European Journal of Plant Pathology **104:** 1–9, 1998.

Mini review

N-acyl-homoserine lactone-mediated gene regulation in biological control by fluorescent pseudomonads: Current knowledge and future work

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Accepted 2 September 1997

Key words: autoinduction, biological control, gene regulation, LuxR/LuxI, *N*-acyl-homoserine lactones, fluorescent pseudomonads, quorum sensing

Abstract

An emerging area within biological control is the role of *N*-acyl-homoserine lactones (*N*-acyl-HSL's) in the regulation of competitive fitness and pathogen suppression. *N*-acyl-HSL regulatory systems utilize two conserved proteins which belong to the LuxR/LuxI regulatory family: one is a transcriptional regulator and the second produces the *N*-acyl-HSL signal. These signals regulate the expression of a diverse range of bacterial traits involved in microbe-microbe and host-microbe interactions. Several fluorescent pseudomonads important in biological control produce *N*-acyl-HSL signals which regulate genes that encode products involved in pathogen suppression. In contrast to pathogenic bacteria, little is known regarding *N*-acyl-HSL-mediated gene regulation in biological control bacteria. This minireview will focus on the current status of the role of *N*-acyl-HSL's in the regulation of phenazine antibiotic and rhamnolipid production in biological control by fluorescent pseudomonads. The potential relevance of this type of regulation in biological control of plant diseases, and areas requiring further research will be addressed.

Abbreviations: LB – Luria Broth; *N*-acyl-HSL – *N*-acyl-L-homoserine lactone.

Introduction

Fluorescent pseudomonads comprise a diverse group of Gram negative bacteria easily distinguished by their broad nutritional capabilities and their ability to produce a water-soluble fluorescent siderophore. During the past twenty years fluorescent pseudomonads have been recognized as potential biological control agents of plant pathogens, both in the phyllosphere and the rhizosphere. Biological control bacteria may ameliorate the effects of plant pathogens by interacting with their plant hosts (i.e. by producing growth hormones or by activating host defense responses) (Handelsman and Stabb, 1997; Kloepper et al., 1993; Thomashow and Weller, 1995), or by directly inhibiting the target pathogen. Examples of these mechanisms that inhibit the ability of plant pathogens to cause disease include the production of antagonistic compounds such as antibiotics, scavenging compounds such as siderophores, toxic metabolites such as ammonia and hydrogen cyanide, and parasitism of the pathogen (reviewed in Weller, 1988; Thomashow and Weller, 1995).

The effective use of biological control would reduce the need for and accumulation of environmentally damaging fungicides and pesticides. However, the consistency and efficiency of microbial biological control must be improved before it becomes commercially feasible. In order to improve biological control, we must consider both the molecular mechanisms that mediate the biological control interaction and the influence of the environment into which the biological control agent is introduced. This includes the physical environment, the plant host, and the effect of the indigenous microbial community, including the pathogen.

Much work has been focused on defining the regulatory systems that control the expression of the competitive mechanisms responsible for pathogen suppression (reviewed in Thomashow and Weller, 1995). This has resulted in the identification of two component regulation involving LemA and GacA (Parkinson and Kofoid, 1992; Willis et al., 1994) and sigma factor mediated (RpoS) regulation of gene expression within biological control bacteria (Sarniguet et al., 1995). A recent and exciting discovery is that *N*-acyl-homoserine lactones (*N*-acyl-HSL's)-mediate gene regulation in several biological control bacteria. This minireview will focus on: 1. the current status of *N*-acyl-HSL-mediated gene regulation in biological control by fluorescent pseudomonads; 2. the interrelationship between *N*-acyl-HSL-mediated gene regulation and other global regulatory systems; and 3. the potential role of the biotic environment in the regulation of biological control mechanisms via *N*-acyl-HSL signals.

