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REVIEW PAPER

Dialogues of root-colonizing biocontrol pseudomonads

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Abstract Among biocontrol agents that are able to suppress root diseases caused by fungal pathogens, root-colonizing fluorescent pseudomonads have received particular attention because many strains of these bacteria trigger systemic resistance in host plants and produce antifungal compounds and exoenzymes. In general, the expression of these plant-beneficial traits is regulated by autoinduction mechanisms and may occur on roots when the pseudomonads form microcolonies. Three major classes of antibiotic compounds reviewed here in detail (2,4-diacetylphloroglucinol, pyoluteorin and various phenazine compounds) are all produced under cell population density-dependent autoinduction control acting at transcriptional and post-transcriptional levels. This regulation can either be reinforced or attenuated by a variety of chemical signals emanating from the pseudomonads themselves, other microorganisms or root exudates. Signals stimulating biocontrol factor expression via the Gac/Rsm signal transduction pathway in the biocontrol strain *Pseudomonas fluorescens* CHA0 are synthesized by many different plant-associated bacteria, warranting a more detailed investigation in the future.

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Abbreviations

AHL	*N*-acyl-homoserine lactone
AI-2	Autoinducer 2
DAPG	2,4-Diacetylphloroglucinol
HCN	Hydrogen cyanide
IAA	Indole-3-acetic acid
PCA	Phenazine-1-carboxylic acid
PLT	Pyoluteorin
PQS	*Pseudomonas* quinolone signal
QS	Quorum sensing

Introduction

Probably all free-living bacteria communicate with one another by synthesizing, secreting and sensing signal molecules, which diffuse in the environment and are detected by specific cellular receptors. The main tasks of these signals are to coordinate metabolic activities and developmental processes in bacterial populations. In general, the higher the cell population densities are in growing cells, the more important signalling becomes. This relationship is known as quorum sensing (QS) and it is assumed that signalling enables the producer bacteria to assess their population densities (Fuqua et al. 1994). To some extent, this is certainly an important aspect. In

many cases, signals amplify their own biosynthesis. Thus, in growing populations, this positive feedback loop results in autoinduction and for this reason, such QS signals are also termed autoinducers (Nealson 1977). However, the physiological condition of the cells and many environmental factors can modulate the concentration of the QS signals and hence the cellular response (Lazdunski et al. 2004; Hense et al. 2007).

In the rhizosphere, bacterial populations preferentially form microcolonies in areas that are particularly rich in root exudates (Bais et al. 2006). Here, the production of QS signals can readily be detected by signal-specific biosensors (Pierson et al. 1998; Steidle et al. 2001). The calling distance, i.e. the range of cell-cell communication via chemical signals, has been experimentally determined on plant roots and estimated to be of the order of 5 μm, but under favourable conditions, e.g. at root tips or root hair junctions, it can extend beyond 50 μm (Gantner et al. 2006). In many rhizobacteria, QS mechanisms induce the synthesis of antimicrobial secondary metabolites and extracellular lytic enzymes that are inhibitory to other bacteria, fungi, protozoa, and nematodes (Haas et al. 2000; Haas and Défago 2005; Siddiqui et al. 2005; Juhas et al. 2005; Jousset et al. 2006). We have argued elsewhere that a primary function of these antimicrobial factors is to protect the producer microcolonies from invasion by other inhabitants of the rhizosphere and hence to preserve the integrity of the producer in its ecological niche on plant roots (Haas and Keel 2003). The measured calling distances of QS signals correspond well to microcolony dimensions. When antibiotic-producing rhizobacteria antagonize the proliferation of root-pathogenic fungi on roots and when, in addition, the defence reactions of the host plant are stimulated, a significant biocontrol effect can result: the plant becomes less susceptible to disease caused by pathogenic fungi. In this review, we will discuss some signal transduction pathways that positively control the expression of biocontrol factors in root-colonizing soil bacteria belonging to the group of fluorescent pseudomonads. These bacteria have been studied in considerable detail for their biocontrol properties and their signalling pathways (Moënne-Loccoz and Défago 2004; Juhas et al. 2005; Haas and Défago 2005).

Signals and nutrients

Signals that are engaged in cell-cell communication of microorganisms meet several criteria: they are produced during a particular growth phase and in response to environmental conditions, they accumulate in the extracellular medium, they interact specifically with cellular receptors and, above a threshold, they trigger expression of a set of genes. However, all these criteria can also be fulfilled by nutrients which are degraded by microorganisms through inducible pathways. For instance, glucose, a sugar present in the rhizosphere (Lugtenberg and Bloemberg 2004), is converted to gluconate by glucose oxidase in fluorescent pseudomonads (Quay et al. 1972). The diffusible product gluconate, which acts as an antifungal agent (Kaur et al. 2006), induces the enzymes of the Entner-Doudoroff pathway leading to the degradation of gluconate and glucose (Quay et al. 1972). Should gluconate be considered a signal? Some authors indeed do not discriminate between rhizosphere signals that primarily serve as nutrients and those that specifically trigger gene expression without having nutritional value (Somers et al. 2004). Other authors emphasize that signals used in cell-cell communication typically regulate gene expression beyond the physiological changes required to metabolize or to inactivate the signals and that, therefore, signals should be distinguished from nutrients (Winzer et al. 2002). In the following, we will adopt the latter view and we will not discuss how nutrients present in exudates chemotactically attract soil bacteria and how these bacteria subsequently attach to root surfaces and form microcolonies. These processes have been reviewed elsewhere (Somers et al. 2004; Lugtenberg and Bloemberg 2004).

Signals that influence the expression of biocontrol traits in pseudomonads can emanate from the biocontrol bacteria themselves, from other soil bacteria or fungi, or from host plants (Fig. 1). Like any biological molecules, signals can be degraded, either by the producing organisms, which thereby avoid an overshooting response (Huang et al. 2006a; Wang et al. 2006), or by other organisms which cause interference of signalling pathways (Zhang and Dong 2004; Xavier and Bassler 2005; González and Keshavan 2006). Signals can have stimulating or inhibitory effects, with repercussion on transcriptional or translational control of biocontrol gene expression.

