Pseudomonadaceae: From Biocontrol to Plant Growth Promotion

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Abstract

Pseudomonas spp. are aerobic, Gram-negative bacteria that are ubiquitously found in soils. They are particularly well suited for plant root colonization and many strains display plant growth-promoting and/or biocontrol activity against various plant pathogens. Their ability to metabolize a wide array of nutrients, their rapidity and ease of growth and their natural abundance in variety of plant-soil environments make them promising organisms for the development of commercial biocontrol and biofertilizer products. In this chapter, we will discuss their diversity, genetics and ecology, while putting special emphasis on the mechanisms involved in biocontrol and/or plant growth promotion. Recent progress in genomics and transcriptomics, as well as future research on these organisms will also be discussed.

3.1 Introduction

The rhizosphere is the narrow zone of soil, influenced by a plant's root system (Rainey 1999). This zone is rich in nutrients when compared with the bulk soil due to the accumulation of a variety of plant exudates, such as amino acids and sugars, providing a rich source of energy and nutrients for bacteria (Gray and Smith 2005). This situation is reflected by the number of bacteria, commonly referred as rhizobacteria (Schroth and Hancock 1982), that are in the rhizosphere, generally 10–100 times higher than that in the bulk soil (Weller and Thomashow 1994). It has been determined that only 1–2 % of bacteria are able to promote plant growth in the rhizosphere (Antoun and Kloepper 2001) and these bacteria are known as plant growth promoting

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rhizobacteria (PGPR). The metabolites produced by PGPR can either directly improve plant growth or indirectly improve plant growth by minimizing the effects of soil-borne plant pathogens, a concept known as biocontrol. In some cases, biocontrol can also be observed alone without plant growth promotion, while both mechanisms often operate together. Some methods of direct plant promotion include production of the phytohormone auxin (Patten and Glick 2002), decrease of plant ethylene levels (Glick 2012) or increase in iron availability through the effects of siderophores (Cézard et al. 2015). Biocontrol mechanisms can include competition, antibiosis (Podile and Kishore 2006) and induced systemic resistance (ISR; Bakker et al. 2007). Bacteria belonging to diverse genera have been identified as PGPR, of which *Pseudomonas* spp. and *Bacillus* spp. are predominant (Podile and Kishore 2006).

3.1.1 The Pseudomonadaceae

Pseudomonas spp. belong to the Pseudomonadaceae, which is a large bacterial family. Created in 1917 by Winslow and colleagues, it belongs to the class of Gammaproteobacteria (Winslow et al. 1917). These organisms are free-living bacteria that are commonly found in water and soil environments. Pseudomonadaceae encompasses four bacterial genera: *Pseudomonas*, *Xanthomonas*, *Gluconobacter* and *Zooglea*. The *Pseudomonas* genus was defined earlier than its family by Migula in 1894 (Migula 1894). At that time, distinction between genera was achieved using bacterial morphological properties. *Pseudomonas* spp. were defined as non-sporulating rod-shape cells which are usually motile. Taxonomy based solely on phenotypical traits was then replaced, due to advances in sequencing technologies, by a classification of *Pseudomonas* species into five "RNA homology" groups (Palleroni et al. 1973). The rRNA group 1 is the largest and encompasses the so called "fluorescent pseudomonads", which will be the focus of this book chapter.

3.1.2 Fluorescent Pseudomonads

Fluorescent pseudomonads are a functional group that comprises *Pseudomonas* species that produce a greenish fluorescent compound, known as pyoverdine, which is a siderophore (Cézard et al. 2015). Pyoverdines are secreted by fluorescent pseudomonads to capture and deliver iron to the cells. Microbial siderophores can also enhance iron uptake by plants that are able to recognize the bacterial ferric-siderophore complex (Masalha et al. 2000; Katiyar and Goel 2004). Over 100 pyoverdines have been identified from different species and strains of *Pseudomonas* (Meyer et al. 2008), representing about 20 % of the microbial siderophores characterized to date (Boukhalfa and Crumbliss 2002).

At the taxonomical level, the fluorescent pseudomonads include phytopathogenic cytochrome *c* oxidase positive species (*Pseudomonas cichorii*, *Pseudomonas marginalis* and *Pseudomonas tolaasii*), non-phytopathogenic, non-necrogenic strains (*Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas chlororaphis*, and

Pseudomonas aeruginosa), and phytopathogenic necrogenic fluorescent *Pseudomonas* species without cytochrome *c* oxidase (*Pseudomonas syringae* and *Pseudomonas viridiflava*) (Choudhary et al. 2009). Various phenotypic methods have been used to cluster and identify bacteria according to several features such as morphology, pigmentation, and nutritional requirements. These methods have shown that *P. fluorescens* and *P. putida* were heterogeneous which led to *P. putida* being subdivided into biotypes A and B (biovars A and B). *P. fluorescens* was also subdivided into seven biotypes. Biotypes A to D and F were named biovars I to V, while biotype D became *P. chlororaphis* and biotype E became *P. chlororaphis* subsp. *aureofaciens* (Palleroni 1984).