N-acyl-homoserine lactone-mediated gene regulation

Each *N*-acyl-HSL regulatory system identified consists of at least two conserved regulatory proteins, an *N*-acyl-HSL synthase and a transcriptional regulator that responds to sufficient levels of recognizable *N*-acyl-HSL. The paradigm for *N*-acyl-HSL-mediated gene regulation is the control of bioluminescence in the marine symbiont *Vibrio fischeri* (reviewed in Dunlap, 1996; Fuqua et al., 1996). Briefly, two adjacent divergent operons control bioluminescence in *V. fischeri*. One operon encodes the transcriptional activator LuxR. The second operon consists of the *N*-acyl-HSL synthase gene *luxI* and six structural genes (*luxCDABEG*). The genes *luxA* and *luxB* encode the subunits of the enzyme luciferase while *luxC*, *luxD* and *luxE* encode the *lux*-specific fatty acid reductase complex which synthesizes and recycles the aldehyde substrate for luciferase (Boylan et al., 1985, 1989). LuxI is responsible for the synthesis of the *N*-(3-oxohexanoyl)-L-HSL signal VAI-1, presumably produced from the cellular precursors S-adenosyl-methionine (AdoMet) and an acyl-Acyl Carrier Protein (acyl-ACP) (Eberhard et al., 1991; More et al., 1996). VAI-1 is freely diffusible through the cellular membrane. Therefore, under the unrestricted conditions found in seawater, VAI-1 does not accumulate intracellularly within the bacterium. Upon colonization of a nutrient-rich and physically confined light organ in a fish or squid, the local bacterial population increases and VAI-1 accumulates. After sufficient VAI-1 has accumulated intracellularly, it interacts with LuxR resulting in activation of the *lux* operon. Since *luxI* is the first gene in the *lux* operon, activation of this operon by LuxR and VAI-1 results in increased *luxI* expression in a positive feedback circuit that leads to an exponential increase in bioluminescence. *N*-acyl-HSL-mediated regulation has been termed 'autoinduction' or 'quorum-sensing' since the accumulation of sufficient signal for gene expression occurs *in vitro* only at relatively high population densities (Nealson et al., 1970; Fuqua et al., 1994).

The LuxR/LuxI system is the best-studied example of an expanding family of proteins that regulate the expression of genes involved in beneficial and pathogenic host-microbe interactions (Fuqua et al., 1996). Examples include: LasR/LasI and RhlR/RhlI in the opportunistic human pathogen *Pseudomonas areuginosa* PAO (Gambello et al., 1991; Passador et al., 1993) and in the bioremediation bacterium *P. aeruginosa* PG201 (Ochsner et al., 1994; Ochsner and Reiser, 1995); RhiR/RhiI in the nitrogen fixing symbiont *Rhizobium leguminosarum* bv. *viciae* (Cubo et al., 1992; Gray et al., 1996); TraR/TraI in the plant pathogen *Agrobacterium tumefaciens* (Piper et al., 1993; Hwang et al., 1994); PhzR/PhzI in the biological control bacterium *Pseudomonas aureofaciens* 30-84 (Pierson et al., 1994; Wood and Pierson, 1996); CarR/CarI and ExpR/ExpI in the plant pathogen *Erwinia caratovora* (McGowan et al., 1995; Pirhonen 1993); and EsaR/EsaI in the plant pathogen *Erwinia stewartii* (Beck von Bodman and Farrand, 1995).

N-acyl-HSL-mediated regulation in biological control by fluorescent pseudomonads

A number of fluorescent pseudomonads involved in biological control are known to produce *N*-acyl-HSL signals (Pierson et al., unpublished results). To date, only a few studies have demonstrated directly the involvement of these signals in the regulation of genes which are essential for pathogen suppression. However, the specific effect of these signals on the outcome of biological control needs to be further analyzed. The best characterized examples of *N*-acyl-HSL-mediated gene regulation in biological control are the regulatory systems which mediate phenazine antibiotic production and rhamnolipid production.

