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REVIEW PAPER

Interactions between plants and beneficial *Pseudomonas* spp.: exploiting bacterial traits for crop protection

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Abstract Specific strains of fluorescent *Pseudomonas* spp. inhabit the environment surrounding plant roots and some even the root interior. Introducing such bacterial strains to plant roots can lead to increased plant growth, usually due to suppression of plant pathogenic microorganisms. We review the modes of action and traits of these beneficial *Pseudomonas* bacteria involved in disease suppression. The complex regulation of biological control traits in relation to the functioning in the root environment is discussed. Understanding the complexity of the interactions is instrumental in the exploitation of beneficial *Pseudomonas* spp. in controlling plant diseases.

Keywords Antibiotics · Biocontrol · Endophytes · Induced resistance · Plant-growth promotion · Siderophores

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Abbreviations

AHL	*N*-acyl-homoserine lactone
DAPG	2,4-diacetylphloroglucinol
ISR	Induced systemic resistance
PCA	Phenazine-1-carboxylic acid
PGPR	Plant growth promoting rhizobacteria
SA	Salicylic acid
SAR	Systemic acquired resistance
TAD	Take-all decline

Introduction

Plant–bacteria interactions are long known and have three well-differentiated manifestations. The first is a direct relation between plants and pathogenic bacteria (for instance, *Agrobacterium* spp., *Erwinia* spp., *Ralstonia* spp., etc.), causing a state of disease. In this case the consequences for the plant are negative. A second type is a direct interaction between plants and non-pathogenic bacteria (for example, *Azorhizobium*, *Bradyrhizobium*, *Rhizobium, Sinorhizobium*, etc.), leading to a beneficial association for both partners. This interaction is a mutualistic symbiosis, yielding positive effects for the plant. These two types of interactions arise as a consequence of fine-tuned molecular signalling between the bacteria and the plants. However, the ultimate boundaries between a mutualistic and a pathogenic interaction can be fuzzy, and the recognition and signal-transduction

processes leading to the plant response may be similar for both types of interactions (Baron and Zambryski 1995; Soto et al. 2006). Thus, studies on the *Sinorhizobium meliloti*-alfalfa mutualistic symbiosis have independently shown: (i) induction by bacteria of a hypersensitive response (HR) in compatible interactions that could be part of a plant mechanism to control the number of successful infections (Vasse et al. 1993); (ii) prevention of host defence response by rhizobia (McKhann et al. 1997); or (iii) accumulation of salicylic acid (SA) and H_2O_2 in roots inoculated with either Nod^- (nodulation) mutants or incompatible rhizobia in contrast with the inoculation with compatible strains, suggesting an involvement of the Nod factors in the inhibition of SA-mediated defence response in legumes (Martínez-Abarca et al. 1998; Bueno et al. 2001). These findings show a resemblance between mutualistic Rhizobia-legume and pathogenic bacteria–plant interactions. The third type of interaction that numerous bacterial genera (e.g. *Alcaligene*s spp., *Bacillus* spp., *Pseudomonas* spp., *Serratia* spp., etc.) establish with plants in principle could be considered as neutral for the plant. Strictly speaking, they do not fit to the definition of pathogenic or mutualistic bacteria, because of the absence of the evident negative or positive effects. However, there has been an increasing body of literature that describes these bacteria as clearly beneficial to plants, either because they directly promote plant growth (Glick 1995; Bashan and Holguin 1998) or they protect plants against a broad range of phytopathogens and pests (Kerry 2000; Ramamoorthy et al. 2001; Gerhardson 2002). Direct plant growth-promoting effects of bacteria that inhabit the rhizosphere have been attributed to provision of nutrients, microelements, hormones, etc., to the plant. These bacteria seem therefore to exert their positive effects by a non-active way, colonizing the surface of plant tissues and providing profitable compounds to the plant. Some of these bacteria go a step further and intimately establish within plant tissues as endophytes (Rosenblueth and Martínez-Romero 2006), without causing any evident damage or morphological changes in the plant. Whether this endophytic establishment is the consequence of an active interaction between plant and bacterium is poorly investigated, although the genome sequencing of some of them, as *Azoarcus* sp., will be instrumental in explaining their behaviour (Krause et al. 2006). Some of these beneficial bacteria do induce plant responses other than growth promotion. For example, they can promote a state of enhanced defensive capacity against pathogen attack (Sticher et al. 1997; van Loon et al. 1998). Protection against plant pathogens can also be a consequence of direct antagonistic interactions between beneficial bacteria and pathogens. In this case, the rhizosphere is just the ecological niche where beneficial bacteria and deleterious microorganisms live, feed and encounter. Eventually, the plant becomes the battle-field where the beneficial and deleterious organisms compete for resources. Obviously the consequences can be of extreme importance for the plant, and it is likely that the plant somehow influences the development and results of these microbial interactions.

This review will focus on the interaction of plant roots with beneficial *Pseudomonas* spp., a group of bacteria that has been studied for direct plant-growth promotion as well as their abilities to control plant diseases (O'Sullivan and O'Gara 1992). Both aspects confer this bacterial group as an alternative for replacing (or reducing) the use of agrochemicals, which fits environmentally-friendly strategies to be implemented in a modern sustainable agriculture framework. Understanding the interactions between the plant, beneficial pseudomonads, and plant pathogens are of crucial importance to overcome practical problems such as the inconsistency of biocontrol performance.

Beneficial (non-deleterious) *Pseudomonas* spp. specified

Pseudomonas spp. form a diverse group of aerobic, Gram-negative, chemoheterotrophic, motile, rod-shaped bacteria. Of particular interest to this review is the subset of RNA group I (Palleroni et al. 1973) species characterized by the distinctive production of yellow-green fluorescent pigments with siderophore activity (pyoverdins or pseudobactins) (see below). They are very versatile bacteria, found abundantly and ubiquitously in nature, and well-adapted to numerous ecological niches due to rather simple nutritional requirements. Members of this group are important as human, animal and plant pathogens and in food spoilage. The genomes of several *Pseudomonas* spp. are available (Stover

et al. 2000; Nelson et al. 2002; Paulsen et al. 2005), revealing genome sizes larger (>5,500 ORFs) than most other sequenced bacterial genomes, although similar or even smaller than genome sizes of other plant-associated bacteria (i.e. *Agrobacterium tumefaciens*, *Sinorhizobium meliloti* or *Mesorhizobium loti*) (Van Sluys et al. 2002). Pseudomonads interacting with plants include phytopathogens grouped in the species *Pseudomonas syringae*. This species is subdivided into pathovars on the basis of host specificity, and they are the aetiological agents of leaf spots, blights, and wilts in susceptible host plants (Gardan et al. 1991, 1999; Young and Triggs 1994). Other members (for example, certain *P. fluorescens P. putida*, or *P. aeruginosa* strains) are known to be beneficial to plants. Some strains have been recognized for a long time as biocontrol agents (Howell and Stipanovic 1980; Lifshitz et al. 1986; Xu and Gross 1986; Weller 1988). They are also known as plant growth promoting rhizobacteria (PGPR), either per se or as a consequence of their abilities to control disease (Burr et al. 1978; Kloepper et al. 1980; Suslow and Schroth 1982; Gardner et al. 1984; Geels et al. 1986; Weller and Cook 1986; Van Peer and Schippers 1988). The number of plant-associated *Pseudomonas* spp. strains that have been described to stimulate plant growth or suppress plant diseases is growing, and knowledge of mechanisms involved is continuously increasing. In this review, we consider as beneficial those species or strains of *Pseudomonas* that improve the fitness of host plants, including those that penetrate and colonize internal tissues without provoking any deleterious effect; that is, establishing an association which *de facto* could be considered as mutualism.