3.1.3 Genomics and Genome Plasticity

During the last decade, several plant-associated fluorescent pseudomonad genomes have been sequenced including *P. putida* (Nelson et al. 2002), *P. fluorescens* (Paulsen et al. 2005) and *P. chlororaphis* (Shen et al. 2012). Recent breakthroughs in high-throughput sequencing technologies have been accompanied by an overwhelming increase in the number of *Pseudomonas* spp. genomes publicly available, which has enabled large scale comparative genomic studies. Such studies have highlighted the tremendous genomic diversity of plant-associated *Pseudomonas* spp. (Loper et al. 2012; Jun et al. 2016).

Plant-associated fluorescent pseudomonads possess a large genome which displays a mosaic structure with genes segregating between the "core genome" and the "accessory genome" (Silby et al. 2011). Genes from the core genome are conserved among the different strains which are thought to be responsible for essential cellular processes, whereas genes from the accessory genome, which are often unique to one or few strains, are responsible for the variability observed among strains. While strains from the fluorescent pseudomonad group share a relatively high number of genes, with 2,789 predicted protein-coding genes present in the genome of ten representative strains, corresponding to between 45 % and 52 % of the total number of genes (Loper et al. 2012), the core genome of the whole *Pseudomonas* genus was estimated to contain 1,224 protein-coding genes (Jun et al. 2016).

The genomic diversity and plasticity of plant-associated fluorescent pseudomonads come from their accessory genomes. Out of ten strains from the fluorescent pseudomonad group, 13,872 putative coding-protein genes were identified (Loper et al. 2012), of which 5,798 had no orthologs with other *Pseudomonas* spp. genomes. This diversity is often reflected in the wide range of secondary metabolites produced (Gross and Loper 2009) and it appears that plant-associated saprophytic *Pseudomonas* spp. genomes are a good place to search for operons involved in the production of new antimicrobial compounds (Gross et al. 2007; Van Der Voort et al. 2015).

Pseudomonas spp. genomes are in perpetual rearrangement as evidenced by the low synteny observed between closely-related strains (Silby et al. 2009; Wu et al. 2011; Loper et al. 2012). Mobile genetic elements, such as genomic islands, transposons or REP-elements are also abundant in the *Pseudomonas* spp. accessory

genomes and often account for horizontal gene transfer acquired genomic material (Silby et al. 2011). The genomic diversity and plasticity of saprophytic plant-associated fluorescent pseudomonads is a key feature that empowers their valuable role in terms of plant growth promotion and biocontrol of plant pathogens.

3.2 Rhizosphere Competence of Plant-Beneficial Fluorescent Pseudomonads

Plant-beneficial fluorescent pseudomonads that possess genetic determinants for plant growth promotion and/or biocontrol of plant pathogens are not always effective when deployed in the field. Despite promising results under controlled condition, many authors have reported on the inconsistency of plant-growth promotion and biocontrol achieved by some *Pseudomonas* spp. strains in the field and have linked these results to an impaired rhizosphere colonization (Kloepper et al. 1980; Weller 1988). Rhizosphere colonization is a crucial step leading to disease-suppression given that: (i) an inverse correlation between population size of plant-beneficial *Pseudomonas* spp. and the disease incidence has been observed in several plant-pathogen systems (Bull et al. 1991; Raaijmakers et al. 1995); (ii) impaired rhizosphere colonization mutants, such as those obtained from *P. chlororaphis* subsp. *piscium* PCL1391, lost their disease suppression capability toward certain pathogens, such as *Fusarium oxysporum* f. sp. *radices-lycopersici* (Chin-A-Woeng et al. 2000); (iii) a linear relationship has been observed between population size of indigenous antibiotics producing *Pseudomonas* spp. and antibiotics accumulation in the rhizosphere (Raaijmakers et al. 1999; Mavrodi et al. 2012a), which is in line with biocontrol capabilities.

We define the rhizosphere competence (or rhizocompetence) of an introduced plant-beneficial *Pseudomonas* spp. strain as its aptitude to establish itself in the rhizosphere of a plant and to persist during several crop cycles while maintaining a high population level. It appeals to the capacity of an organism to forge a successful trophic relationship with the plant as well to its ability to compete with indigenous microorganisms coveting the very same ecological niches. Various approaches have been undertaken to identify traits involved in the rhizosphere competence of plant-beneficial *Pseudomonas* spp. including site-directed mutagenesis (Lugtenberg et al. 2001), rhizosphere-induced gene monitoring with promoter-trapping technology (IVET; Rainey 1999) and broader population-based approaches by assessing traits which distinguish fluorescent pseudomonads from the rhizosphere to those isolated from the bulk soil (Latour et al. 2003). Many traits indispensable for rhizosphere colonization have been characterized, such as flagella, chemotaxis, adhesion, etc. (Lugtenberg et al. 2001) often by monitoring the speed at which bacteria reach the root tip of seedlings grown under gnotobiotic conditions with total disregard for the key role of competition in rhizosphere colonization. Nonetheless, comparative studies carried out in agricultural soils have highlighted the superior root-colonizing ability of certain genotypes over others (Raaijmakers and Weller 2001; Ghirardi et al. 2012), and have successfully identified major competitive enhancing traits for rhizosphere colonization (Ghirardi et al. 2012).