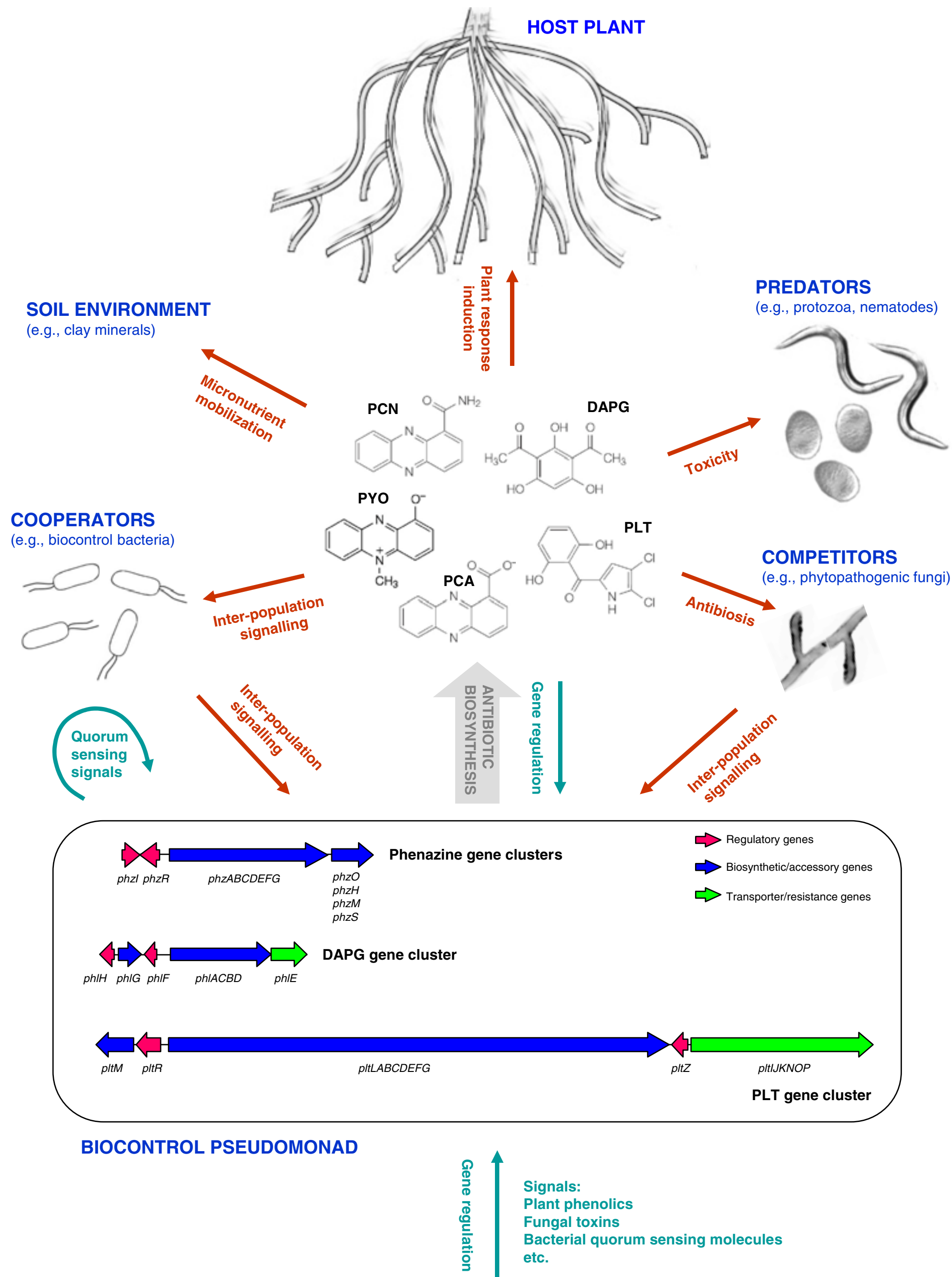

Fig. 1 Overview of interactions between biocontrol strains, plants, pathogens, predators, co-operators, and soil. The antibiotics (phenazines, DAPG, PLT) produced by the biocontrol strains play a central role between all the elements of the complex network of interactions. The organization of the *phz*, *phl* and *plt* biosynthetic genes in fluorescent pseudomonads is according to Mavrodi et al. (2006); Bangera and Thomashow (1999); Schnider-Keel et al. (2000); and Brodhagen et al. (2005). Red arrows designate interactions, green arrows designate transcriptional gene regulation

Signals affecting biocontrol factor expression at a transcriptional level

Control of root diseases by biocontrol pseudomonads involves a blend of complementary mechanisms, the most prominent being antibiosis towards plant pathogens, degradation of virulence factors produced by pathogens and induction of defence mechanisms in host plants. Efficient competition for colonization sites and micro- and macro-nutrients in the rhizosphere is an important prerequisite for effective biocontrol (van Loon et al. 1998; Lugtenberg et al. 2001; Compant et al. 2005; Haas and Défago 2005). Root-colonizing plant-beneficial pseudomonads release a remarkable diversity of metabolites with antibiotic activity. There is compelling experimental evidence from genetic analyses involving antibiotic-negative mutants and from detection of antibiotic production in the rhizosphere by analytical and reporter gene techniques that these compounds are important for biocontrol activity (Raaijmakers et al. 2002; Haas and Keel 2003; Haas and Défago 2005). Furthermore, it has been established that populations of antibiotic-producing, rhizosphere-associated pseudomonads are key biological components in natural soils that are suppressive to major root diseases such as take-all of wheat and black root of tobacco (Keel et al. 1996; Weller et al. 2002; Ramette et al. 2003; de Souza et al. 2003a; Weller et al. 2007).