First steps in the beneficial *Pseudomonas*–plant interaction: plant colonization

All beneficial traits that *Pseudomonas* spp. can potentially provide to plants would be worthless if a fundamental prerequisite is not fulfilled: the successful establishment and persistence of the bacteria in the rhizosphere or within plant root tissues. Successful colonization by a given soil inhabiting pseudomonad is a consequence of a complex, continuous and delicate balance among a large array of biotic (the plant, the beneficial colonizer, other microorganisms, etc.) and abiotic (soil type, water and mineral contents, pH, temperature, composition of root exudates, nutrient availability, etc.) factors (Loper et al. 1985; Acea and Alexander 1988; Bahme and Schroth 1987; Howie et al. 1987; Kwok et al. 1987; Stephens et al. 1987; Lam 1990). To determine the contributions of these factors as well as their interactions is of crucial importance, and gaps in this knowledge may explain why practical application of PGPR suffers from inconsistent performance (Thomashow 1996). In the hostile and nutrient-limiting bulk soil environment, plant roots and their immediate vicinity are extremely attractive places for both beneficial and deleterious soil borne microbes. The roots and the rhizosphere offer an ecological niche that provides an important source of a wide range of nutrients (Degenhardt et al. 2003). The establishment of the microbe–plant interaction is preceded by movement of the free-living microorganisms toward the plant roots. Chemotaxis to attractants which are present in plant root exudates can be important in the establishment of bacterial cells in the rhizosphere (Bais et al. 2004; Welbaum 2004). Beneficial *Pseudomonas* spp. chemotactically reach root surfaces thanks to flagellar motility (De Weger et al. 1987; Turnbull et al. 2001a, b; De Weert et al. 2002). Thus motility appears an essential trait involved in root colonization, as was revealed by the use of motility mutants (Simons et al. 1996; Dekkers et al. 1998b; Capdevila et al. 2004; Martínez-Granero et al. 2006). In contrast, other studies have indicated that flagella may have only a minor role in colonization (Howie et al. 1987; Scher et al. 1988). Subsequently competition for nutrients selects the best adapted individuals, giving them advantage in successive root colonization steps. The major study in understanding how beneficial *Pseudomonas* strains colonize plant roots is the one carried out by Lugtenberg and associates (Lugtenberg and Dekkers 1999; Lugtenberg et al. 2001). Indeed, in-depth knowledge has been gathered over the last decades on environmental factors and bacterial traits involved in root colonization of several PGPR *Pseudomonas* spp. strains. One of the best studied examples is *P. fluorescens* WCS365. This strain

originates from potato (*Solanum tuberosum* L.) (Geels and Schippers 1983), and is a good colonizer of both potato (Brand et al. 1991) and tomato (*Lycopersicon esculentum* L.) (Simons et al. 1996) roots. Strain WCS365 controls tomato foot and root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Dekkers et al. 2000). In addition to flagellar motility, bacterial lipopolysaccharides (LPS), particularly the O-antigen, a high bacterial growth rate, vitamin B_1 synthesis ability, and exudation of NADH dehydrogenase are also contributing factors in root colonization of *P. fluorescens* WCS365 (Simons et al. 1996; Dekkers et al. 1998b; Camacho-Carvajal et al. 2002). As for the use of carbon sources present in root exudates, tomato root colonization by WCS365 is not determined by its ability to use specific sugars (Lugtenberg et al. 1999). However, amino acid (leucine, arginine, histidine, valine, isoleucine and tryptophan) synthesis is essential for effective root colonization of *P. fluorescens* WCS365 (Simons et al. 1997). On the contrary, the presence of the polyamine putrescine negatively affects the competitive root colonization ability of this strain (Kuiper et al. 2001). Finally, strain WCS365 has a *sss* (*P. aeruginosa*)/*xerC* (*Escherichia coli*) homologue that encodes a site-specific recombinase that has been shown to be important for competitive colonization of potato, tomato and wheat (*Triticum aestivum* L.) rhizospheres (Dekkers et al. 1998a). This site-specific recombinase plays a key role in a regulatory process for DNA rearrangements that causes phase variation (Höfte et al. 1994). The action of site-specific recombinases influencing colonization traits has been also demonstrated in *P. fluorescens* biocontrol strains F113 (Martínez-Granero et al. 2005) and Q8r1-96 (Mavrodi et al. 2006). Phase variation (reviewed by Van den Broek et al. 2005a) can affect bacterial traits related to root colonization (Sánchez-Contreras et al. 2002; Martínez-Granero et al. 2006) and biocontrol activity (Van den Broek et al. 2003). Taken together these findings, it can be concluded that root colonization, and the bacterial traits implicated in this process, plays a crucial role in biocontrol (Chin-A-Woeng et al. 2000). In addition, improvement of the colonization ability of poor root colonizing *Pseudomonas* strains can be achieved, for example, by the transfer of the site-specific recombinase gene (Dekkers et al. 2000).

Getting intimate: endophytic beneficial *Pseudomonas* spp.

Some plant-associated microorganisms can go a step further in their colonization abilities. Endophytism can be considered as a universal phenomenon, and probably all plants harbour endophytic bacterial species (Strobel et al. 2004; Rosenblueth and Martínez-Romero 2006). Several excellent reviews have been published on bacterial endophytes and prospects on utilizing them for plant-growth promotion, biological control of pathogens and pests, and exploitation of their natural products in agriculture, industry and/or medicine (Hallmann et al. 1997; Sturz et al. 2000; Lodewyckx et al. 2002; Strobel and Daisy 2003; Strobel et al. 2004; Compant et al. 2005; Gray and Smith 2005; Rosenblueth and Martínez-Romero 2006). Here, we will only refer to PGPR pseudomonads that can penetrate into the roots establishing an endophytic relation. Reinhold-Hurek and Hurek (1998) have indicated the criteria for proper recognition of endophytic bacteria which go beyond isolation from surface-disinfected plant tissues. A correct assessment of endophytic status must be supported, for example, by microscopic proof and by the capacity of the endophyte to re-infect disinfected seedlings (Rosenblueth and Martínez-Romero 2006). The current known endophytic bacteria only represent a minor part of endophytic populations inhabiting plant tissues. This fact becomes evident after compiling the continuously growing list of results obtained from culturing-independent identification methods. These reports usually show larger and more diverse bacterial communities than those obtained from culturing on microbiological media (Sessitsch et al. 2002; Conn and Franco 2004; Miyamoto et al. 2004). However, there are some exceptions, and results obtained from culturing or culture-independent methods may show no significant differences, as Cankar et al. (2005) have shown for *Pseudomonas* spp. and other endophytic bacteria in Norway spruce (*Picea abies* L. Karst) seeds. Non-pathogenic, endophytic *Pseudomonas* spp. have been identified and/or isolated from numerous plant species (cultivated or

not, herbaceous or woody), from diverse geographical origins, as well as from different plant organs and tissues. Some examples of plant species in which endophytic populations of *Pseudomonas* spp. have been detected are listed in Table 1. Endophytic beneficial or neutral *Pseudomonas* spp. are detected as part of complex bacterial consortia present in different plant tissues. Sometimes, they are the predominant bacteria, in other cases they are just minor components of the endophytic microflora.