3.2.1 Interactions Between Plant-Beneficial *Pseudomonas* spp. and Their Host

3.2.1.1 Rhizoplane Colonization by Plant-Beneficial *Pseudomonas* spp.

Some root exudates, such as malic acid and citric acid, act as chemoattractants for beneficial (and deleterious) bacteria. Hence, motility (De Weger et al. 1987) and especially flagella-driven chemotaxis towards exudate components (De Weert et al. 2002), is an important trait for the rhizosphere competence of *Pseudomonas* spp. In fact, the rhizosphere is not considered as a homogeneous environment but rather as a succession of favourable and less favourable ecological niches; chemotaxis allows *Pseudomonas* spp. to set a course to the most advantageous locations which are generally located at the junctions between epidermal root cells and sites of side roots appearance (Chin-A-Woeng et al. 1997). Adhesion to the root surface is an important mechanism in root colonization, and several determinants have been described. The hair-like structures pili and a root-adhesion outer membrane protein, homologous to OprF from the plant pathogen *P. syringae*, have been shown to be involved in the adhesion to the root surface of several plants by *Pseudomonas* spp. (Vesper 1987; De Mot et al. 1992). The plant root surface glycoprotein agglutinin has been implicated in the adhesion of *P. putida* to the root, an adhesion mediated by the coding-protein gene *aggA* (Anderson 1983; Buell and Anderson 1992). Transition from transient adhesion to irreversible attachment to root surfaces constitutes the first step to the formation of a microcolony (or biofilm), which will soon become a mature biofilm. Lap (large adhesion proteins) has been shown to be involved in this transition in *P. putida* (Hinsa et al. 2003). Biofilms are multicellular aggregates encased in a complex matrix mainly composed of extracellular polymeric substances (EPS), proteins and eDNA (extracellular DNA; Flemming and Wingender 2010). Biofilms enable plant-beneficial *Pseudomonas* spp. to resist harsh conditions including desiccation and high concentrations of toxic compounds (Danhorn and Fuqua 2007).

3.2.1.2 Antibiotic Production

Large populations of antibiotic-producing *Pseudomonas* spp. have been observed in several fields (Raaijmakers et al. 1997; Mazurier et al. 2009; Parejko et al. 2012) and have been frequently associated with disease-suppressiveness (Raaijmakers and Weller 1998; Weller et al. 2002; Mazurier et al. 2009). One may think that the capacity to produce broad-spectrum antibiotics, such as 2,4-diacetylphloroglucinol (DAPG) or phenazine derivatives might enhance the ecological competence throughout antagonism towards competitors. However, it remains to be demonstrated. Carroll et al. (1995) showed that the incapacity to produce DAPG did not reduce the rhizosphere competence of strain *P. fluorescens* F113 in the rhizosphere of sugarbeets. In contrast, phenazine defective mutants of strains *P. synxantha* 2–79 and *P. chlororaphis* subsp. *aureofaciens* 30–84 were not able to maintain high population levels in the rhizosphere of wheat in the presence of indigenous microorganisms, whereas a *P. chlororaphis subsp. aureofaciens* 30–84 phenazine defective mutant colonized to the

same extent as its parent strain when inoculated in the rhizosphere of wheat grown in pasteurized soil (Mazzola et al. 1992). These results suggest the involvement of phenazine production in competitive rhizosphere colonization by plant-beneficial *Pseudomonas* spp., but do not demonstrate that antibiosis is the mechanism involved. It has been suggested that phenazines do not affect the immediate competitors of plant-beneficial *Pseudomonas* spp. (Mavrodi et al. 2006; Pierson and Pierson 2010) and could serve other purposes (Price-Whelan et al. 2006).

3.3 Fluorescent Pseudomonad Mechanisms Leading to Plant Growth Promotion

With regards to plant growth promotion, fluorescent pseudomonads are often divided in two groups, based on their mode of action. The first group, which will be covered in this section, consists of fluorescent pseudomonads that directly influence plant growth, seed emergence or improve crop yields and are often referred as biofertilizers (Glick et al. 1999). The second group is known as biocontrol fluorescent pseudomonads that are able to indirectly influence plant growth by reducing the negative pressure that plant pathogens put on the plant's growth and development.

3.3.1 Direct Plant Growth Promotion

Various mechanisms of direct plant growth promotion have been studied, such as the production of phytohormones, including auxins, cytokinins and gibberellins, the reduction of ethylene levels in plants through the action of ACC-deaminase enzyme and mechanisms to increase nutrient availability in the plant, such as increasing phosphorus uptake by solubilisation of inorganic phosphates, the production of iron-chelating siderophores to increase iron uptake and nitrogen fixation. Only few species of *Pseudomonas* have shown the ability to fix nitrogen, including *Pseudomonas stutzeri* (Krotzky and Werner 1987) and *Pseudomonas azotifigens* (Hatayama et al. 2005). As these species are not part of the fluorescent pseudomonad group, nitrogen fixation will not be further discussed.

Plant growth hormones (e.g. auxins, cytokinins and gibberellins) are synthesized in extremely low concentrations in plants and act as chemical messengers and growth and development regulators in plants (Martínez-Viveros et al. 2010). In addition to being synthesized by plants, these phytohormones are synthesized by a number of bacteria associated with plants and soil (Martinez-Toledo et al. 1988; Bottini et al. 1989). The production of phytohormones by *Pseudomonas* species is considered to be one of their main mechanisms of plant growth promotion (Egamberdieva 2005). To date, auxins are the most well studied phytohormones in rhizobacteria (Karadeniz et al. 2006; Spaepen et al. 2007). Bacterial production of phytohormones is interesting as there is currently no evidence for metabolic effects of phytohormones in bacteria (Persello-Cartiaux et al. 2003).