Phenazine gene expression

Pseudomonas aureofaciens 30-84 is an effective biological control agent for take-all disease of wheat caused by the ascomycete *Gaeumannomyces graminis* var. *tritici* (*Ggt*). *P. aureofaciens* 30-84 produces three phenazine antibiotics; phenazine-1-carboxylic acid (PCA), 2-hydroxy-phenazine-1-carboxylic acid (2-OH-PCA), and 2-hydroxy-phenazine (2-OH-PZ), which are responsible for ca. 90% of this strain's ability to inhibit *Ggt* (Pierson and Thomashow, 1992). Phenazines are broad-spectrum antibiotics that uncouple oxidative phosphorylation in target organisms and generate toxic intracellular oxygen species (Turner and Messenger, 1986).

Phenazine production is critical to the biological control ability of *P. aureofaciens* in soil. Mutants defective in phenazine production do not inhibit *Ggt in vitro* and fail to suppress take-all. In addition, phenazine production contributes to the rhizosphere competence of strain 30-84. Loss of phenazine production resulted in up to a 10^4-fold decrease in the survival of strain 30-84 in rhizosphere soil containing indigenous microorganisms (Mazzola et al., 1992).

Model for regulation of phenazines by N-acyl-HSL's

The genetics of phenazine biosynthesis have been well characterized in *P. aureofaciens*. Five contiguous open reading frames (*phzFABCD*) are required for phenazine biosynthesis (Pierson et al., 1995) and two additional regulatory genes are directly involved in *N*-acyl-HSL-mediated control of phenazine expression (Figure 1). The first of these regulatory genes, *phzR*, encodes a LuxR-like transcriptional activator of the five phenazine biosynthetic genes (Pierson et al., 1994). The second gene, *phzI*, encodes a *N*-acyl-HSL synthase similar to LuxI (Wood and Pierson, 1996). The structure of the signal produced by PhzI in strain 30-84 was recently determined to be hexanoyl-homoserine lactone (Wood et al., in press). When this signal reaches a specific concentration, it is believed to interact with PhzR. This interaction presumably allows PhzR to bind to a specific sequence within the *phz* promoter, commonly referred to as a *lux* box (Devine et al., 1989; Fuqua et al., 1994), resulting in the transcription of the *phz* biosynthetic genes.

PhzR/PhzI is part of a sensory transduction pathway

The PhzR/PhzI *N*-acyl-HSL system in *P. aureofaciens* 30-84 is regulated by a two component sensory transduction pathway (Pierson et al., 1996). Pleiotrophic mutants of strain 30-84 were identified which were defective in phenazine, hydrogen cyanide, protease, and the production of the endogenous *N*-acyl-HSL signal. These mutants are not complemented by *phzR*, *phzI* or the *phz* biosynthetic region. However, some of these mutants were complemented by *lemA* while others were complemented by *gacA*, members of a two component sensory transduction pathway previously identified in a number of pseudomonads (Parkinson and Kofoid, 1992). LemA (*le*sion *m*anifestation) is a membrane associated sensor-kinase first identified in the plant pathogen *P. syringae* pv. *syrnigae* B728a (Hrabak and Willis, 1992). GacA (*g*lobal regulation of *a*ntibiotic and *c*yanide) is a response regulator first identified in the biological control bacterium *P. fluorescens* CHA0 (LaVille et al., 1992). In strain 30-84, loss of either *lemA* or *gacA* results in a ca. 40-fold reduction in the level of transcription of *phzI* as measured by a *phzI*::*uidA* reporter fusion, suggesting that LemA/GacA controls *N*-acyl-HSL production and phenazine production at least partly via the transcriptional control of *phzI* (Figure 1) (Pierson et al., 1996). Recently, a linkage between GacA and the production of another *N*-acyl-HSL signal, *N*-butyryl-homoserine lactone (BHL), was demonstrated in *P. aeruginosa* PAO (Reimmann et al., 1997). In this system, inactivation of *gacA* resulted in the delayed and reduced production of BHL and a reduction in the transcription of the two *luxR* homologues *rhlR* and *lasR*, which are required for the expression of extracellular virulence factors by this strain. The introduction of additional copies of *gacA in trans* resulted in increased production of BHL, LasR and RhlR. Thus, a linkage between two component regulatory pathways and quorum-sensing may be a common phenomenon.