Among the antimicrobial compounds released by plant-beneficial pseudomonads, 2,4-diacetylphloroglucinol (DAPG), pyoluteorin (PLT), phenazines, hydrogen cyanide (HCN), and cyclic lipopeptides (e.g., viscosinamide, amphisin, putisolvin) have received particular attention for their major contribution to biocontrol of root diseases that are caused by agronomically important fungal and oomycete pathogens including *Gaeumannomyces*, *Thielaviopsis*, *Fusarium*, and *Pythium* species (Haas and Keel 2003; Chin-A-Woeng et al. 2003; Andersen et al. 2003; Kuiper et al. 2004; Mavrodi et al. 2006; Raaijmakers et al. 2006; Rezzonico et al. 2007; Weller et al. 2007). Most effective biocontrol pseudomonads produce at least one of the above-mentioned diffusible or volatile antibiotics and some strains, e.g. *Pseudomonas fluorescens* strains CHA0 and Pf-5, produce multiple antibiotics. In general, these antibiotics have broad-spectrum toxic activity against fungi, bacteria, protozoa, nematodes, and sometimes also against plants or even viruses (Raaijmakers et al. 2002, 2006; Haas and Keel 2003; Jousset et al. 2006). However, for DAPG and PLT the precise modes of action are largely unknown. Phenazines being redox-active antibiotics are thought to harm phytopathogenic fungi by generation of reactive oxygen species (Chin-A-Woeng et al. 2003; Mavrodi et al. 2006; Price-Whelan et al. 2006). HCN is an inhibitor of metalloenzymes, in particular of most terminal oxidases (Blumer and Haas 2000). Cyclic lipopeptides are surfactants promoting surface motility of the producer organism; at high concentrations, they are detrimental to the integrity of phospholipid membranes in various organisms (Raaijmakers et al. 2006).

Some of the antibiotic metabolites have remarkably diverse functions, besides their toxic activity (Fig. 1). DAPG, PLT, and phenazines can function as signal molecules that affect gene expression not only in the producer bacteria, but also in other organisms (Schnider-Keel et al. 2000; Maurhofer et al. 2004; Brodhagen et al. 2004; Baehler et al. 2005; Dietrich et al. 2006; Price-Whelan et al. 2006). DAPG has been described as an inducer of systemic plant resistance (Iavicoli et al. 2003; Weller et al. 2007) and as a stimulant of amino acid exudation from roots (Phillips et al. 2004). Phenazines, in their reduced form, might enable the producing bacteria to mobilize micronutrients such as iron (Fe^{3+}) from the rhizosphere environment (Hernandez et al. 2004; Price-Whelan et al. 2006). In the following, we will highlight some examples of signal perception and exchange involving transcriptional control mechanisms, with a focus on the biosyntheses of DAPG, PLT, and phenazines (Fig. 1).

DAPG and PLT as signals

DAPG has been shown to be particularly important in the suppression of root diseases caused by *Thielaviopis basicola* (Keel et al. 1990), *Pythium ultimum* (Fenton et al. 1992; Baehler et al. 2006) and *Gaeumannomyces graminis* (Keel et al. 1992). The DAPG biosynthetic locus has been identified and analysed in some detail in *P. fluorescens* strains Q2-87, CHA0, and F113 (Bangera and Thomashow 1999; Schnider-Keel et al. 2000; Delany et al. 2000). The DAPG locus comprises the biosynthetic genes *phlACBD*; *phlD* encodes a type III polyketide

synthase catalysing the synthesis of phloroglucinol from malonyl-CoA and *phlACB* encodes enzymes that presumably are required for the subsequent acetylation reactions to mono-acetylphloroglucinol and DAPG (Achkar et al. 2005; Zha et al. 2006). The *phlE* gene located downstream of the *phlACBD* cluster codes for a putative transport/resistance protein (Bangera and Thomashow 1999; Abbas et al. 2004). The *phlF, phlG*, and *phlH* genes located upstream of the biosynthetic genes encode, respectively, a pathway-specific transcriptional repressor belonging to the TetR family, a hydrolase that specifically degrades DAPG to less toxic mono-acetylphloroglucinol, and a second TetR-type transcriptional regulator that activates the expression of the *phlACBD* genes and negatively controls *phlG* (Bangera and Thomashow 1999; Schnider-Keel et al. 2000; Delany et al. 2000; Abbas et al. 2004; Bottiglieri and Keel 2006). Pathway-specific control in strains CHA0 and F113 is brought about by the PhlF protein which represses the expression of the *phlACBD* operon by binding to two conserved operator sites in the *phlA* leader region (Schnider-Keel et al. 2000; Delany et al. 2000; Haas and Keel 2003; Abbas et al. 2004; D. Haas and C. Gigot-Bonnefoy, unpublished results). DAPG itself acts as the derepressing signal by dissociating the repressor PhlF from the *phlA* promoter, thereby acting as an autoinducer of its own biosynthesis (Schnider-Keel et al. 2000; Abbas et al. 2004). PhlH, the second pathway-associated transcriptional regulator, is postulated to antagonize the repressive effect of PhlF, but the precise mechanism remains to be determined (Haas and Keel 2003).

Pyoluteorin is another important antimicrobial metabolite that is produced by some *Pseudomonas* strains and that contributes in particular to the suppression of root diseases caused by the oomycete *P. ultimum* (Howell and Stipanovic 1980; Maurhofer et al. 1992, 1994). The PLT biosynthetic locus has been identified in *P. fluorescens* Pf-5 (Kraus and Loper 1995; Nowak-Thompson et al. 1999), in *P. fluorescens* CHA0 (Péchy-Tarr et al. 2005) and in *Pseudomonas* sp. M18, which appears to belong to *P. aeruginosa* (Ge et al. 2004). The biosynthetic locus comprises the *pltLABCDEFG* and *pltM* genes; *pltB* and *pltC* encode polyketide synthases with core functions in PLT biosynthesis (Nowak-Thompson et al. 1999). Pathway-specific positive control of PLT biosynthetic gene expression in strains CHA0 and Pf-5 is brought about by a LysR-type transcriptional regulator encoded by the divergently transcribed *pltR* gene (Nowak-Thompson et al. 1999; M. Bottiglieri and C. Keel, unpublished data). PLT induces its own biosynthesis via an autoinduction mechanism similar to that described for DAPG (Brodhagen et al. 2004; Baehler et al. 2005) and there is some experimental evidence that the transcriptional activator PltR may mediate this mechanism (M. Bottiglieri and C. Keel, unpublished data). Recently, a second regulatory gene, *pltZ*, linked to the PLT biosynthetic locus has been identified in strain M18 (Huang et al. 2004) and *P. fluorescens* strain Pf-5 (Brodhagen et al. 2005). This gene encodes a TetR-type transcriptional repressor of PLT gene expression (Huang et al. 2004) and is located upstream of a genomic region (*pltHIJKNO* in strain M18 and *pltIJKNOP* in strain Pf-5) encoding an ABC-type transport apparatus for PLT and accessory membrane-bound proteins (Brodhagen et al. 2005; Huang et al. 2006b). Exogenous PLT induces the expression of both the PLT biosynthetic and transport operons, suggesting that PLT production and export are coordinated (Brodhagen et al. 2005; Huang et al. 2006a, b).