3.3.1.1 Indole-3-Acetic Acid

Auxin is a phytohormone produced by plants and involved in growth regulation. It was discovered that a majority of bacteria in the rhizosphere are able to produce the auxin indole-3-acetic acid (IAA) and auxins are able to influence plant growth in beneficial and deleterious ways (Patten and Glick 1996). Several pathways for IAA synthesis from L-tryptophan have been investigated, such as the indole-3-pyruvic acid pathway (Costacurta et al. 1994; Patten and Glick 1996), the indole-3-acetamide pathway (Patten and Glick 1996) and the side chain oxidase pathway (Oberhänsli et al. 1991; Patten and Glick 1996).

Many factors can influence the production of IAA levels including the IAA production pathway (Persello-Cartiaux et al. 2003) and the localization of the IAA synthesis genes, either in the bacterial chromosome or on a plasmid (Patten and Glick 1996). The impact of bacterial IAA on plants has been either beneficial or deleterious and its effect seems to depend on the level of IAA produced inherently by the plant (Dubeikovsky et al. 1993; Persello-Cartiaux et al. 2003). In cases where plants produce low levels of IAA, the addition of bacterial IAA can be beneficial on the plant roots. Beneficial effects of bacterial IAA have been shown to stimulate root hair formation and increase the number and length of lateral and primary roots (Davies 1995). When the plant is producing adequate levels of IAA, the addition of bacterial IAA can be detrimental on root length. At deleterious levels, IAA has been shown to be inhibitory to primary root growth (Davies 1995). Cucumber plants inoculated with a wild type IAA producing *P. protegens* CHA0 strain demonstrated enhanced growth, while inoculation with an IAA overproducing mutant stunted the cucumber growth (Beyeler et al. 1999). The IAA overproducing strain *P. fluorescens* BSP53a stimulated root development in black currant, but suppressed root development in sour cherry cuttings (Dubeikovsky et al. 1993). The authors suggest that their results indicate that the amount of IAA produced by black currant (*Ribes nigrum* L.) plants was suboptimal while the amount secreted by sour cherry (*Prunus cerasus* L.) cuttings was already optimal for the plant and the additional level of IAA produced by *P. fluorescens* BSP53a was inhibitory. The range of optimal IAA concentration for plants may be small as *P. putida* GR12-2, a low level producer of IAA, resulted in a two to three fold increase in the length of canola (*Brassica napus* L.) seedling roots (Glick 1995; Caron et al. 1996) while an over-producing mutant of *P. putida* GR12-2 (producing four times the amount of the wild type) significantly inhibited the growth of canola roots (Xie et al. 1996).

3.3.1.2 Cytokinins

Cytokinins are N^6-substituted aminopurines that are synthesized in plant roots and are translocated to the shoots through the xylem. They are involved in multiple functions and act as plant growth regulators and influence plant physiological and developmental processes such as cell division, seed germination, root development, accumulation of chlorophyll, leaf expansion, and delay of leaf and chloroplast senescence (Patrick 1987; Salisbury and Ross 1992; Arshad and Frankerberger 1993; Chernyad'ev 2009). Several natural cytokinins are known and include isopentenyladenine and compounds differing in the presence or absence and location of a

hydroxyl group: zeatin, trans-zeatin, cis-zeatin and dihydrozeatin (Chernyad'ev 2009). At very low concentrations (as low as 10^{-8} M) cytokinin use in plant growth promotion can be efficient, environmentally safe and inexpensive (Chernyad'ev 2009). They have been shown to act in conjunction with auxins. In *in vitro* plant cell cultures, a high cytokinin/auxin ratio promoted shoot production while auxin alone initiated root growth and equimolar amounts of cytokinin and auxin caused undifferentiated callus cells to proliferate (Crozier et al. 2000).

Cytokinins may also be produced by rhizosphere microorganisms that live in close proximity to the root and these cytokinins also may influence plant growth and development (Nieto and Frankenberger 1990; Arshad and Frankerberger 1993; De Salamone et al. 2001). Inoculation of plants with bacteria producing cytokinins has been shown to stimulate shoot growth and reduce root/shoot ratio in plants suffering due to drought (Arkipova et al. 2007).

3.3.1.3 Gibberellins

Gibberellins (GAs) are a large group of important tetracyclic diterpenoid acids and are produced by plants and influence a range of developmental processes in plants including stem elongation, seed germination, seedling emergence, and flower and fruit growth (Davies 1995; Crozier et al. 2000; King and Evans 2003; Sponsel 2003). In most of these processes, gibberellins act in combination with other phytohormones and other regulatory factors, demonstrating highly integrated signaling pathways (Trewavas 2000).