Other phenazine producers also make N-acyl-HSL's

All phenazine-producing pseudomonads tested synthesized *N*-acyl-HSL signals which may regulate phenazine gene expression, including: *P. fluorescens* strain 2-79, *P. aureofaciens* type strain ATCC13985, *P. chlororaphis* type strain ATCC9446, *P. phenazinium* type strain ATCC33666, and the pyocyanin-producing *P. aeruginosa* strain ATCC9027 (Pierson and Wood, unpublished results).

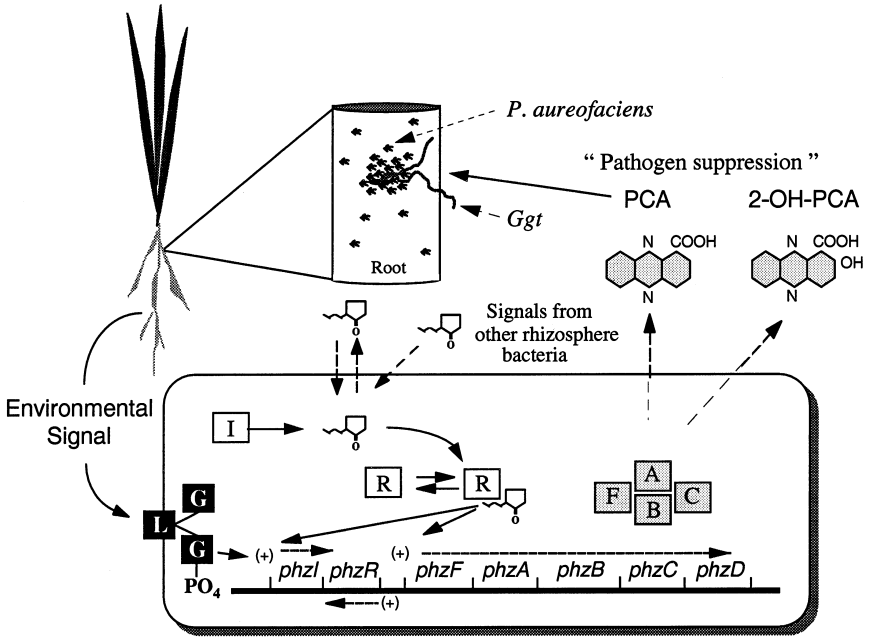

Figure 1. Model of phenazine gene regulation in *P. aureofaciens* 30-84. Specific features of the model are described in the text. PCA, and 2-OH-PCA indicate phenazine-1-carboxylic acid and 2-hydroxy-phenazine-1-carboxylic acid, respectively. The boxed L and G and the boxed R and I represent the LemA/GacA sensory transduction system and the PhzR/PhzI *N*-acyl-HSL system, respectively. The symbol (~⍰) represents *N*-acyl-HSL signals in general. *phzFABCD* is the phenazine biosynthetic operon. *Ggt* represents the take-all pathogen *Gaeumannomyces graminis* var. *tritici*.