In *P. fluorescens* strains CHA0 and Pf-5, a mechanism of molecular cross-talk between the DAPG and PLT biosynthetic pathways has been identified that enables the bacteria to maintain production of these key antibiotics at balanced levels. In this molecular balance, DAPG and PLT act as signalling molecules inducing the expression of their own biosynthetic genes while strongly repressing the expression of the biosynthetic genes of the other antibiotic (Schnider-Keel et al. 2000; Brodhagen et al. 2004; Baehler et al. 2005, 2006). The pathway-specific transcriptional regulators PhlF and PltR appear to function as receptors of these signals. However, the underlying molecular mechanisms still need to be substantiated. The housekeeping sigma factor RpoD, the alternative sigma factors RpoS, RpoN and RpoE (Schnider et al. 1995; Sarniguet et al. 1995; Johansen et al. 2002; Haas and Keel 2003; Péchy-Tarr et al. 2005), Lon protease (Whistler et al. 2000), and the H-NS-like proteins MvaT and MvaV (Baehler et al. 2006) influence the DAPG/PLT balance. In the rhizosphere environment, these global transcriptional regulators might allow the bacteria to coordinate antibiotic production with other cellular

functions, such as nutrition acquisition and response to environmental stress.

As exogenous signals, DAPG and PLT mediate both intra- and interpopulation communication. In the genetically distinct biocontrol strains CHA0 and Q2-87, DAPG produced by either strain on wheat roots is perceived as a positive signal boosting in situ expression of *phl* genes in the other strain (Maurhofer et al. 2004). Similarly, PLT produced by *P. fluorescens* Pf-5 acts as a signal activating *plt* gene expression in a PLT-negative derivative of Pf-5 in the rhizosphere of cucumber (Brodhagen et al. 2004). Salicylate, a metabolite produced by strain CHA0, numerous other pseudomonads and plants, strongly represses the production of DAPG and PLT and the expression levels of the corresponding biosynthetic genes (Schnider-Keel et al. 2000; Baehler et al. 2005). It is noteworthy that QS molecules of the *N*-acyl-homoserine lactone (AHL) type, which are synthesized by some DAPG producers such as *P. fluorescens* F113 (Laue et al. 2000) and 2P24 (Wei and Zhang 2006), do not contribute to the regulation of antibiotic biosynthesis in these bacteria, although AHLs function as key signals in cell-density dependent regulation of phenazine antibiotic production in other biocontrol pseudomonads (see below).

Whereas pathogenic fungi are major targets of biocontrol pseudomonads, only few examples of specific signalling interactions between these organisms have been documented. Fusaric acid, a toxin and pathogenicity factor of the root pathogen *Fusarium oxysporum*, is probably the most prominent example of a fungal compound that interferes with bacterial expression of a biocontrol trait. Fusaric acid strongly represses DAPG production in *P. fluorescens* CHA0 (Duffy and Défago 1997; Schnider-Keel et al. 2000; Notz et al. 2002). This repression may ultimately result in failure of the bacterium to suppress the root disease caused by the fungus (Duffy and Défago 1997; Duffy et al. 2004). An example of positive signalling is the stimulation of DAPG gene expression in strain CHA0 by unidentified signals from culture filtrates and volatiles of the biocontrol fungus *Trichoderma atroviride* (Lutz et al. 2004). Several fungal pathogens including *Gaeumannomyces*, *Pythium*, and *Fusarium* cope with DAPG by detoxifying it or by developing other mechanisms of resistance to it (Mazzola et al. 1995; de Souza et al. 2003b; Duffy et al. 2003; Schouten et al. 2004).

Little is known about plant signals affecting the expression of DAPG and PLT biosynthetic genes in *P. fluorescens*. Clearly, the plant species has an important role in determining the extent of disease suppression provided by *P. fluorescens* strain CHA0, suggesting that signals present in root exudates may modulate the production of biocontrol factors (Maurhofer et al. 1994, 1995; Schmidli-Sacherer et al. 1997). For instance, *phl* gene expression in strain CHA0 is consistently enhanced in the rhizosphere of cucumber, wheat and maize when *Pythium* infests the roots (Notz et al. 2001). Since neither the fungus itself nor its culture filtrates stimulate DAPG expression in vitro, increased *phl* gene expression is presumably due to alterations in root exudates caused by pathogen attack (Notz et al. 2001; Maurhofer et al. 2002). Furthermore, the *phl* genes are more strongly expressed on wheat and maize roots than on cucumber and bean roots, pointing to differences in root exudate composition between these monocotyledonous and dicotyledonous plants (Notz et al. 2001). However, the signals involved are largely unknown. Possible candidates are phenolic metabolites that are released by the plants as root exudates or as cell wall degradation products. Phenolic compounds have a number of ecological functions, e.g. as growth inhibitors of other plant species, as antimicrobial agents, or as specific signals guiding interactions between plants and rhizobia or *Agrobacterium* spp. (Siqueira et al. 1991; Phillips and Kapulnik 1995; Hirsch et al. 2004; Bais et al. 2006). Among several compounds of plant origin, indole-3-acetic acid (IAA) stimulates *phl* gene expression, resorcinol represses *plt* gene expression and salicylate downregulates the expression of both *phl* and *plt* genes in strain CHA0 (Schnider-Keel et al. 2000; Baehler et al. 2005; de Werra et al. 2006).

Phenazines and AHLs as signals

Phenazine compounds are coloured heterocycles derived from chorismate. They are synthesized by Gram-negative and -positive bacteria. Phenazine-1-carboxylic acid (PCA) is the primary product of a set of enzymes encoded by the *phzABCDEFG* operon in *P. fluorescens* 2-79, *P. chlororaphis* PCL1391, *P. aeruginosa* PAO and *P. aureofaciens* 30-84 (although in the last organism a different nomenclature is used for the *phz* operon) (Pierson et al. 1995; Mavrodi et al. 1998, 2006; McDonald et al. 2001).