Many GAs have been identified using modern analytical techniques and 136 GAs have been identified in plants, fungi and bacteria (Arshad and Frankerberger 1993; Bottini et al. 2004). Three β-hydroxylated, C19 gibberellins GA_1, GA_3 and GA_4, have all been reported as being directly involved in promotion of shoot elongation in plants (Crozier et al. 2000). Gibberellic acid (GA_3) is the main product of gibberellins in bacteria (Bruckner and Blecschmidt 1991). It is a terpenoid hormone involved in regulating plant growth and development (Karakoç and Aksöz 2006). Gibberellin production has been observed in various *Pseudomonas* spp. *P. putida* H-2-3 that produces bioactive GA_1 and GA_4 significantly increased the growth of a GA-deficient rice (*Oryza sativa* L.) cultivar *Waito-C* (Kang et al. 2014). This strain was also able to enhance plant growth as well as tolerance to drought and salt stresses in soybean (*Glycine max* (L.) Merr.) plants through various mechanisms, including GA production (Kang et al. 2014). Inoculation of GA-producing *Pseudomonas* sp. 54RB led to increased growth and yield in soybean plants (Afzal et al. 2010). GA_3 production is influenced by cultural conditions and these factors include pH, temperature and incubation time (Kahlon and Malhotra 1986).

3.3.1.4 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase

Ethylene is a gaseous phytohormone that acts at low concentrations in the regulation of all processes of plant growth, development and senescence (Shaharoona et al. 2006; Saleem et al. 2007). In addition to acting as a plant growth regulator, it has also been identified as a stress hormone. At high concentrations, ethylene can be harmful as it induces defoliation, premature senescence and cellular processes that inhibit stem and root growth (Li et al. 2005). In response to various environmental

stressors, plants will synthesize 1-aminocyclopropane-1-carboxylate (ACC) which is the immediate precursor for ethylene (Chen et al. 2002; Glick et al. 2007). Some of the ACC produced by the plants is secreted into the rhizosphere and reabsorbed by the plant roots, where it will be converted to ethylene. Accumulation of ethylene in the roots leads to poor root growth and further stress. The ability to degrade ACC by bacteria in the rhizosphere helps in the re-establishment of a healthy root system that can surmount environmental stresses (Martinez-Viveros et al. 2010).

Many bacteria synthesize the enzyme ACC deaminase that will degrade ethylene to α-ketobutyrate and ammonia (Glick et al. 1998; Glick 2005). A significant amount of ACC might be excreted by the plants roots and taken up by soil microorganisms and hydrolyzed by ACC deaminase, decreasing the amount of ACC in the environment, preventing ethylene accumulation in plants and allows the bacteria to use ACC as a nitrogen source (Penrose and Glick 2003; Persello-Cartiaux et al. 2003; Glick 2005).

Pseudomonas strains demonstrating ACC deaminase activity have been isolated in soil (Govindasamy et al. 2008). Reed and Glick (2005) inoculated canola seeds with ACC-deaminase producing *Pseudomonas asplenii* and observed an increase in dry matter content of the root and aerial parts. Arshad et al. (2008) demonstrated that a strain of *Pseudomonas* sp. with ACC deaminase activity was able to partially eliminate the effect of drought stress on the growth of peas. Tomato plants pretreated with *P. fluorescens* and *P. migulae* (both displaying ACC deaminase activity) were healthier and demonstrated better growth under high salinity stress compared to plants pretreated with an ACC deaminase deficient mutant or without bacterial treatment (Ali et al. 2014). *P. fluorescens* strains transformed with ACC deaminase gene and its regulatory region increased length of canola plants (Wang et al. 2000).

3.3.1.5 Phosphate Solubilizing *Pseudomonas* spp.

Phosphorus (P) is an essential plant nutrient for growth and development with low availability in many agricultural soils (Martínez-Viveros et al. 2010). Many soils have a high total P content due to the application of P fertilizers over long periods of time (Dey 1988), however, a large portion of P is present in insoluble forms and is not available for plant nutrition (Mullen 2005).

Phosphate solubilizing bacteria constitute between 1 % and 50 % of the total population of cultivable bacteria in soil (Chabot et al. 1993; Khan et al. 2009). A considerably higher concentration of phosphate solubilizing bacteria is found in the rhizosphere as compared to bulk soil (Katznelson et al. 1962; Raghu and MacRae 1966). The ability of rhizosphere bacteria to solubilize insoluble P minerals has been attributed to their secretion of organic acids (e.g. gluconate, citrate, lactate, and succinate) and phosphatases (Gyaneshwar et al. 1999; Rodríguez and Fraga 1999) to convert the insoluble phosphate into soluble ions (Podile and Kishore 2006). These bacteria solubilize quantities in excess of their nutritional demands, thereby making it available for plants (Chen et al. 2006).

Increased plant growth and phosphate uptake have been reported in many crop species as a result of the inoculation of phosphate solubilizing *Pseudomonas* species, for example in rice (Gusain et al. 2015), in soybean (Fankem et al. 2015; Afzal et al. 2010), in pea (Oteino et al. 2015) and in wheat (Babana and Antoun 2006).

Additionally, Afzal et al. (2010) found increased nodulation in soybean plants that were co-inoculated with *Bradyrhizobium* strain TAL 377 and *Pseudomonas* sp. strain 54RB as compared to only *Bradyrhizobium* TAL 377. They suggest that the increase in nodulation could be due to *Pseudomonas*-induced phosphate solubilisation (as well as an increase in gibberellic acid), which increased root proliferation and stimulated plant growth (Afzal et al. 2010).

3.3.1.6 Siderophores

Hundreds of siderophores have been identified and reported for cultivable microorganisms, some of which are recognized and used by different microorganisms, while other are species-specific (Crowley 2006). These compounds are produced by various types of bacteria in response to iron deficiency, normally occurring in neutral to alkaline pH soils, due to low iron solubility at high pH (Sharma and Johri 2003). Many plants can use various bacterial siderophores as iron sources, although the total concentrations may be too low to significantly contribute to plant iron uptake.