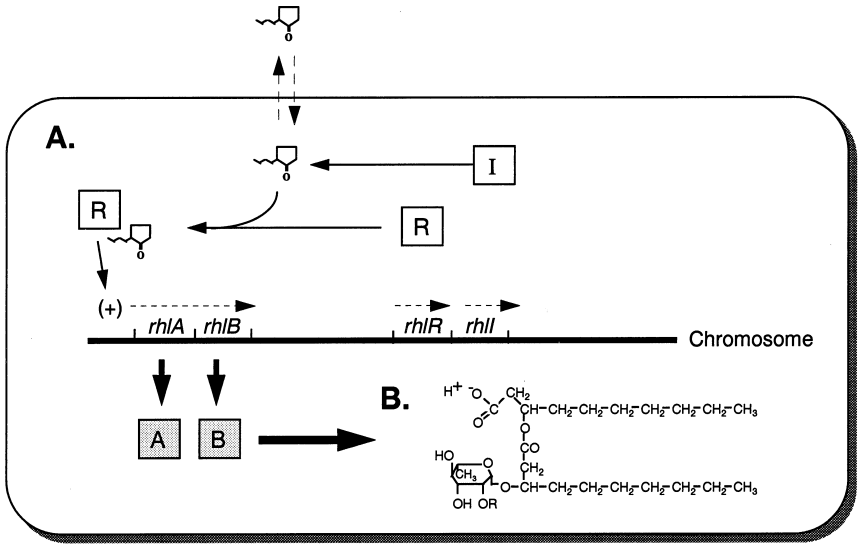

Figure 2. A. Model of the regulation of rhamnolipid production in *P. aeruginosa*. Details of the model are described in the text. Symbols are the same as in Figure 1 except the boxed R and I and the boxed A and B represent the RhlR/RhlI *N*-acyl-HSL system and the rhamnolipid biosynthetic enzymes RhlA and RhlB, respectively. B. Structure of rhamnosylβ3hydroxydecanoyl3-hydroxydecanoate (a monorhamnolipid) (figure adapted from Stanghellini and Miller, 1997).

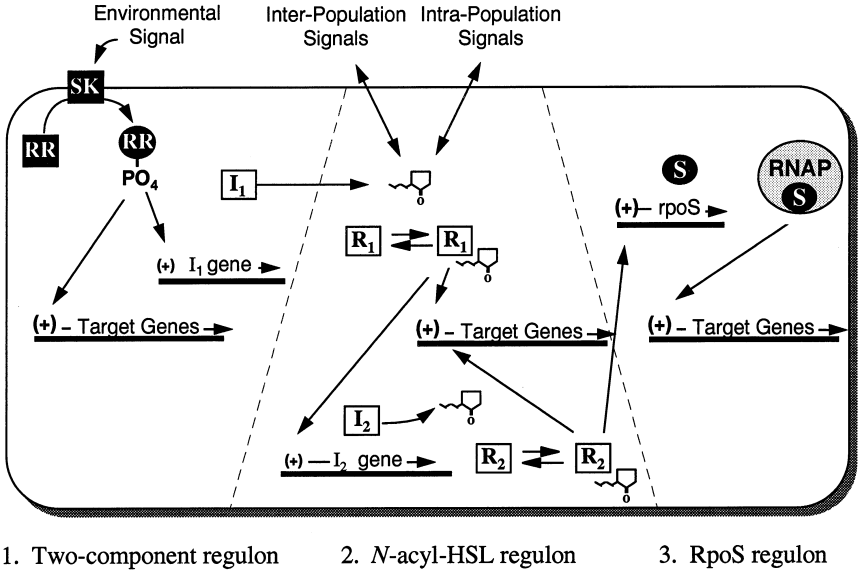

Figure 3. Summary of known interactions between *N*-acyl-HSL regulatory systems and other global regulatory systems. This figure represents a composite of what is currently known in several bacterial systems, especially *P. aeruginosa* strain PAO. The numbered headings under the figure refer to the subheadings under section IV in the text. SK and RR represent the sensor-kinase and the response-regulator components of two component regulatory systems, respectively. I_1 and I_2 represent different LuxI *N*-acyl-HSL synthases and R_1 and R_2 represent different LuxR transcriptional activators. The symbol (~○) represents *N*-acyl-HSL signals in general. The circled S represents the stationary phase sigma factor RpoS and RNAP represents the RNA polymerase core enzyme.

Rhamnolipid gene expression

Rhamnolipids are glycolipids belonging to a class of compounds known as biosurfactants produced by pseudomonads and other bacteria (Figure 2). Biosurfactants are amphipathic molecules that modify the properties of a liquid medium by reducing the surface tension (Stanghellini and Miller, 1997). Most rhamnolipids are anionic and are secreted from cells during late exponential and stationary phase growth (Rendell et al., 1990).