Modification reactions lead from PCA to phenazine-1-carboxamide (with *phzH*), 2-hydroxyphenazine-1-carboxylic acid (with *phzO*), 1-hydroxyphenazine (with *phzS*) or pyocyanine (=1-hydroxy-5-methyl-phenazinium betaine, with *phzM* and *phzS*) (Chin-A-Woeng et al. 2001a; Delaney et al. 2001; Mavrodi et al. 2001; Parsons et al. 2007). The first demonstration of PCA being a biocontrol determinant came from a study on the suppression of take-all by *P. fluorescens* 2-79 (Thomashow and Weller 1988); take-all is an important disease of wheat and barley caused by *G. graminis* var. *tritici* (Ggt). Both phenazine-1-carboxamide and 2-hydroxyphenazine-1-carboxylic acid, which have stronger antifungal activity against *Fusarium* spp. and Ggt than has PCA, are particularly important for biocontrol activity of *P. chlororaphis* PCL1391 and *P. aureofaciens* 30-84, respectively (Chin-A-Woeng et al. 2001a; Delaney et al. 2001). A combination of PCA and DAPG produced by a genetically engineered derivative of *P. fluorescens* Q8r1-96 has proved effective against Rhizoctonia root rot of wheat (Huang et al. 2003). We note in passing that certain strains of *P. aeruginosa*, while opportunistic animal and human pathogens, are well adapted to the rhizosphere (Berg et al. 2005) where they can display biocontrol properties (Troxler et al. 1997; Ge et al. 2004) and stimulate induced systemic resistance (Audenaert et al. 2002). In the root-colonizing biocontrol strain *P. aeruginosa* 7NSK2, a combination of pyocyanine and the iron chelator pyochelin induces systemic resistance against the leaf pathogens *Botrytis cinerea* on tomato and *Magnaporte grisea* on rice (Audenaert et al. 2002; de Vleesschauwer et al. 2006).

At the transcriptional level, phenazine biosynthesis is controlled by the PhzR-PhzI quorum sensing system. PhzR is a transcriptional activator of the *phz* operons in *P. fluorescens* 2-79, *P. chlororaphis* PCL1391 and *P. aureofaciens* 30-84 and PhzI is an autoinducer synthase producing mostly *N*-(3-hydroxy-hexanoyl)-homoserine lactone (in strain 2-79) and *N*-hexanoyl-homoserine lactone (in the other two strains). These AHLs activate PhzR (Wood and Pierson 1996; Chancey et al. 1999; Chin-A-Woeng et al. 2001b; Khan et al. 2005). Additional regulators of phenazine production have been identified, e.g. RpeA, a repressor of phenazine production in *P. aureofaciens* 30-84 (Whistler and Pierson 2003), and Pip, which activates phenazine-1-carboxamide production in *P. chlororaphis* PCL1391, together with the stress and stationary phase sigma factor RpoS (Girard et al. 2006). In *P. aeruginosa*, QS regulation of secondary metabolism including phenazine biosynthesis is highly complex, involving a hierarchically organized system with two autoinducers, i.e. *N*-(3-oxododecanoyl)-homoserine lactone (the reaction product of the LasI enzyme and principal activator of the LasR transcription factor) and *N*-butanoyl-homoserine lactone (the reaction product of the RhlI enzyme and principal activator of the RhlR transcription factor), and the *Pseudomonas* quinolone signal (PQS) (Lazdunski et al. 2004; Juhas et al. 2005; Price-Whelan et al. 2006). Despite differences in regulatory elements, the pseudomonads described here all share the principle of QS-dependent control of phenazine biosynthesis (Mavrodi et al. 2006). Phenazines themselves may act as late QS signals in the sense that they regulate several dozens of genes that are not directly related to phenazine biosynthesis, during late growth phases (Dietrich et al. 2006).

AHLs regulate phenazine gene expression on roots (Wood et al. 1997) and can serve as interpopulation signals in the wheat rhizosphere (Pierson et al. 1998). AHL 'mimics' extracted from *Medicago truncatula* positively influence the expression of QS reporter constructs (Gao et al. 2003), but it is not known whether these compounds have an impact on biocontrol strains in the rhizosphere. By contrast, interference with AHL signalling has been observed in biocontrol bacteria: the phenazine-producer *P. chlororaphis* PCL1391 lost its ability to protect tomato against Fusarium wilt in the presence of AHL-degrading rhizobacteria (Molina et al. 2003) and unidentified signal molecules from a subpopulation of wheat rhizosphere-associated bacteria were found to affect phenazine gene expression negatively in *P. aureofaciens* 30-84 (Morello et al. 2004). Other interference may come from soil fungi which are a potential reservoir of QS 'quenchers'. For instance, penicillic acid and patulin are secondary metabolites of *Penicillium* spp. that inhibit the expression of QS reporter constructs (Rasmussen et al. 2005). Fusaric acid, a fungal metabolite, represses phenazine-1-carboxamide biosynthesis and *N*-hexanoyl-homoserine lactone production in *P. chlororaphis* PCL1391 (van Rij et al. 2005). Interestingly, salicylate, a plant defence signal, down-regulates pyocyanine formation by *P. aeruginosa* on plant roots (Prithiviraj et al. 2005).

Signals affecting biocontrol factor expression at a post-transcriptional level

Small RNAs serve multiple regulatory purposes in bacteria, mostly at the level of mRNA stability and translation initiation. In many saprophytic bacteria, QS-dependent gene regulation can involve several small RNAs at critical checkpoints. *Vibrio cholerae* provides an excellent example: in this human pathogen the expression of the central virulence regulator HapR is determined by three converging QS pathways. All together, seven small RNAs are involved in these signal transduction pathways (Lenz et al. 2005). In one branch, the VarS/VarA two-component system positively controls the expression of three small RNAs termed CsrB, CsrC and CsrD. VarS is a sensor protein located in the cytoplasmic membrane and VarA is the cognate response regulator. Interaction of VarS with an external signal is assumed to result in autophosphorylation of VarS and in subsequent phosphotransfer to VarA. The three VarA-dependent small RNAs have a strong affinity for the small dimeric RNA-binding protein CsrA (Lenz et al. 2005), a translational regulator whose structure and function is conserved in a wide range of Gram-negative bacteria (Babitzke and Romeo 2007). Thus, at high cell densities, the small RNAs CsrB-CsrC-CsrD sequester CsrA and this ultimately favours the expression of HapR, resulting in repression of virulence and biofilm genes (Bejerano-Sagie and Xavier 2007).