Plant health management can take advantage of mutualistic endophytic microorganisms to establish, for example, *in planta* suppressiveness (Sikora 2006). It is not well established whether endophytism is advantageous to the bacterium (Rosenblueth and Martínez-Romero 2006), although it is assumed that once endophytic the cells are less exposed to biotic and abiotic stresses (Hallmann et al. 1997). Benefits for the plant provided by endophytic, non-pathogenic *Pseudomonas* spp. have been demonstrated. For example, plant growth promotion exerted by *Pseudomonas*, usually in combination with other endophytic bacterial genera, has been reported in soybean (Kuklinsky-Sobral et al. 2004), rice (Adhikari et al. 2001), oilseed rape and tomato (Nejad and Johnson 2000), and hybrid spruce (*Picea glauca* × *engelmannii*) (Chanway et al. 2000). It might well be that the presence of mutualistic endophytic bacterial consortia enhance plant growth, although the contribution of each bacterium is unknown. Thus, presence of a large number of bacterial genera (including *Pseudomonas*) in root nodules of spontaneous legumes has been reported, although they do not induce nodule formation (Zakhia et al. 2006). The mechanisms by which endophytic *Pseudomonas* spp. promote plant growth can be diverse. They could be a direct consequence of providing (micro)nutrients, minerals, phytohormones, etc. to the plant. For example, growth promotion of

Table 1 Examples of plant species and plant parts in which endophytic *Pseudomonas* spp. have been detected

Plants species	Plant part	Reference
Brassica napus L. (canola, oilseed rape)	Roots	Misko and Germida (2002)
Calystegia soldanella L.	Roots, rhizosphere	Park et al. (2005)
Citrus sinensis [L.] Osbeck (orange)	Stems	Lacava et al. (2006)
Coffea arabica L. (coffee)	Leaves, berries, etc.	Vega et al. (2005)
Croccus albiflorus Kit (crocus)	Aerial parts	Reiter and Sessitsch (2006)
Daucus carota L. (carrot)	Roots	Surette et al. (2003)
Elymus mollis Trin.	Roots, rhizosphere	Park et al. (2005)
Glycine max L. (soybean)	Leaves, stems, roots	Kuklinsky-Sobral et al. (2005)
Gossypium hirsutum L. (cotton)	Roots	Mcinroy and Kloepper (1995)
Hedysarum spp.	Root nodules	Benhizia et al. (2004)
Oryza sativa L. (rice)	Stems, roots	Yang et al. (1999) and Adhikari et al. (2001)
Picea abies L. Karst (Norway spruce)	Seeds	Cankar et al. (2005)
Picea glauca x engelmannii (hybrid spruce)	Stems, roots	Chanway et al. (2000)
Pinus sylvestris L. (Scots pine)	Meristematic bud tissues	Pirttilä et al. (2004)
Pisum sativum L. (pea)	Stems	Elvira-Recuenco and Van Vuurde (2000)
Populus trichocarpa x deltoides cv. Hoogvorst (poplar tree)	Xylem sap	Germaine et al. (2004)
Solanum sp.	Rootstocks	Long et al. (2004)
Solanum tuberosum L. (potato)	Stems, roots, endorhiza, endosphere	Garbeva et al. (2001) and Berg et al. (2005)
Tagetes spp. (marigolds)	Roots	Sturz and Kimpiski (2004)
Ulmus spp. (elm trees)	Stems, roots	Mocali et al. (2003)
Vitis vinifera L. (grapevines)	Xylem sap	Bell et al. (1995)
Diverse spontaneous legumes	Root nodules	Zakhia et al. (2006)

two soybean cultivars has been associated to production of indole acetic acid (IAA) and solubilization of mineral phosphate by endophytic bacteria, including *Pseudomonas* representatives (Kuklinsky-Sobral et al. 2004). On the other hand, plant growth promotion by endophytes can be due to control of phytopathogens. However, a contrasted *in planta* endophytic biocontrol activity is not always demonstrated, and some studies only provide data from in vitro antagonism bioassays between the isolated endophyte and the pathogen. Nonetheless, there are good examples of biocontrol activity promoted by endophytic pseudomonads in different pathosystems, although the mechanism(s) involved still need to be elucidated. For example, three endophytic *Pseudomonas* spp. strains (*P. fluorescens*, *P. tolaasii* and *P. veronii*) significantly enhance plant growth in the absence of pathogens, but also reduce seedling diseases of rice caused by *Achlya klebsiana* and *Pythium spinosum* (Adhikari et al. 2001). An endophytic isolate of *P. corrugata* controls population numbers of *Agrobacterium vitis*, the causal agent of grape crown gall disease (Bell et al. 1995). Two *P. putida* strains were effective in reducing disease severity of cotton vascular wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* (Chen et al. 1995). Endophytic isolates of *P. denitrificans* and *P. putida* from *Quercus fusiformis* Small. can reduce oak wilt disease (*Ceratocystis fagacearum*) and/or crown loss (Brooks et al. 1994). Finally, potato endophytic *P. fluorescens* strains are effective as biological agents against *Rhizoctonia solani* in potato and lettuce (*Lactuca sativa* L.) (Grosch et al. 2005). In all these cases the in situ activity of endophytes as related to biological control is very difficult to demonstrate. In this sense, it is very interesting that *in planta* synthesis and deposition of 2,4-diacetylphloroglucinol (DAPG) crystals in tomato roots by the endophytic *P. fluorescens* strain FPT9601 has been reported (Aino et al. 1997), suggesting that an important biocontrol trait like production of antibiotics is actively expressed in this environment.