Among most of the bacterial siderophores studied, those produced by *Pseudomonas* species are known for their high affinity to iron. The most abundant siderophore in *Pseudomonas* sp. is pyoverdine. Carrillo-Castañeda et al. (2002) reported positive effects on alfalfa (*Medicago sativa* L.) plantlet growth after the inoculation of siderophore producing *Pseudomonas* sp. grown in iron limited cultures. The inoculated alfalfa seeds increased their germination as well as the root and stem dry weight.

3.3.1.7 Pyrroloquinoline Quinone

Pyrroloquinoline quinone (PQQ) is the main cofactor in redox enzymes names quinoproteins and was first identified in 1979 as a cofactor in bacterial methanol dehydrogenase (Salisbury et al. 1979) and glucose dehydrogenase (Duine et al. 1979). The production of the PQQ molecule is encoded by the *pqq* operon which consists of six core genes, *pqqABCDEF* (Goldstein et al. 2003; Oteino et al. 2015). Additional genes in the PQQ operon (*pqqHIJKM*) have been identified in *P. fluorescens* B16 (Choi et al. 2008). PQQ has antioxidant properties and is involved in plant growth promotion through phosphate solubilisation. Glucose dehydrogenase uses PQQ as a redox cofactor for the oxidation of glucose to gluconic acid. This acid is then diffused into the areas surrounding the bacteria and helps in the acidic solubilisation of insoluble phosphates in soil (Duine et al. 1990; Stites et al. 2000; Misra et al. 2012). Other studies have shown that PQQ is also involved in the biocontrol ability in certain *P. fluorescens* strains (James and Gutterson 1986; de Werra et al. 2009).

In addition to its role in phosphate solubilisation, PQQ is suspected to be directly involved in plant growth promotion. A PQQ mutant of *P. fluorescens* B16 lost its growth promoting ability in tomato, cucumber, Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.), and hot pepper (*Capsicum annuum* (L.)), which were restored when the PQQ genes were complemented in the B16 mutant (Choi et al. 2008). This group also directly applied synthetic PQQ to cucumber plants and saw an increase in the fresh weight of the plants (Choi et al. 2008). They also applied synthetic PQQ to germinating seedlings of Arabidopsis and hot pepper and observed increases in the

fresh and dry weight of Arabidopsis and the size of the cotyledons of the hot peppers, indicating that PQQ is directly involved in plant growth promotion (Choi et al. 2008).

3.3.2 Plant-Beneficial Fluorescent Pseudomonads in Biocontrol

The fluorescent pseudomonads group contains numerous organisms that have the capacity to suppress diseases in several plant-pathogen systems and that can act as effective biological control agents (BCAs; Haas and Défago 2005). In this section, we will attempt to summarize the knowledge gathered on biocontrol of plant pathogens using fluorescent pseudomonads.

3.3.2.1 Plant-Pathogens and Systems Controlled by Fluorescent Pseudomonads

Since the publication of an important review by Weller in 2007, a large number of studies have focused on plant beneficial fluorescent pseudomonads and their antagonistic activity toward plant pathogens. The biocontrol capability of fluorescent pseudomonads is particularly interesting as they exhibit a wide activity and are able to target a broad spectrum of plant pathogens. Among these, the fungus *Gaeumannomyces graminis* var. *tritici*, responsible for the take-all disease of wheat, is the most studied and described plant pathogen system and serves as a model system for *Pseudomonas* spp./pathogen interactions (Kwak and Weller 2013). In this system, *P. protegens* CHA0 has been shown to control the disease through characterized antifungal activity determinants, such as the production of DAPG (Keel et al. 1992). When comparing the biocontrol activity of *P. protegens* CHA0 and a mutant unable to produce DAPG, Keel et al. (1992) found that the DAPG-mutant showed less inhibition of *G. graminis* var. *tritici in vitro*, and less suppression effect on take-all of wheat as compared to the wild-type. However, although various roles of DAPG are known, such as an inducer of plant resistance and a signal molecule that affects gene expression (Dubuis et al. 2007), the precise mode of action of DAPG in disease suppression is still a matter of debate.

An increasing number of papers have been published about new fluorescent pseudomonads demonstrating biocontrol activity, so we present a table listing studies that have occurred since Weller's review published in 2007 (Table 3.1). Most of these studies have focused on fungal diseases, whereas studies describing the biocontrol of bacterial and viral diseases using fluorescent pseudomonads are rare. In this context, in the last 10 years, *Rhizoctonia solani* has been the most investigated pathogen, while the majority of biocontrol stains described belong to the species *P. fluorescens*.

With the recent developments and costs reductions associated with next-generation genome sequencing, genome sequencing of fluorescent pseudomonads of biocontrol interest has been significantly increasing. Next-generation sequencing not only allows the comparison of different biocontrol strains and exploring their functional heterogeneity against their origins (Loper et al. 2012; Rong et al. 2012) but it is also increasingly used to identify genes of biocontrol interest, such as those involved in secondary metabolite biosynthesis (Massart et al. 2015; Roquigny et al. 2015).