In fluorescent pseudomonads, as in other Gram-negative bacteria, the same (VarS/VarA-like) QS pathway is conserved although the output can vary. In *P. fluorescens* CHA0, this QS pathway has a major role in positively regulating biocontrol factor expression in various host-pathogen systems (Laville et al. 1992; Siddiqui et al. 2005). It is initiated by the GacS/GacA two-component system, which is homologous to VarS/VarA, and it involves three functional homologs of CsrB-CsrC-CsrD, termed RsmX, RsmY and RsmZ, and two homologs of CsrA, termed RsmA and RsmE (Laville et al. 1992; Zuber et al. 2003; Reimmann et al. 2005; Kay et al. 2005) (Fig. 2a). The importance of the GacS/GacA pathway is supported by studies on *gacS* and *gacA* mutants of *P. fluorescens* BL915 and *Pseudomonas* sp. PCL1171 which have lost biocontrol properties (Ligon et al. 1999; van den Broek et al. 2003).

In strains of *P. aeruginosa*, the conserved GacS/GacA system directs the synthesis of two small RNAs, designated RsmY and RsmZ, which antagonize one CsrA homolog, named RsmA (Reimmann et al. 1997; Pessi et al. 2001; Heurlier et al. 2004; Kay et al. 2006) (Fig. 2b). In both *P. aeruginosa* PAO and *P. fluorescens* CHA0, the sequestration of the RNA-binding proteins (RsmA, and RsmE in the latter bacterium) by the small RNAs leads to translation of target mRNAs. Many target mRNAs specify enzymes for secondary metabolism or lytic exoenzymes. Typical target genes of *P. fluorescens* CHA0 are *hcnA* (for an HCN synthase subunit), *phlA* (for a subunit of the DAPG biosynthetic enzyme complex) and *aprA* (for exoprotease AprA) (Blumer et al. 1999; Zuber et al. 2003; Kay et al. 2005) (Fig. 2a). In *P. aeruginosa*, target genes include *hcnA* and the *phz* genes (Reimmann et al. 1997; Pessi et al. 2001) (Fig. 2b). Thus, there is in fact considerable overlap between virulence factors of *P. aeruginosa* and biocontrol traits of *P. fluorescens*. For instance, HCN, phenazines and alkaline protease qualify for both attributes (Haas et al. 2004).

In *P. aeruginosa*, there are two sensors, RetS and LadS, in addition to GacS, all of which appear to determine the activity of GacA. RetS is an antagonist of GacA and might function as a phosphatase removing the phosphate group from phosphorylated GacA (Goodman et al. 2004; Laskowski and Kazmierczak 2006; Yahr and Wolfgang 2006). LadS, like GacS, appears to activate GacA (Ventre et al. 2006). Stimulation of *rsmY* and *rsmZ* transcription by phosphorylated GacA requires RsmA and probably further unknown factors (Fig. 2b). According to genomic sequence data, RetS and LadS also occur in *P. fluorescens* and *P. putida* strains, but the roles of these sensors remain to be determined. In strain CHA0, both RsmA and RsmE are involved in the expression of RsmX, RsmY and RsmZ (Fig. 2a).

In *P. aeruginosa*, the GacS/GacA system positively regulates the expression of the *lasR*, *rhlR* and *rhlI* genes and thereby stimulates the synthesis of *N*-butanoyl-homoserine lactone (Reimmann et al. 1997; Kay et al. 2006) (Fig. 2b). A similar positive effect of the GacS/GacA system on AHL synthesis is also found in biocontrol strains of *P. chlororaphis*, *P. aureofaciens* and *P. putida* (Chancey et al. 1999; Chin-A-Woeng et al. 2001b; Bertani and Venturi 2004; Han et al. 2006). In all fluorescent pseudomonads examined,

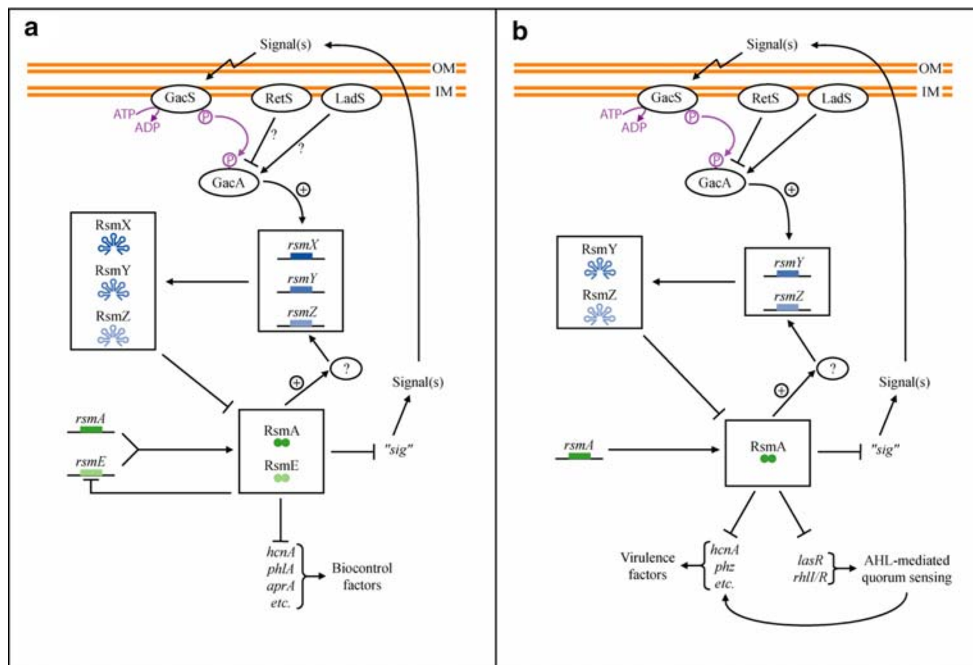

Fig. 2 Post-transcriptional signal transduction pathway downstream of the GacA response regulator in *P. fluorescens* CHA0 (**a**) and *P. aeruginosa* PAO1 (**b**). For a description of the mechanisms, see text. OM, outer membrane; IM, inner membrane; *sig*, hypothetical signal biosynthetic genes. Violet arrows indicate phosphotransfer

GacA function appears to favour the biofilm mode of growth, as opposed to planktonic life of single cells (Heeb and Haas 2001; Goodman et al. 2004; Ventre et al. 2006).