Mechanisms involved in the endophytic colonization by beneficial *Pseudomonas* spp. are also largely unknown. As for other endophytes, it could be assumed that diverse cell wall-degrading enzymes are involved (Hallmann et al. 1997; Compant et al. 2005). Similarly, bacterial penetration at sites of root injury or zones of emergence of lateral roots or zones of elongation and differentiation of the root seems likely (Rosenblueth and Martínez-Romero 2006). In *P. fluorescens* biocontrol strain WCS417, it has been reported that the O-antigen of LPS is involved in endophytic colonization of tomato roots (Duijff et al. 1997). These authors suggested that colonization of internal root tissues is related to the induction of resistance exerted by this strain. Once the internal colonization is established, little is known about changes that non-deleterious endophytic *Pseudomonas* induce in the host plant, except for the thoroughly studied phenomenon of induction of disease resistance (see below). Recently, Wang and associates (2005) have studied the gene expression profile induced by the plant growth-promoting strain *P. fluorescens* FPT9601-T5 in the model plant *Arabidopsis thaliana*. This strain is endophytic on both tomato and *Arabidopsis* roots. Interestingly, this work revealed that upon root colonization by strain FPT9601-T5, 95 *Arabidopsis* genes were up-regulated (involved in metabolism, signal transduction, stress response, and particularly interesting, putative auxin-regulated genes and nodulin-like genes) and 105 genes were down-regulated (among them, some ethylene-responsive genes). The microarray analysis presented by these authors suggested similarities among rhizobacteria, endophytic PGPR and, in certain aspects, to rhizobia. A similar approach was used by Verhagen and associates (2004) who studied transcriptome changes in *Arabidopsis* upon induction of systemic resistance (see below) by *P. fluorescens* WCS417r. However, although WCS417r has been reported as endophytic in tomato, possible endophytic behaviour of this strain has not been studied in *Arabidopsis*. So far information on bacterial gene expression in the endosphere is not available. However, it is expected that in vivo expression technology (IVET) and additional genomic tools will develop for endophytic studies, similarly as they have been used for studying in vivo gene expression in rhizosphere *Pseudomonas* strains (Rainey 1999; Ramos-González et al. 2005).

From the initial sites of penetration (either by natural ways or by artificial inoculation), endophytic pseudomonads may spread internally to distant parts of the plant, colonizing both under- and aboveground tissues and organs. Rapid vascular transport from the roots to aerial tissues was suggested as an explanation for the extensive internal colonization in several plant

species by *P. aureofaciens* strain L11 (Lamb et al. 1996). The fate, composition and distribution of the endophytic bacterial community can be affected by different parameters. For example, certain crop management strategies can strongly influence the resident endophytic microflora. The use of glyphosate herbicide before planting soybean modified endophytic bacterial population, *P. oryzihabitants* being one of the two bacterial species that could be recovered from soybean plants by glyphosate enrichment isolation (Kuklinsky-Sobral et al. 2005). Moreover, anthropogenic influences may determine the composition of bacterial endophytes, the interaction among them, and between them and the host plant. In this sense, Benhizia and associates (2004) suggested that management of legumes could explain differences observed for the endophytic bacteria, including *Pseudomonas* sp., hosted in nodules of several wild *Hedysarum* spp. compared with those of the related crop plant *H. coronarium* L. In addition, the presence, composition, diversity, abundance and functionality of endophytic *Pseudomonas* spp. are determined by factors such as the genotype of the host plant (Siciliano and Germida 1999; Germida and Siciliano 2001; Reiter et al. 2003), growth temperature (Pillay and Nowak 1997), presence and interaction with plant pathogens (Hallmann et al. 1998; Reiter et al. 2003), seasonal temperature variations (Mocali et al. 2003), plant organ (Mocali et al. 2003) or tissue growth stage (Pirttilä et al. 2005).

Pseudomonas spp. traits involved in plant growth promotion and plant protection

Once a beneficial *Pseudomonas* strain has been able to colonize a host plant, it might be able to display a wide array of activities contributing to plant fitness. As mentioned, the consequences are promotion of plant growth per se or protection against plant pathogens, resulting in enhanced plant growth (Fig. 1). Suppression of disease by pseudomonads is mediated by direct antagonistic effects on the pathogen or by induced systemic resistance (ISR). Competition for nutrients, microelements (predominantly Fe^{3+}), and antibiosis can be exerted without interacting with the plant. Expression of *Pseudomonas* antibiotic and siderophore biosynthesis genes in the rhizosphere has been demonstrated (for example, Wood et al. 1997; Loper and Henkels 1999; Notz et al. 2001; Seveno et al. 2001). Similarly, it has been reported that this expression can be strongly affected by environmental, microbial or plant factors (Slininger and Sheawilbur 1995; Duffy and Défago 1999; Loper and Henkels 1999; Notz et al. 2001). The involvement of ISR in disease suppression implicates a direct communication between the bacteria and the plant. It is interesting to remark, that signal transduction pathways in plants upon *Pseudomonas* spp. contact are much better known (van Loon et al. 1998; Pieterse et al. 2003) than the bacterial traits involved in triggering such defence responses (Bakker et al. 2003, 2007). We will review the current knowledge on *Pseudomonas* traits that play a role in suppression of plant diseases.

Competition for iron: production of siderophores by beneficial *Pseudomonas* spp.

Due to the fact that iron, despite its abundance in Earth's crust, is largely unavailable for microbial assimilation, microorganisms have developed a strategy to scavenge available iron. This is particularly important for bacteria living in soil, an environment that provides iron (Fe^{3+}) at only about 10^{-18} M. This strategy involves the carefully iron-regulated biosynthesis and secretion of high-affinity, low-molecular-weight iron-chelating ligands called siderophores, as well as the production of proteins receptors for the recognition of the ferric siderophores. Production of both receptors and cognate siderophores are induced during iron-limiting growth and repressed by high concentrations of iron. Siderophores show a wide structural diversity and can be classified according to their main iron chelating groups (Höfte 1993). The production of siderophores by plant-associated bacteria has received major attention because of their role in both biological control of diseases and in virulence of plant-pathogens (Neilands and Leong 1986; Loper and Buyer 1991). This is particularly true for siderophores of *Pseudomonas* spp., which are produced in a large variety to sustain survival and growth of bacterial cells under iron-limiting conditions. Possibly, production and utilization of siderophores are evolutionary responses to the diverse and often adverse habitats in which these bacteria live (Ishimaru and Loper 1993). Pyoverdines (or pseudobactins) are the prevalent class of siderophores

Fig. 1 Increased radish growth after seed treatment with *P. fluorescens* WCS374 in a commercial greenhouse naturally infested with *Fusarium oxysporum* f.sp. *raphani*. The middle and right field plots were sown with non-treated and coating-treated radish seeds, respectively, whereas in the plot on the left seeds sown were coated with cells of WCS374 (Leeman et al. 1995b)

produced by fluorescent *Pseudomonas* spp. They are yellow-green water-soluble chromopeptides, fluorescent under ultraviolet irradiation (λ = 366 nm), and with a rather complex structure compared to that of most of the microbial siderophores described. They have both catechol and hydroxamate groups that chelate iron (Leong 1986). Their molecular structures, gene clusters responsible for biosynthesis, excretion and uptake, and their regulation have been extensively studied and reviewed (Crosa 1997; Meyer 2000; Ravel and Cornelis 2003). Differences in the number and composition of the amino acids present in the peptide chain of a given pyoverdine-type siderophore are characteristics of the *Pseudomonas* species or strain that biosynthesizes it (Höfte 1993; Fuchs et al. 2001). Environmental conditions and composition of root exudates may influence pyoverdine (as well as other siderophores) production on/within plant tissues, thus stimulating or abolishing any effect on either plant-growth promotion or plant protection potential capabilities exerted by them.