Synthetic biosurfactants have been shown to effectively control many zoosporic plant pathogens, including *Olpidium brassicae* (big vein of lettuce), *Pythium aphanidermatum* (root rot of cucumbers), and *Phytopthora capsici* (root rot of peppers) (Stanghellini and Rasmussen, 1994; Stanghellini et al., 1996). Although zoosporic plant pathogens are taxonomically diverse, they all utilize a unique unicellular motile zoospore for dispersal and infection which serves as the target for rhamnolipids. These zoospores have one or two flagella and are encased by a plasma membrane which is sensitive to disruption by biosurfactants. Recently, a strain of *P. aeruginosa* was identified in a hydroponic system that controls *Plasmopara lactucae-radicis* (root rot of lettuce) through the production of rhamnolipids (Stanghellini and Miller, 1997).

Regulation of rhamnolipid production

The genetics and regulation of rhamnolipid production have been best studied in *Pseudomonas aeruginosa* strains PG201 and PAO1 (Brint and Ohman 1995; Ocshner et al., 1994). For both strains, rhamnolipid production is controlled by an *N*-acyl-HSL-mediated regulatory system similar to that found in *P. aureofaciens* (Figure 2). Two biosynthetic genes, *rhlA* and *rhlB*, encode rhamnosyltransferase I, an enzyme that converts thymidine-diphospho-rhamnose and β-hydroxydecanol-β-hydroxydecanoate into L-rhamnosyl-β-hydroxydecanol-β-hydroxydecanoate, the first rhamnolipid produced by this strain. Two regulatory genes, *rhlR* and *rhlI*, which belong to the *LuxR*/*LuxI N*-acyl-HSL regulatory family, are located downstream of *rhlA* and *rhlB* in both strains and control transcription of these two biosynthetic genes (Ocshner et al., 1994; Ochsner and Reiser, 1995; Brint and Ohman, 1995). RhlI is also involved in the regulation of the phenazine pyocyanin produced by *P. aeruginosa* (Brint and Ohman, 1995).

RhlR/RhlI are components of a larger sensory transduction cascade

Just as in the case of phenazine gene regulation, the RhlR/RhlI system does not operate independently. Latifi et al. (1996) identified a hierarchical *N*-acyl-HSL cascade in *P. aeruginosa* PAO1 in which the RhlR/RhlI system is regulated by a second *N*-acyl-HSL system, LasR/LasI. In this bacterium, a *rhlR*::*lacZ* transcriptional fusion was not expressed in a *lasR* mutant, indicating that the LasR/LasI *N*-acyl-HSL system is required for the expression of the RhlR/RhlI *N*-acyl-HSL system. Even more interesting is their finding that the RhlR/RhlI system can regulate the expression of the stationary phase sigma factor RpoS. An uncharacterized pleiotrophic *rhlRI* mutant of *P. aeruginosa* (strain PANO67) fails to express a *rpoS*::*lacZ* transcriptional reporter, suggesting that this two tier *N*-acyl-HSL sensory transduction pathway regulates *rpoS* expression.

Discussion

N-*acyl-HSL's are components of larger sensory transduction pathways*

The data presented suggest that *N*-acyl-HSL regulatory systems generally do not function independently, but are essential components of integrated bacterial sensory transduction systems. Therefore, any discussion of *N*-acyl-HSL-mediated regulation must consider the role of these systems in the context of the overall sensory transduction systems of the cell.

N-*acyl-HSL's and two component regulatory systems*
In *P. aureofaciens*, the PhzR/PhzI system is active only when the LemA/GacA two component sensory system is functional (Pierson et al., 1996; Chancey et al., in prep). It appears that the LemA/GacA system acts as a master switch controlling other regulatory pathways. If LemA/GacA behave analogously to other two component systems, then the PhzR/PhzI system would only be active in the presence of the correct environmental signals (Parkinson and Kofoid, 1992). Interestingly, although the first *lemA* gene was reported in 1992 (Hrabak and Willis, 1992) and homologs have been found in many bacteria, no specific signal has yet been identified. The connection between a two component and an *N*-acyl-HSL-mediated regulatory system may allow *N*-acyl-HSL regulation to occur only under appropriate conditions. However, under the appropriate conditions, the *N*-acyl-HSL-response system may further allow the propagation of low level stimuli throughout a population (Piper et al., 1993).