The signal molecules that activate the GacS/GacA cascade in bacteria have not been identified chemically nor are the signal biosynthetic genes known. The signals are unrelated to AHLs and some other well-known QS signals such as PQS, autoinducer 2 (AI-2; a furanosyl diester), or 3-hydroxy-palmitate methyl ester of *Ralstonia* spp. (Dubuis et al. 2006). In both *P. fluorescens* CHA0 and *P. aeruginosa* PAO, the signals activating the GacS/GacA cascade are produced at high cell population densities and under positive GacA control. Mutants that are defective for GacA or the small RNAs (RsmX-RsmY-RsmZ and RsmY-RsmZ, respectively) synthesize $\leq$5% of signal, by comparison with the wild-type (Kay et al. 2005, 2006). Thus, the Gac signals have the characteristics of autoinducers (Fig. 2). In strain CHA0, thiamine is necessary for signal activity, biocontrol factor expression and suppression of *P. ultimum* on cress (Dubuis et al. 2006). Two tentative explanations can be offered for this effect at present. Either signal biosynthesis requires relatively elevated levels of thiamine pyrophosphate as a cofactor such that a limiting thiamine supply will support central metabolism and growth, but not signal production, or thiamine could be a component of the Gac/Rsm signal transduction pathway. There is the intriguing possibility that thiamine triphosphate, a derivative of thiamine pyrophosphate, could be an intracellular signal. In *Escherichia coli*, amino acid starvation leads to transient accumulation of thiamine triphosphate (Lakaye et al. 2004).

AHLs are not the only QS signals that different species or genera of microorganisms can share to establish cross-talk (Riedel et al. 2001). The same has also been observed for AI-2 and for the Gac signals (Bassler et al. 1997; Dubuis and Haas 2007). A survey of beneficial and pathogenic plant-associated pseudomonads reveals that many species produce and release signal molecules activating the Gac/Rsm cascade in *P. fluorescens* and that this signal activity does not correlate with AHL production (Table 1). The bioassay used to detect Gac signals is illustrated in Fig. 3: The expression of the small RNA gene *rsmZ* and that of the biocontrol genes *hcnA* and *phlA*

Table 1 Bacterial strains producing signal molecules activating the Gac/Rsm cascade in strain CHA0

Species	Strain	Gac signal[a]	AHLs	Comments	Reference or source
Pseudomonas aeruginosa	PAO1	++	+[b,c]	Pathogen, biocontrol[e,f]	Holloway (1955)
Pseudomonas corrugata	LMG 2172	++	+[b,c]	Pathogen[f]	Sutra et al. (1997)
Pseudomonas fluorescens	CHA0	++	–	Biocontrol	Stutz et al. (1986)
	CM1′A2	++	–	Biocontrol	Fuchs and Défago (1991)
	F113	++	+[d]	Biocontrol	Fenton et al. (1992)
	Pf-5	++	–	Biocontrol	Paulsen et al. (2005)
	PITR2	++	–	Biocontrol	Harrison et al. (1993)
	Q37-87	++	+[b]	Biocontrol	Keel et al. (1996)
	Q65c-80	++	–	Biocontrol	Harrison et al. (1993)
Vibrio harveyi	BB120	++	+[d]	Marine bacterium	Bassler et al. (1997)
Vibrio natriegens		++	–	Marine bacterium	A. Kukangara
Pseudomonas aureofaciens	30-84	+	+[b,c]	Biocontrol	Pierson et al. (1995)
Pseudomonas caricapapayae	LMG 2152	+	+[b,c]	Pathogen[f]	Sutra et al. (1997)
Pseudomonas chlororaphis	LMG 1245	+	+[b,c]	Type strain	Sutra et al. (1997)
	LMG 5004	+	+[b,c]	Type strain	Sutra et al. (1997)
Pseudomonas fluorescens	LMG 1794	+	–	Type strain	Sutra et al. (1997)
	2-79	+	+[b,c]	Biocontrol	Weller (1983)
	M114	+	–	Biocontrol	Fenton et al. (1992)
	P3	+	+[b]	Soil bacterium	Sharifi-Tehrani et al. (1998)
	P12	+	+[b,c]	Biocontrol	Keel et al. (1996)
	Pf0-1	+	–	Soil bacterium	Compeau et al. (1988)
	PILH1	+	–	Biocontrol	Keel et al. (1996)
	Q69c-80	+	–	Biocontrol	Pierson and Weller (1994)
	SBW25	+	–	Biocontrol	Rainey and Bailey (1996)
Pseudomonas putida	LMG 2257	+	–	Type strain	Sutra et al. (1997)
	KD	+	–	Biocontrol	Sharifi-Tehrani et al. (1998)
	KT2440	+	–	Soil bacterium	Franklin et al. (1981)
Xanthomonas campestris	8004	+	–	Pathogen[f]	Turner et al. (1984)
Escherichia coli	DH5α	–	–	Laboratory strain	Sambrook and Russell (2001)
Pseudomonas alcaligenes	Ps93	–	–	Lipase producing strain	Gerritse et al. (1998)

[a] ++, high signal activity, similar to that of *P. fluorescens* CHA0; +, intermediate signal activity, below that of *P. fluorescens* CHA0; –, no or insignificant signal activity detectable. Extracts containing the signals were obtained from 50-ml cultures as described before (Dubuis et al. 2006) and tested with *hcnA′-′lacZ*, *rsmZ-lacZ* and *phlA′-′lacZ* reporter constructs as shown in Fig. 4. Adapted from Dubuis (2005)

[b] +, Long chain AHLs detected by the reporter strain *Agrobacterium tumefaciens* NTL4/pZLR4 (Shaw et al. 1997)

[c] +, Short chain AHLs detected by the reporter strain *Chromobacterium violaceum* CV026 (McClean et al. 1997)

[d] AHLs produced by *P. fluorescens* F113 (*N*-(3-hydroxy-7-*cis*-tetradecenoyl)-homoserine lactone; Laue et al. 2000 and *V. harveyi* *N*-3-hydroxybutanoyl-homoserine lactone; Bassler et al. 1997) were not detected by the reporter strains *A. tumefaciens* NTL4/pZLR4 and *C. violaceum* CV026