Certain *Pseudomonas* strains are also capable of using heterologous siderophores for their iron supply (Bakker et al. 1988; Jurkevitch et al. 1992; Mirleau et al. 2000). This strategy confers them important selective advantages in iron-limiting conditions: economization of metabolic efforts, increased ecological fitness and competitiveness in the rhizosphere, better root and soil colonization ability, and, as a derived consequence, enhanced capabilities for suppressing plant diseases. This strategy also implies the induction of outer membrane proteins specific for the exogenous siderophores. For example, the PGPR *P. putida* strain WCS358 (Geels and Schippers 1983) has the ability of using its own siderophore (pseudobactin-358) through a receptor that is highly specific for it (Bitter et al. 1991), as well as to utilize a large variety of heterologous siderophores through additional receptors (Koster et al. 1993). In contrast, pseudobactin-358 can only be utilized by a small number of pseudomonads (Marugg et al. 1989; Bakker et al. 1990; Raaijmakers et al. 1994). Obviously, this confers an enhanced rhizosphere competence to strain WCS358 against other soil-inhabiting pseudomonads. In addition, the ability to use exogenous siderophores can be engineered by the transfer and expression of siderophore receptor or iron-regulated siderophore promoter genes in heterologous genetic backgrounds (Loper and Henkels 1999). By implementing this strategy, rhizosphere competence or ecological fitness of the bacteria could be enhanced in some cases (Raaijmakers et al. 1995b).

Besides pyoverdines, some plant beneficial *Pseudomonas* spp. strains may produce one or more different types of siderophores (Meyer et al. 1992; Buysens et al. 1996; Boopathi and Rao 1999; Mercado-Blanco et al. 2001; Lim et al. 2002). Another compound with siderophore activity that can be produced under iron limitation by *Pseudomonas* spp. is salicylic acid (SA; 2-hydroxybenzoic acid) (Akenbauer and Cox 1988; Meyer et al. 1992; Visca et al. 1993; Anthoni et al. 1995; Leeman et al. 1996). SA is also the precursor or intermediate in the biosynthesis of siderophores, such as pyochelin and dihydroaeruginoic acid in *P. aeruginosa* (Cox et al. 1981; Serino et al. 1995, 1997) or pseudomonine in *P. fluorescens* (Anthoni et al. 1995; Mercado-Blanco

et al. 2001). In the PGPR *P. fluorescens* WCS374 (Geels and Schippers 1983) genes responsible for SA biosynthesis (*pmsB* and *pmsC*) have been identified (Mercado-Blanco et al. 2001). Interestingly, the expression of the *pmsB* gene (coding for an isochorismate pyruvate lyase) in chloroplasts of tobacco transgenic plants, produced increased accumulation of SA and SA glucoside, constitutive expression of acidic pathogenesis-related (PR) proteins, and enhanced pathogen resistance in these plants (Verberne et al. 2000).

Involvement of siderophores produced by *Pseudomonas* strains in suppression of diseases is variable and sometimes even controversial. It has been suggested that siderophores are antagonistic by means of sequestering iron from the environment, thereby limiting iron availability for the pathogen (Bakker et al. 1986; Loper and Buyer 1991). Lemanceau et al. (1992, 1993) attributed pseudobactin-358 production to the capacity of *P. putida* WCS358 to suppress Fusarium wilt of carnation. Moreover, this pyoverdine siderophore seemed to be responsible of the enhanced disease suppression exerted by a combination of WCS358 with a non-pathogenic *F. oxysporum* strain. The involvement of *Pseudomonas* siderophores in disease suppression has also been demonstrated, for example, against Fusarium wilt of radish (*Raphanus sativus* L.) (Raaijmakers et al. 1995a), *Pythium* damping-off (Buysens et al. 1996) and *Botrytis cinerea* (Audenaert et al. 2002) in tomato. In some pathosystems like Pythium damping-off of cucumber (*Cucumis sativus* L.), (Kraus and Loper 1992), *Pythium aphanidermatum* root rot of cucumber (Ongena et al. 1999), and take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici* (Thomashow and Weller 1990; Hamdan et al. 1991), *Pseudomonas* siderophores play no or only a minor role in disease suppression.

Besides its involvement in biocontrol activity, production of siderophores by beneficial *Pseudomonads* spp. can also affect iron plant nutrition. These effects are dependent on the plant species, the siderophore-producer *Pseudomonas* strain, and the experimental conditions. For example, in experiments performed under gnotobiotic conditions iron uptake in pea and maize was inhibited by purified pseudobactin (Becker et al. 1985). In other experiments, lime-induced chlorosis amelioration was achieved when ferric pyoverdines were amended in pot bioassays (Jurkevitch et al. 1986, 1988). Dicot and monocot plants may vary in their iron uptake rates from *P. putida* Fe-pseudobactin (Barness et al. 1991). None or little influence on Fe acquisition by oat (*Avena sativa* L.) plants grown in a calcareous soil was found when the roots were inoculated with *Pseudomonas* strains that produce high amounts of siderophores (Alexander and Zuberer 1993). In hydroponically-grown barley seedlings (*Hordeum vulgare* L.), ferric pseudobactin-358 was efficiently used as an iron source, and the chlorophyll synthesis was stimulated (Duijff et al. 1994b). In carnation (*Dianthus caryophyllus* L.) ferric pseudobactin-358 had differential effects on two cultivars that differed in their ferric reducing activity, and stimulated chlorophyll synthesis in the cultivar with the highest activity (Duijff et al. 1994a). Pot-grown mung bean plants (*Vigna radiata* L. Wilzeck) shown enhanced chlorophyll levels, reduction of chlorotic symptoms, and significant increases in total and physiological available iron when plants were treated with a siderophore-producing *Pseudomonas* sp. strain (Sharma et al. 2003). Promotion of rootlet elongation was achieved when cucumber seedlings were inoculated with siderophore-producer *Pseudomonas* strains (De Bellis and Ercolani 2001). Finally, siderophore producing pseudomonads can also be influenced by plant phytosiderophores. For example, the degree of iron stress in different plant rhizospheres of an engineered derivative of *P. fluorescens* Pf-5 was shown to be dependent on the iron-efficiency of the plant and on the amounts of plant-produced phytosiderophores (Marschner and Crowly 1998).