Table 3.1 Plant pathogens controlled by plant-beneficial *Pseudomonas* spp. and the mode of action involved in biocontrol

Category of pathogen	Pathogens	Plant system	Biocontrol strain	Biocontrol mechanism	References
Fungi	*Alternaria tenuissima*	Cardoon	*Pseudomons* sp. PS2	Antibiosis (PCA, 2-OH-PHZ, IAA)[a]	Jošić et al. (2012a, b)
	Botrytis cinerea	Tobacco	*P. putida* B001	ISR	Park et al. (2011)
		Alfalfa	*P. fluorescens* UM270	Antibiosis (phenazines, DAPG, HCN, IAA), competition (siderophores), ISR (ACC deaminase, auxin)	Hernández-León et al. (2015)
	Fusarium oxysporum	Mungbean	*Pseudomonas* sp. NAFP-19, NAFP-31 and NAFP-32	Antibiosis, competition	Noreen et al. (2015)
	F. oxysporum f. sp. *radicids-cucumerinum*	Cucumber	*P. aeruginosa* P23	Antibiosis (DAPG), competition (siderophores)	Bradley and Punja (2010)
	F. oxysporum f. sp. *radices-lycopersici*	Tomato	*P. chlororaphis* M71	Antibiosis (potential phenazines and DAPG), competition (siderophores)	Puopolo et al. (2011)
	F. oxysporum	Cymbidium orchids	*Pseudomonas* sp. BRL-1	Competition (siderophores)	Sen et al. (2009)
	Fusarium solani	Mungbean	*Pseudomonas* sp. NAFP-19, NAFP-31 and NAFP-32	Antibiosis, competition	Noreen et al. (2015)
	Gaeumannomyces graminis var. *tritici*	Wheat	*P. fluorescens* JC14-07, HC9-07, and HC13-07	Antibiosis (PCA)	Yang et al. (2011)
		Wheat	*P. fluorescens* VUPf5	Antibiosis (HCN, PCA), competition (siderophores)	Lagzian et al. (2013)
		Barley	*Pseudomonas* sp. DSMZ 13134	Antibiosis, competition, ISR	Frölich et al. (2012)

	Macrophomina phaseolina	Sorghum	*P. fluorescens* SRI-156	Antibiosis (IAA), siderophores	Gopalakrishnan et al. (2011)
		Broadbean	*P. fluorescens* RF36	Antibiosis (IAA)	Devi et al. (2011)
		Mungbean	*Pseudomonas* sp. NAFP-19, NAFP-31 and NAFP-32	Antibiosis, competition	Noreen et al. (2015)
		Safflower	*P. fluorescens* CTPF31	Competition (siderophores)	Govindappa et al. (2011)
		Sunflower	*P. aeruginosa* PF23	Potential antibiosis (biosurfactants)	Tewari and Arora (2014)
	Mucor hiemalis f. sp. *hiemalis*	Cymbidium orchids	*Pseudomonas* sp. BRL-1	Competition (siderophores)	Sen et al. (2009)
	Phytophthora drechsleri	Cucumber	*P. fluorescens* strains	Antibiosis (DAPG, PCA, PLT)	Shirzad et al. (2012)
			P. fluorescens CV6	Antibiosis (HCN), competition (siderophores)	Maleki et al. (2010)
	Phytophthora infestans	Potato	*P. chlororaphis* R47	Antibiosis (HCN, phenazines, PRN, HPR), competition (siderophores)	Guyer et al. (2015)
	Polymyxa betae	Sugar beet	P. putida	Currently unknown mechanism	Aksoy and Kutluk Yilmaz (2008)
	Pyrenophora teres	Barley	*P. fluorescens* MKB156	ISR	Khan et al. (2010)
	Pythium irregulare	Soybean	*P. protogens* WayneR1	Antibiosis (DAPG, HCN, PLT)	McSpadden Gardener et al. (2007) and Rong et al. (2012)
	Pythium myriotylum	Cocoyam	*Pseudomonas* sp. CMR12a	Antibiosis (PCN, HCN, biosurfactants)	Perneel et al. (2007)
	Pythium ultimum	Alfalfa	*P. fluorescens* UP61.2, UP143.8 and UP148.3	Antibiosis (DAPG, HCN, PLT, PRN)	Quagliotto et al. (2009)
	Ralstonia solanacearum	Tomato	*P. brassicacearum* J12	Antibiosis (DAPG, HCN), siderophores	Zhou et al. (2012)
		Eggplant	*Pseudomonas* sp. EB67	Antibiosis (DAPG, IAA) siderophores	Ramesh et al. (2009)

(continued)

Table 3.1 (continued)