[e] Pathogens for vertebrates

[f] Pathogens for plants

is stimulated about three-fold in *P. fluorescens* CHA0 by saturating amounts of the homologous signals. No stimulation is observed in a *gacA* mutant background (Dubuis and Haas 2007). Signals extracted from culture supernatants of several biocontrol pseudomonads or from the pathogens *P. aeruginosa*, *P. corrug-*

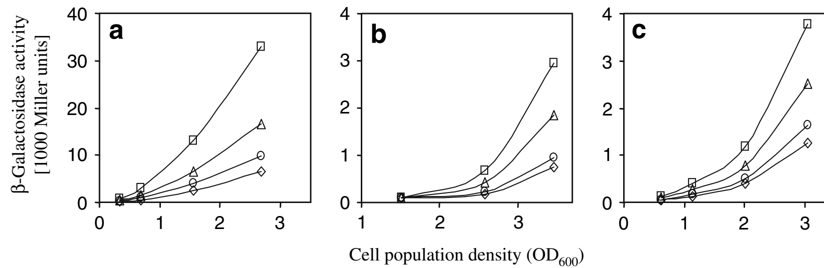

Fig. 3 Bioassay of signal activity. Dichloromethane-extracted supernatants from bacterial strains were tested with *hcnA* (**a**), *rsmZ* (**b**) and *phlA* (**c**) reporter strains as described elsewhere (Dubuis et al. 2006). ß-Galactosidase measurements were carried out in triplicate using cells from the reporter strains *P. fluorescens* CHA0/pME6530 (*hcnA′-′lacZ*; a), CHA0/pME6091 (*rsmZ-lacZ*; b) and CHA0/pME6702 (*phlA′-′lacZ*; c). ◇, control without added extract; ◯, plus extract from strain having weak or no signal activity; △, plus extract from strain having intermediate signal activity; □, plus extract from strain having strong, CHA0-like activity; OD_{600}, optical density at 600 nm. Adapted from Dubuis (2005)

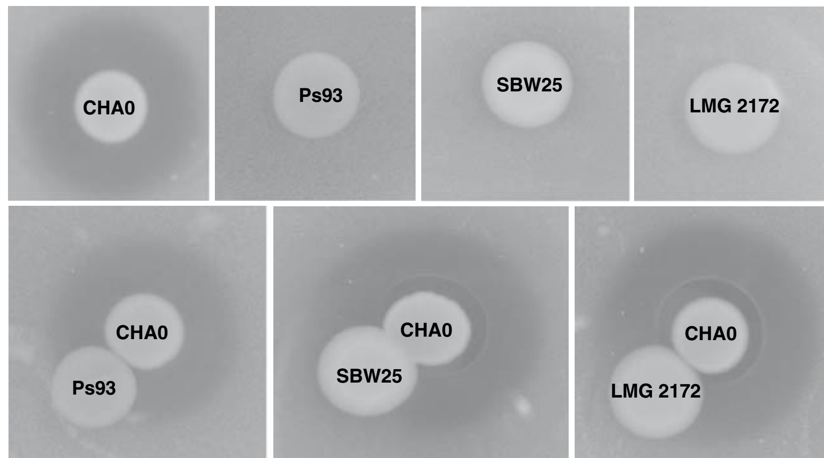

Fig. 4 Antibiotic production by *P. fluorescens* CHA0 in the presence of *P. fluorescens* SBW25, *P. corrugata* LMG 2172 or *P. alcaligenes* Ps93. Strains SBW25, LMG 2172 and Ps93 were inoculated by placing 5-µl drops of overnight cultures onto nutrient agar. After incubation at 30°C for 24 h, 5-µl drops of a CHA0 culture were added and incubation was continued for 24 h. An overlay with *Bacillus subtilis* revealed antibiotic production by growth inhibition zones

ata, *P. caricapapayae* and *Xanthomonas campestris* have similarly high or somewhat lower inducing activities (Table 1; Fig. 3). In this test, a *rpfF* mutant of *X. campestris*, which does not produce the diffusible signal factor (DSF; *cis*-11-methyl-dodecenoate), is still active. Interestingly, two *Vibrio* spp. have been found to have good signal activity, whereas *P. alcaligenes* and *E. coli* DH5α are devoid of it (Dubuis and Haas 2007) (Table 1). Signal activity can be visualized as stimulation of antibiotic production in strain CHA0. When colonies of *P. fluorescens* SBW25 or *P. corrugata* LMG 2172 (both of which do not produce detectable antibiotic compounds) grow next to a colony of strain CHA0, greatly enhanced halos of growth inhibition of a sensitive indicator, *Bacillus subtilis*, can be observed. By contrast, the signal-negative strain Ps93 of *P. alcaligenes* has no effect on antibiotic production (Fig. 4). These experiments demonstrate the ability of various plant-associated bacteria to stimulate post-transcriptionally the expression of biocontrol factors (here: essentially DAPG) in *P. fluorescens* CHA0. Although inhibitory compounds would also be detected by the bioassay shown in Fig. 3, none of the microorganisms tested in Table 1 appears to have such activity.

Conclusions

Biocontrol of root diseases by fluorescent pseudomonads is multifactorial; important mechanisms include antagonism between the biocontrol agents and the pathogens, degradation of virulence factors and induction of systemic resistance in the plant. Not surprisingly, both the biocontrol agents and the pathogens have developed defence mechanisms to minimize the impact of antagonism and some of these strategies have been shown to be active in the rhizosphere. Unlike predation, the biocontrol strategies that we have reviewed here work at a distance (of several micrometers) and rely on the emission or destruction of chemical signals. Root exudates critically influence the outcome, via signals that are mostly unknown and merit to be investigated. However, not all biocontrol interactions are of the antagonistic kind. Some previously unsuspected, positive interactions between rhizosphere bacteria stimulate the expression of biocontrol traits via the Gac/Rsm signal transduction pathway. This pathway operates essentially at a post-transcriptional level and in this respect differs from the better-characterized, AHL-dependent signal transduction pathways, whose outputs manifest themselves at the level of transcription. When activated, the Gac/Rsm cascade favours the biofilm mode of growth and the expression of multiple biocontrol factors in root-colonizing bacteria. Available reporter constructs facilitate the detection of activating signal molecules. In future research, it will be interesting to investigate which factors prevailing in the rhizosphere influence the activity of this signal transduction pathway as well as that of other key regulatory elements in plant-beneficial rhizobacteria.

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