M., Schell, M. A. and Denny, T. P. 1997a. Hierarchical autoinduction in *Ralstonia solanacearum*: control of acyl-homoserine lactone production by a novel autoregulatory systems responsive to 3-hydroxypalmitic acid methyl ester. *J. Bacteriol.* 179:7089-7097.

Flavier, A. B., L. M., Schell, M. A. and Denny, T. P. 1997b. Identification of β-hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in *Ralstonia solanacearum*. *Mol. Microbiol.* 26:251-259.

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

Gilson, L., Kuo, A. and Dunlap, P. V. 1995. AinS and a new family of autoinducer synthesis protein. *J. Bacteriol.* 177:6946-6951.

Gray, K. M., Pearson, J. P., Downie, J. A., Boboye, B. E. A. and Greenberg, E. P. 1996. Cell-to-cell signaling in the symbiotic nitrogen-fixing bacterium *Rhizobium leguminosarum*: Autoin-

duction of a stationary phase and rhizosphere-expressed genes. *J. Bacteriol.* 178:372-376.

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

Hwang, I., Li, P.-L., Zhang, L., Piper, K. R., Cook, D. M., Tate, D. E. and Farrand, S. K. 1994. TraI, a LuxI homologue, is responsible for production of conjugation factor, Ti plasmid *N*-acyl homoserine lactone autoinducer. *Proc. Natl. Acad. Sci. USA* 91:4639-4643.

Jones, B., Yu, B., Bainton, N. J., Birdsall, M., Bycroft, B. W., Chhabra, S. R., Cox, A. J. R., Golby, P., Reeves, P. J., Stephens, S., Winson, M. K., Salmond, G. P. C., Stewart G. S. A. B. and Williams, P. 1993. The *lux* autoinducer regulates the production of exoenzyme virulence determinants in *Erwinia carotovora* and *Pseudomonas aeruginosa*. *EMBO J.* 12:2477-2482.

Latifi, A., Foglino, M., Foglino, K., Williams, P. and Lazdunski, A. 1996. A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhlR (VsmR) to expression of the stationary-phase sigma factor RpoS. *Mol Microbiol.* 21:1137-1146.

Li, P.-L, Hwang I., Miyagi H., True-Krob, H. and Farrand, S. K. 1999. Essential components of the Ti plasmid *trb* system, a type IV macromolecular transporter. *J. Bacteriol.* (In press).

McGowan, S., Sebaihia, M., Jones, S., Yu, B., Bainton, N., Chan, P. F., Bycroft, B., Stewart G. S. A. B., Williams, P. and Salmond, G. P. C. 1995. Carbapenem antibiotic production in *Erwinia carotovora* is regulated by CarR, a homologue of the LuxR transcriptional activator. *Microbiology* 141:541-550.

Moré, M. I., Finger, L. D., Stryker, J. L., Fuqua, C., Eberhard, A. and Winans, S. C. 1996. Enzymatic synthesis of a quorum-sensing autoinducer through use of defined substrates. *Science* 272:1655-1658.

Nealson, K. H. 1977. Autoinduction of bacterial luciferase: occurrence, mechanism, and significance. *Arch. Microbiol.* 112:73-79.

Ochsner, U. A. and Reiser, J. 1995. Autoinducer-mediated regulation of rhamnolipid biosurfactant synthesis in *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* 92:6424-6428.

Oger, P., Kim, K.-S., Sackett, R. L., Piper, K. R. and Farrand, S. K. 1998. Octopine-type Ti plasmids code for a mannopine-inducible dominant-negative allele of *traR*, the quorum-sensing activator that regulates Ti plasmid conjugal transfer. *Mol. Microbiol.* 27:277-288.

Parkinson, J. S. and Kofoid, E. C. 1992. Communication modules in bacterial signaling proteins. *Ann. Rev. Genet.* 26:71-112.

Passador, L., Cook, J. M., Gambello, M. J., Rust, L. and Iglewski, B. H. 1993. Expression of *Pseudomonas aeruginosa* virulence genes requires cell-cell communication. *Science* 260:1127-1130.

Pearson, J. P., Gray, K. M., Passador, L., Tucker, K. D., Eberhard, A., Iglewski,B. H. and Greenberg, E. P. 1994. Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. *Proc. Natl. Acad. Sci. USA* 91:197-201.

Pearson, J. P., Pesci, E. C. and Iglewski, B. H. 1997. Roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *J. Bacteriol.* 179:5756-5767.