Interactions between multiple N-*acyl-HSL systems*
In *P. aeruginosa*, the RhlR/RhlI system is functional only when the LasR/LasI system is active, indicating a hierarchy in these *N*-acyl-HSL-mediated regulatory systems (Latifi et al., 1996). This suggests that multiple *N*-acyl-HSL-mediated regulatory systems may be present in bacteria and that their function may be interdependent. Since each of these systems recognizes a structurally different *N*-acyl-HSL, and LuxR-type proteins have the potential to recognize a limited range of related signals (Fuqua et al., 1996), the presence of multiple response systems may serve to extend the range of recognizable *N*-acyl-HSL's. Whether such hierarchical signaling systems exist in most biological control bacteria and the role these systems play in pathogen suppression needs to be determined.

N-*acyl-HSL's and RpoS*
The work by Latifi et al. (1996) suggests that, in addition to the other factors previously shown to regulate *rpoS* expression and/or function (cAMP, growth rate, osmolarity, and starvation) (Loewen and Hennge-Aronis, 1994), *N*-acyl-HSL signals also play an integral role in *rpoS* expression in *P. aeruginosa*. RpoS is a sigma factor that regulates gene expression in response to nutrient starvation and high cell densities. These gene products are essential for stationary growth and long term survival of bacteria (Hengge-Aronis, 1993; Loewen and Hennge-Aronis, 1994; Huisman and Kolter, 1994). In the biological control bacterium *P. fluorescens* Pf-5, mutations in *rpoS* resulted in increased production of the antifungal metabolites pyoluteorin and 2,4-diacetylphloroglucinol while production of a third antifungal metabolite, pyrrolnitrin, was abolished (Sarniquet et al., 1995). These mutants also showed reduced survival on wheat straw over time. Thus, RpoS can determine the success of a biological control bacterium at two levels: survival and pathogen inhibition. The role of *N*-acyl-HSL signals in *rpoS* expression in other biological control bacteria remains to be determined.

Potential role of the biotic environment in mediating the regulation of biological control mechanisms via *N*-acyl-HSL signals

Most research on the role of *N*-acyl-HSL's has focused on its role in gene regulation in pure cultures under *in vitro* conditions. However, recent work [reviewed in Fuqua et al., 1996; Pierson and Pierson, 1996] suggests that the biotic environment may play an important role in influencing *N*-acyl-HSL-mediated gene regulation. The biotic environment includes signals produced by the bacterial population itself (intrapopulation signaling), those produced by other microorganisms that share similar ecological niches (interpopulation signaling), and any signals produced by the host. The net result of these interactions may drastically affect the ability of an introduced biological control bacterium to persist and to suppress the pathogen.

Intrapopulation signaling in the rhizosphere

In *Vibrio* spp., effective *N*-acyl-HSL-mediated gene regulation requires a specific host organelle. The host organelle: a) provides a physical barrier to the diffusion of the *N*-acyl-HSL signal and b) supplies sufficient nutrients to support large populations, leading to high concentrations of the diffusible *N*-acyl-HSL signal. It appears that the rhizosphere may also satisfy these same two criteria. It is generally accepted that roots provide sufficient nutrients in the form of root exudates for microbial growth (Curl and Truelove, 1986). Bacteria grow on roots as microcolonies located primarily within root cell junctions (Rovira, 1956; Bowen and Rovira, 1976), which may allow concentration of the signal molecule. These microcolonies are often embedded within the mucigel of the root. Chin-A-Woeng et al. (1997) showed the presence of bacterial microcolonies located underneath a mucilaginous layer, presumably of plant origin. This layer could provide a physical containment to facilitate *N*-acyl-HSL accumulation, although the permeability of the layer to *N*-acyl-HSL's has not been tested.