Antibiosis

One of the few modes of action of fluorescent *Pseudomonas* spp. that have been documented to be involved in natural suppression of disease in so called ''disease suppressive soils'' is inhibition of the pathogen through the production of antibiotics. Take-all is a devastating disease in wheat caused by *G. graminis* var. *tritici*. When wheat is grown in monoculture every year in the same field and severe outbreaks of take-all have been observed, a spontaneous decrease in take-all incidence and severity occurs (take-all decline, TAD), and this state of TAD is maintained over long time periods (Weller et al. 2002). An explanation for TAD is the build up of populations of DAPG producing

fluorescent *Pseudomonas* spp. (Raaijmakers and Weller 1998). High populations of DAPG producing pseudomonads are detected in TAD soils collected worldwide, whereas in disease conducive soils their populations are below population densities of 10^5 colony forming units (cfu) per gram of root. The threshold level for root colonization by DAPG producing pseudomonads to protect against the take-all fungus is 10^5 cfu/g of root (Raaijmakers and Weller 1998). Moreover, DAPG could be detected on wheat roots from TAD soils by HPLC mass spectrometry in effective quantities (Raaijmakers et al. 1999). Transfer of TAD to disease conducive soils by mixing in TAD soil correlated with transfer of DAPG producing pseudomonads above the threshold level for disease suppression (Weller et al. 2002). Taken together these results strongly suggest that build up of DAPG producing fluorescent pseudomonads can explain the phenomenon of TAD. Interestingly the involvement of antibiotic production in protection of wheat against the take-all fungus after inundative application of fluorescent pseudomonads had been presented in earlier work from several groups. Thomashow and associates (Thomashow and Weller 1988; Thomashow et al. 1990) demonstrated that the production of phenazine-1-carboxylic acid (PCA) is responsible for suppression of take-all by *P. fluorescens*. Their approach was to make mutants of the bacterial strain that are defective in PCA production and to subsequently complement the mutants by inserting the wild type genes on a plasmid, resulting in restoration of PCA production. The wild-type strain effectively suppressed disease whereas the mutants did not, and the complemented mutants again suppressed disease to wild-type levels (Thomashow and Weller 1988). These results were elegantly complemented by showing that PCA was actually produced in the rhizosphere of plants grown from seed treated with PCA producing pseudomonads (Thomashow et al. 1990). Since then similar studies have shown the involvement of a variety of antibiotic compounds including pyoluterin, phenazine-1-carboxamide, DAPG, pyrrolnitrin, pyocyanine, hydrogen cyanide, and viscosanamide (excellently reviewed by Haas and Défago 2005). Recently, cyclic lipopeptide production by *Pseudomonas* spp. received major attention (Raaijmakers et al. 2006) and no doubt many more new metabolites of *Pseudomonas* spp. with antimicrobial activity will be discovered.

Interestingly, metagenomics approaches reveal that there is a wide array of effective antibiotic molecules to be discovered in soil ecosystems (Handelsman 2004). Exploitation of such information for control of plant pathogens could be achieved by genetically modifying fluorescent *Pseudomonas* spp. strains to produce these antibiotics. Indeed, the approach to modify strains with antibiotic biosynthesis genes from heterologous strains has resulted in improved biological control (Voisard et al. 1989; Timms-Wilson et al. 2000; Bakker et al. 2002). Implementation of such genetically modified microorganisms in agricultural practices raises governmental and public concern about possible effects on nontarget organisms and functioning of ecosystems. Several studies, including long-term field studies, have been undertaken to address such questions and all studies basically deliver the same answer. Application of engineered *Pseudomonas* spp. strains can lead to changes, but these are always minor, especially when compared to common agricultural practices like crop rotation, and these minor changes are transient (Glandorf et al. 2001; Bakker et al. 2002; Viebahn et al. 2003, 2005; Blouin-Bankhead et al. 2004; Timms-Wilson et al. 2004).

Thus, antibiosis is an attractive and powerful mode of action of PGPR *Pseudomonas* spp. strains, but one concern is obviously the occurrence of resistance in the target pathogen against the particular antibiotic, which would lead to loss of biocontrol effectiveness. Indeed, isolates of *G. graminis* var. *tritici* that varied in their sensitivity to PCA and DAPG, were also differentially sensitive to biocontrol by *Pseudomonas* spp. strains producing these antibiotics (Mazzola et al. 1995). Nevertheless, the phenomenon of TAD appears to be based solely on production of the antibiotic DAPG but it is very robust. One explanation could be that the antibiotics do more than just inhibiting growth and/or activity of the pathogen. As a matter of fact we will see below that antibiotic production by fluorescent *Pseudomonas* spp. has been implicated in their ability to induce systemic resistance in plants.

Induced systemic resistance (ISR) and *Pseudomonas* spp. determinants

When appropriately stimulated, plants develop a state of enhanced defensive capacity that is called induced

resistance (Van Loon et al. 1998). ISR was discovered as a mode of action of disease suppression by PGPR *Pseudomonas* spp. independently by two research groups (Van Peer et al. 1991; Wei et al. 1991). In these experiments the PGPR strain and the pathogen were inoculated, and remained, spatially separated on the plant (for instance the PGPR on the root and the pathogen on aboveground plant parts), thereby excluding direct interactions between the two populations. Thus, suppression of disease has to be mediated by the plant. Phenotypically, ISR is similar to systemic acquired resistance (SAR) that is triggered by necrotizing pathogens. SAR requires accumulation of salicylic acid (SA) in the plant (Sticher et al. 1997), since in transgenic plants that constitutively express the *nah*G gene, a *P. putida* salicylate hydroxylase gene, SA cannot accumulate and SAR is not expressed (Gaffney et al. 1993). ISR by *P. fluorescens* WCS417r in *Arabidopsis thaliana* does not depend on accumulation of SA but is dependent on intact responses to ethylene and jasmonic acid (JA) (Pieterse et al. 1998). For *P. aeruginosa* 7NSK2 on tomato it was demonstrated however that the signal transduction pathway that is triggered and leads to ISR against *Botrytis cinerea* is SA dependent, as ISR was abolished in NahG tomato (Audenaert et al. 2002). Interestingly, simultaneously triggering the SA and the ethylene/JA signaling pathway in *A. thaliana*, leads to enhanced disease suppression (Van Wees et al. 2000). Therefore it is suggested that combining bacterial traits that trigger either the SA or the ethylene/JA dependent response can improve biological control. Exploitation of this knowledge in a sensible way requires elucidation of the nature of bacterial triggers of ISR.

Salicylic acid is a *Pseudomonas* metabolite that was suggested to trigger induced resistance (Leeman et al. 1996; De Meyer and Hofte 1997; Maurhofer et al. 1998). For numerous strains of fluorescent pseudomonads, SA production under iron limited conditions has been observed. It is well known that exogenous application of SA to plants leads to induced resistance (Sticher et al. 1997). However, most studies that investigated a role of bacterially produced SA in induced resistance conclude that it is not SA itself that is the microbial signal (Press et al. 1997; Audenaert et al. 2002; Ran et al. 2005b). Interestingly, SA biosynthesis is often linked to the production of SA containing siderophores, like pyochelin in *P. aeruginosa* (Audenaert et al. 2002) or pseudomonine in *P. fluorescens* (Mercado-Blanco et al. 2001), and instead of excreting SA in the rhizosphere these bacteria may well produce only the SA containing siderophore. Cases in which SA is the bacterial signal that triggers induced resistance were observed when strains were genetically modified to produce SA. For instance the *P. aeruginosa* mutant KMPCH no longer produces pyochelin, but can still produce SA. For this mutant Audenaert and associates (2002) clearly demonstrated that production of SA is the main determinant. Likewise, SA biosynthetic genes expressed in a non SA producing *P. fluorescens* strain resulted in improved ISR (Maurhofer et al. 1998).