Pesci, E. C., Pearson, J. P., Seed, P. C. and Iglewski, B. H. 1997. Regulation of *las* and *rhl* quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* 179:3127-3132.

Pierson, L. S. and Thomashow, L. S. 1992. Cloning and heterologous expression of the phenazine biosynthetic locus *of Pseudomonas aureofaciens* 30-84. *Mol. Plant-Microbe Interact.* 5:330-339.

Pierson, L. S., Keppenne, V. D. and Wood, D. W. 1994. Phenazine antibiotic biosynthesis in *Pseudomonas aureofaciens* 30-84 is regulated by PhzR in response to cell density. *J. Bacteriol.* 176:3966-3974.

Piper, K. P., Beck von Bodman, S. and Farrand, S. K. 1993. Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid conjugal transfer by autoinduction. *Nature* 362:448-450.

Piper, K. R., Beck von Bodman, S., Hwang, I. and Farrand, S. K. 1999. Hierarchical gene regulatory systems arising from fortuitous gene associations: Controlling quorum-sensing by the opine regulon in *Agrobacterium*. *Mol. Microbiol.* 32:1077-1089.

Pirhonen, M., Flego, D., Heikinheimo, R. and Palva, E. T. 1993. A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in the plant pathogen *Erwinia carotovora*. *EMBO J.* 12:2467-2476.

Poellinger, K. A., Lee, J. P., Parales, J. V. and Greenberg, E. P. 1995. Intragenic suppression of a *luxR* mutation: Characterization of an autoinducer-independent LuxR. *FEMS Microbiol. Lett.* 129:97-102.

Poplawsky, A. R, Chun, W., Slater H., Daniels, M. J. and Dow, J. M. 1998. Synthesis of extracellular polysaccharide. extracellular enzymes, and Xanthomonadin in *Xanthomonas campestris*: Evidence for the involvement of two intercellular regulatory signals. *Mol. Plant-Microbe Interact.* 11:68-70.

Rosemeyer, V., Michiels, J., Verreth, C. and Vanderleyden, J. 1998. *luxI*- and *luxR*-homologous genes of *Rhizobium etli* CNPAF512 contribute to synthesis of autoinducer molecules and nodulation of *Phaseolus vulgaris*. *J. Bacteriol.* 180:815-821.

Schell, M. A. 1996. To be or not to be: how *Pseudomonas solanacearum* decides whether of not to express virulence genes. *Eur. J. Plant Pathol.* 102:459-469.

Schripsema, J., De Rudder, K. E. E., Van Vlit, T. B., Lankhorst, P. P., De Vroom, E., Kijne, J. W. and Van Brussel, A. A. N. 1996. Bacteriocin *small* of *Rhizobium leguminosarum* belongs to the class of *N*-acyl-L-homoserine lactone molecules, known as autoinducers and as quorum sensing co-transcription factors. *J. Bacteriol.* 178:366-371.

Shaw, P. D., Ping, G., Daly, S. L., Cha, C., Cronan, J. E., Rinerhart, K. L.and Farrand, S. K. 1997. Detecting and characterizing *N*-acyl-homoserine lactone signal molecules by thin-layer chromatography. *Proc. Natl. Acad. Sci. USA* 94:6036-6041.

Stevens, A. M., Dolan, K. M. and Greenberg, E. P. 1994. Synergistic binding of the *Vibrio fischeri* LuxR transcriptional acti-

vator domain and RNA polymerase to the *lux* promoter region. *Proc. Natl. Acad. Sci. USA* 91:12619-12623.

Swift, S., Throup, J. P., Williams, P., Salmond, G. P. C. and Stewart, G. S. A. B. 1996. Quorum sensing: a population-density component in the determination of bacterial phenotype. *TIBS* 21:214-219.

Tang, J. L., Liu, Y.-N., Barber, C. E., Dow, J. M., Wootton, J. C. and Daniels, M. J. 1991. Genetic and molecular analysis of a cluster of *rpf* genes involved in positive regulation of synthesis of extracellular enzymes and polysaccharide in *Xanthomonas campestris* pathovar *campestris*. *Mol. Gen. Genet.* 226:409-417.

Zhang, L., Murphy, P. J., Kerr, A. and Tate, M. E. 1993. *Agrobacterium* conjugation and gene regulation by *N*-acyl-L-homoserine lactones. *Nature* 362:446-448.

Zhu, J. and Winans, S. C. 1998. Activity of the quorum-sensing regulator TraR of *Agrobacterium tumefaciens* is inhibited by a truncated, dominant defective TraR-like protein. *Mol. Microbiol.* 27:289-297.