Although *N*-acyl-HSL signals have been hypothesized to effect cell-to-cell communication within microbial populations (Dunlap, 1996; Fuqua et al., 1994; Swift et al., 1994), evidence that these signals function *in situ* has been lacking. Recent work has shown that *N*-acyl-HSL-mediated crosstalk between isogenic bacterial populations occurs in the wheat rhizosphere (Pierson et al., 1996; Wood et al., in press). In these studies, a PhzI$^-$ phenazine reporter strain was restored for phenazine gene expression *in situ* on axenic wheat roots by the introduction of a second isogenic PhzI$^+$ strain. These results demonstrated that: 1) *N*-acyl-HSL production is required for *phz* expression on roots and; 2) *N*-acyl-HSLs can serve as regulatory signals in the rhizosphere. Thus, *N*-acyl-HSL signals are required for phenazine production which is essential for the ability of *P. aureofaciens* to persist and suppress the take-all pathogen in the rhizosphere.

Interpopulation signaling- a new twist on quorum sensing

In the rhizosphere, bacteria may recognize not only their own signals but also signals produced by other organisms. Theoretically, even a single bacterial cell could activate gene expression if a sufficient concentration of recognizable *N*-acyl-HSL was available from other organisms. These exogenous signals could influence the outcome of competition between microbial populations by modifying the temporal pattern of competitive gene expression. Indeed, the indigenous microbial community may influence the success of an introduced biological control agent by contributing toward the 'total amount' of recognizable signal, or by acting as sinks, reducing the level of signal either by utilizing the signal(s) themselves or by degrading it. In addition, it is possible that exogenous signals could inhibit the action of the endogenous signal by competing for binding to the transcriptional activator (Eberhard et al., 1986). Therefore, competition among microorganisms for *N*-acyl-HSL signals and the potential for these signals to affect gene expression in unexpected ways may play an important role in determining the success of a biological control bacterium.

The potential for interspecies signaling was first suggested by Greenberg et al. (1979), who called it 'alloinduction.' McKenney et al. (1995) suggested that interspecies communication occurs between the opportunistic human pathogens *P. aeruginosa* and *Burkholderia cepacia in vitro*, however, no evidence for the involvement of *N*-acyl-HSL's was presented. More recently, an *Agrobacterium tumefaciens N*-acyl-HSL-dependent reporter strain was used to detect *N*-acyl-HSL's produced by other plant pathogenic soil bacteria *in vitro* (Fuqua et al., 1996). We found that many wheat rhizosphere-colonizing bacteria produce diffusible signals that complement a *phzI* mutant of *P. aureofaciens* 30–84 *in vitro* (Pierson and Pierson, 1996). These data suggest that other microorganisms represent potential sources of additional *N*-acyl-HSL

signals. In addition, since it has been suggested that 90–99% of the total microflora on and around plant roots can not be cultured *in vitro* and therefore can not be tested, the potential for interpopulation signaling in the rhizosphere may be even greater.

Summary

An introduced biological control agent must contend with a myriad of environmental inputs. Many regulatory mechanisms have evolved to allow bacteria to detect and rapidly respond to key signals, including two component, sigma factor, and *N*-acyl-HSL-mediated gene regulation. Together, these regulatory systems allow bacterial cells to respond to a variety of signals, including environmental and population-based signals. Future research needs to focus not only on *N*-acyl-HSL regulatory systems, but on how these regulatory systems are integrated into overall sensory transduction pathways. This approach will result in an enhanced understanding of how a biological control agent interacts with its environment and will answer key questions regarding the timing and level of expression of the mechanisms responsible for successful biological control. This information may allow the success of an introduced biological control bacterium to be predicted and may also suggest ways in which environmental conditions could be manipulated to increase disease control.

Acknowledgements

The authors would like to thank Dr. Martha Hawes, Dr. Christina Kennedy, Dr. Ben Lugtenberg and Dr. Paul Williams for valuable discussions. Some of the work described was supported by grant No. 9514074 from the National Science Foundation.

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