Other bacterial determinants of *Pseudomonas* spp.-mediated ISR have been reviewed recently (Bakker et al. 2007) and will only be briefly discussed here. Like SA, siderophores are produced by fluorescent pseudomonads under conditions of iron limitation. Whereas their role in disease suppression was thought to be mainly competition for iron with the pathogen (see Sect. ''Competition for Iron...''), several studies have suggested that they can be bacterial signals that trigger ISR. In these studies purified siderophores triggered ISR (Leeman et al. 1996; Meziane et al. 2005; Ran et al. 2005a), and mutants defective in siderophore production were less or noneffective in triggering ISR. In some cases purified siderophores could trigger ISR, but siderophore mutants were as effective as the wild-type strain in ISR induction (Leeman et al. 1996; Meziane et al. 2005). Apparently, multiple determinants of one *Pseudomonas* strain can trigger ISR, and this redundancy becomes clear when one trait is knocked out but the other still leads to effective ISR (Bakker et al. 2003). On the one hand this redundancy hampers mutant studies on bacterial triggers of ISR, on the other hand presence of multiple inducing traits leads to robustness of the system. Additional *Pseudomonas* traits that are involved in ISR include, an iron regulated *N*-alkylated benzylamine derivative (Ongena et al. 2005), the O-antigen of the lipopolysaccharides (LPS) (Van Peer and Schippers 1992; Leeman et al. 1995a, 1996; Van Wees et al. 1997; Meziane et al. 2005), and flagella (Meziane et al. 2005). Like siderophores, the role of antibiotics in biological control was solely associated with direct inhibition of the pathogen. For *P. aeruginosa* 7NSK2 it is now

apparent that the phenazine antibiotic pyocyanin is involved in ISR against *B. cinerea* in tomato (Audenaert et al. 2002). A very interesting recent finding for strain 7NSK2 is that the pyocyanin can have both positive and negative effects on disease development in rice, depending on the pathogen. Whereas pyocyanin production by 7NSK2 elicits ISR against *Magnaporte grisea* it actually enhances susceptibility for *Rhizoctonia solani*, since the wild type did not protect against *R. solani* whereas a pyocyanin mutant did (De Vleesschauwer et al. 2006). The *Pseudomonas* metabolite DAPG, that plays a key role in TAD, was also demonstrated to effectively induce ISR in *A. thaliana* against *Peronospora parasitica* (Iavicoli et al. 2003) and against *P. syringae* pv. *tomato* (Weller et al. 2004), and in tomato against the root-knot nematode *Meloidogyne javanica* (Siddiqui and Shoukat 2003). Recently, additional determinants that influence ISR by fluorescent *Pseudomonas* spp. were reported, like production of *N*-acyl-l-homoserine lactone (Schuhegger et al. 2006), 2,3-butanediol (Han et al. 2006b), and genes involved in diverse functions like a methyl-accepting chemotaxis protein, biosynthesis of purines, phospholipase C, transport of branched-chain amino acids, an ABC transporter, and genes of unknown function (Han et al. 2006a). The multitude of determinants of ISR described in a relatively short time period suggests that many more bacterial determinants will be discovered.

Who is in charge? Quorum-sensing and *gac*A*gac*S regulatory systems

Many of the beneficial *Pseudomonas* spp. traits displayed in interactions with plants are influenced by major regulatory systems. Quorum-sensing (QS) is a widespread gene regulatory mechanism identified in a large number of bacterial species. It plays a crucial role in the physiology, development and environmental behaviour of bacterial communities. It is based on the production and utilization of specific, small signalling molecules called autoinducers, allowing bacterial cells to communicate. By producing such signals, and by ''sensing'' their accumulation, bacteria are able to monitor their population density, and modify the expression of target genes. This response may confer, for example, enhanced environmental and improved defence capabilities to the entire bacterial community. QS systems regulate processes such as antibiotic biosynthesis, biofilm formation, bioluminescence, sporulation, virulence factor gene expression, etc. (Miller and Bassler 2001; Bassler 2002; Von Bodman et al. 2003; Venturi 2006). The most common QS molecular signals in Gram-negative bacteria are *N*-acyl-homoserine lactones (AHLs), which may differ in the length of the acyl-chain moiety and the nature of the substitution at the C3 position (Cámara et al. 1998). However, other signal molecules have been detected, as the designated *Pseudomonas* quinolone signal (PQS) found in the ubiquitous environmental and opportunistic human pathogen *P. aeruginosa* (Pesci et al. 1999). QS systems are known to be based in the activities of two proteins belonging to the LuxI and LuxR families. LuxI-type proteins are cytoplasmic enzymes responsible for the biosynthesis of the intercellular AHLs signals, and LuxR-type proteins are transcriptional regulators. How this mechanism operates has been described and reviewed elsewhere (Fuqua et al. 2001; Miller and Bassler 2001). QS systems have been found in *Pseudomonas* spp., and their regulation has been studied in detail for some cases (excellently reviewed by Venturi 2006). Several plant growth-promoting and biocontrol *Pseudomonas* species also produce QS signals. For example, in *P. aureofaciens* 30-84 two AHL QS systems have been found. The first one to be reported is the PhzI–PhzR QS system (Pierson et al. 1994; Wood et al. 1997) which is involved in the regulation of the phenazine antibiotic biosynthesis operon *phzFABCD*. This antibiotic, when produced in the rhizosphere of wheat, constitutes a key trait for controlling take-all disease. In addition, strain 30-84 has a second QS system, CsaI–CsaR, which is not involved in phenazine regulation, but in both rhizosphere competitiveness and regulation of biosynthesis of cell-surface components (Zhang and Pierson 2001). The PhzI–PhzR QS system also regulates the production of the antifungal metabolite phenazine-1-carboxamide in the plant-beneficial strain *P. chlororaphis* PCL1391. Strain PCL1391 produces additional AHL molecules, but the genetic determinants for the production and response of these AHLs remain unidentified (Chin-A-Woeng et al. 2001, 2005). AHL QS systems have also been reported in biocontrol strain *P. fluorescens* 2P24. In this case, the QS system is composed of the

autoinducer synthase PcoI and the signal receptor PcoR, which has been shown to be involved in biofilm formation, colonization of wheat rhizosphere and in suppressing wheat take-all. Nevertheless, the biocontrol mechanisms regulated are not yet known (Wei and Zhang 2006). Finally, AHL QS systems have been found in *P. putida* WCS358 and IsoF (a PGPR and bioremediation strain). The system identified (PupI–PupR) has shown to be involved in the positive regulation of biofilm development in strain IsoF (Steidle et al. 2002), whereas phenotypes regulated by this system has not yet been found for strain WCS358 (Bertani and Venturi 2004). Attention should be given here to the interesting findings from a broad survey study performed by Elasri and associates (2001). This work shows that autoinducer signal production is common among plant-associated *Pseudomonas* isolates, but was not present in free-living ones. This suggests an important role of AHL QS systems in plant-*Pseudomonas* interactions. Whether the plant can be an active player on some of the process that bacterial QS system regulate is not known, but considering their implication on biofilm formation or on root and/or rhizosphere colonization, it is tempting to speculate on its influence. For example, the ability of beneficial *Pseudomonas* to colonize and to persist in such ecological niches, could be somehow interfered with, favoured, or disrupted by plant determinants. In that sense, it is known that higher plants can produce and secrete AHL-interfering compounds, mostly with stimulatory effects on LuxR-type proteins (Teplitski et al. 2000; Gao et al. 2003; Bauer and Mathesius 2004). However, nothing is known about either the nature of this mimic compounds or their putative interfering effects with bacterial QS systems. Similarly, for beneficial endophytic *Pseudomonas* spp., nothing is currently known about the possible production of QS signals inside the plants, or whether this regulatory mechanism may operate in any process related to endophytic-mediated plant fitness.

Having shown the involvement of QS systems in controlling important traits of beneficial pseudomonads, attention should be called here about an upper level of regulation. Thus, within the regulatory hierarchy of *Pseudomonas* spp. biocontrol traits, the top controlling mechanism is likely the GacS/GacA two-component system. Downstream to this one, QS systems and RNA-binding proteins (such as RsmA or RsmE) and small regulatory RNAs would play leading roles in the GacS/GacA signal transduction pathway (Haas and Keel 2003). Indeed, it has been shown that the GacS/GacA system regulates the expression of QS systems in, for example, the above-mentioned strains PCL1391 (Chin-A-Woeng et al. 2000) and 30-84 (Chancey et al. 1999). However, it must be said that the GacS/GacA system also controls the expression of important pseudomonads biocontrol factors, in which AHL signals have not been detected (for example, Whistler et al. 1998; Bull et al. 2001). This two-component system consists of a sensor kinase (GacS) and a cognate response regulator (GacA). The GacS/GacA system has a decisive influence in controlling (positively or negatively) the expression of a number of beneficial *Pseudomonas* spp. genes involved in plant protection: synthesis of secondary metabolites with antimicrobial activities, synthesis and secretion of enzymes, synthesis of siderophores, impact on cell surface composition, etc., (reviewed by Heeb and Haas 2001; Haas et al. 2002; Haas and Keel 2003). Although, the information lately gathered has improved our knowledge on how GasS/GacA system may regulate biocontrol activities in beneficial *Pseudomonas*, there are still many questions to be answered (Haas et al. 2002). Even so, these authors have proposed a signal transduction pathway model where the GacS/GacA system, upon GacS sensing of certain signals, upregulates the production of certain regulatory RNAs. These regulators may subsequently ease the translational repression of target mRNAs exerted by small RNA-binding proteins. This would take place when bacteria are reaching the exponential growth. Indeed, it has been recently demonstrated that three small GacA-dependent RNAs (RsmX, RsmY and RsmZ) are responsible of the posttranscriptional derepression of biocontrol factors and motility of *P. fluorescens* CHA0, with the positive consequence of protecting cucumber against the oomycete *Pythium ultimun*. This effect is produced when RsmA and RsmE RNA-binding proteins are sequestered by the three small RNAs. Interestingly, expression of these RNAs takes place at different bacterial growth moments: RmsX and RsmY increase along with cell growth, whereas RsmZ does it during the late growth phase (Kay et al. 2005).

Finally, it is also worthy to mention that different sigma factors have been shown to play important

roles in regulating diverse pseudomonads biocontrol traits. For example, the *rpoD* gene encoding the housekeeping sigma factor (σ^{70}) in *P. fluorescens* CHA0 regulates pyoluteorin and DAPG antibiotics production, which improves protection of cucumber against *P. ultimun* (Schnider et al. 1995). Also for strain CHA0, Pechy-Tarr and associates (2005) have shown that the environmental sigma factor RpoN (σ^{54}) is a major regulator of diverse traits important for biocontrol activity in the same pathosystem. Likewise, the stationary-phase sigma factor RpoS (σ^{S}) of strain *P. fluorescens* Pf-5 decisively influences antibiotics production, biological control activity, and survival of this strain on surfaces of developing cucumber seedlings (Sarniguet et al. 1995).

Recent studies are providing new insights into the complex regulatory network controlling important biocontrol traits. In the end, the emerging picture is that new complexities are continuously being added. An illustrative example of this comes from the work of Van den Broek and associates (2005b), which shows that a functional GacA/GacS system, together with RpoS and MutS (involved in mutation repair), play a role in phase variation of *Pseudomonas* sp. PCL1171. This has important consequences since phase variation (mediated by *sss/XerC*-homologues), whose influence in plant root colonization has already been mentioned in this review, has also a relevant regulatory role in biocontrol, both directly (for example, in siderophore production) and indirectly (as in motility and root colonization). In addition, spontaneous mutation in *gacA/gacS* genes is considered one of the mechanisms of phenotypic variation which affects the expression of secondary metabolism, inhibiting, for example, the production of antimicrobial compounds (Van den Broek et al. 2005a).

Concluding remarks

As discussed in this review, rhizosphere inhabiting *Pseudomonas* spp. can reduce plant diseases significantly and both the modes of action and the bacterial traits involved are diverse. Some strains of fluorescent pseudomonads can establish a mutualistic type of interaction by colonizing the plant endophytically. Both the mechanism of entry and unravelling the specific *in planta* activities displayed by these endophytes are as yet unexplored research areas. Exploring them will undoubtedly yield exciting new insights in the interactions between plants and beneficial *Pseudomonas* spp. Exploitation of the beneficial effects of these plant growth promoting *Pseudomonas* bacteria requires a more thorough understanding of their functioning in the highly complex and dynamic rhizosphere environment. As discussed in the last section, expression of bacterial traits involved in biological control of plant pathogens is tightly regulated and AHL signal molecules play an intriguing role in this respect. These AHL molecules have recently also been implicated in the sensing of bacteria by animals, more specifically *Caenorhabditis elegans* (Beale et al 2006). Thus, these molecules play a role in communication within and between bacterial populations (Pierson et al. 1998), in communication between bacteria and plants (Schuhegger et al. 2006) and vice versa (Teplitski et al. 2000), and between bacteria and a nematode. In an environment containing all these organisms, like the rhizosphere, studying these interactions and predicting their outcome undoubtedly constitutes an exciting challenge.

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