Category of pathogen	Pathogens	Plant system	Biocontrol strain	Biocontrol mechanism	References
	Rhizoctonia solani	Potato	*P. aeruginosa* RZ9	Currently unknown mechanism	Mrabet et al. (2015)
		Isabgol	*P. aeruginosa* SD12	Antibiosis (HCN, 1-OH-PHZ), competition (siderophores)	Patra (2012)
		Lettuce	*P. jessenji* RU47	ISR (siderophores)	Adesina et al. (2009)
		Sugar beet	*Pseudomonas poae* RE*1-1-14	Currently unknown mechanism	Müller et al. (2013)
		Bean	*P. fluorescens* UTPF5 (formerly *P. fluorescens* P-5)	Antibiosis (DAPG, HCN)	Afsharmanesh et al. (2010)
		Mungbean	*Pseudomonas* sp. NAFP-19, NAFP-31 and NAFP-32	Antibiosis, competition	Noreen et al. (2015)
		Broad bean	*P. fluorescens* RF36	Antibiosis (IAA)	Devi et al. (2011)
		French bean	*P. fluorescens* strains	Currently unknown mechanism	Negi et al. (2011)
	Rhizoctonia cerealis	Wheat	*P. chlororaphis* subsp. *aurantiaca* Pa40	Antibiosis (2-OH-PHZ, PRN, HCN), competition (siderophores), ISR	Jiao et al. (2013)
	Rhizoctonia oryzae	Wheat	*P. fluorescens* 29G9 and Wood3R, *P. chlororaphis* 48G9	Secondary metabolites (DAPG, PRN, CLP, PCA)	Mavrodi et al. (2012b)
	Sclerotinia sclerotiorum	Tomato	*Pseudomonas* sp. Psf5	Antibiosis, siderophores	Hammami et al. (2013)
	Sclerotium rolfsii	Tomato	*Pseudomonas* sp. PCI2	Antibiosis (IAA)	Pastor et al. (2010, 2012)

Bacteria	*Clavibacter michiganensis* subsp. *Michiganensis*	Tomato	*P. fluorescens* LBUM300	Antibiosis (DAPG, HCN)	Lanteigne et al. (2012)
	Pectobacterium carotovorum subsp. *carotovorum*	Orchids	*Pseudomonas* sp. BRL-1	Competition (IAA, siderophores)	Sen et al. (2009)
	P. carotovorum	Tobacco	*P. putida* B001	ISR	Park et al. (2011)
	Pseudomonas savastanoi	Olive	*P. fluorescens* PCIF7	Unknown antibiosis mechanism	Maldonado-González et al. (2013)
	Streptomyces scabies	Potato	*P. fluorescens* LBUM223	Antibiosis (PCA)	Arseneault et al. (2013, 2014)
Virus	Tobacco mosaic virus	Tobacco	*P. putida* B001	ISR	Park et al. (2011)

[a]*PCA* phenazine 1-carboxylic acid, *2-OH-PHZ* 2-hydroxyphenazine, *IAA* Indole acetic acid, *HCN* hydrogen cyanide, *DAPG* 2,4-diacetylphloroglucinol, *PLT* pyoluteorin, *PRN* pyrrolnitrin, *HPR* 2-hexyl-5-propyl-alkylresorcinol

3.3.2.2 Mechanisms Involved in Disease Suppression

Over the years, it has been demonstrated that fluorescent pseudomonads display numerous capabilities to suppress plant diseases due to various genetic and phenotypic characteristics. To date, several mechanisms of disease suppression have been detected in *Pseudomonas* spp. and the main ones are competition for iron (Berg 2009), plant induced systemic resistance (ISR; Bakker et al. 2007) and antibiosis (Raaijmakers et al. 2002). Once fluorescent pseudomonads are established in the plant rhizosphere, more than one mechanisms of biocontrol may be used in parallel.

3.3.2.2.1 Competition

Previously described as one of the primary ways for bacteria to establish themselves in the rhizosphere, competition is also one of the main mechanisms used by BCAs to compete with plant pathogens for space and nutrients, leading to reduced disease development (Haas and Défago 2005). One of the main nutrients that leads to competition is iron due to its limiting presence in soil (Loper and Buyer 1991). Fluorescent pseudomonads will compete for iron through the production of the siderophore pyoverdine. Siderophores have demonstrated biocontrol capacity under *in vitro* and *in vivo* conditions on pathogenic fungi or fungal-like organisms, including *Pythium* spp. and *Fusarium* spp. (Loper and Buyer 1991; León et al. 2009; Sen et al. 2009). Since the 1990s, it was suggested that siderophores production was dependent on a large range of biotic and abiotic factors (Loper and Buyer 1991; O'Sullivan and O'Gara 1992); notably, the nature and the concentration of nitrogen and carbon sources, the level of phosphate, and the soil's pH and temperature (O'Sullivan and O'Gara 1992). For example, in the case of *P. aeruginosa* PAO1, a high phosphate concentration inhibits pyoverdine production. Succinic acid and ammonium sulphate have been identified to be the best sources of carbon and nitrogen for pyoverdine production with an optimum carbon to nitrogen ratio of 4 to 1 (Barbhaiya and Rao 1985). Loper and Buyer (1991) concluded that among these factors, pH might be the most important for iron availability in soil. More recently, similar studies were performed and demonstrated the importance of minerals and carbon sources for siderophore biosynthesis and therefore for microbial competition (Duffy and Défago 1999; Guiñazú et al. 2010, 2013).

3.3.2.2.2 Induced Resistance

Some physical or chemical stresses have been shown to be responsible for an induced state of resistance in plants which protects against pathogenic infections (Pieterse et al. 2014). Resistance can be triggered either by pathogenic or non-pathogenic microorganisms, therefore two main types of resistance have been identified: systemic acquired resistance (SAR), which is usually triggered by pathogenic microorganisms infecting the plant but to a level that does not cause disease development, and induced systemic resistance (ISR) generally triggered by beneficial microorganisms. This distinction between the agents responsible for inducing either SAR or ISR is not always clear and it has been shown that a beneficial microorganism may induce SAR response (Van De Mortel et al. 2012), and a pathogenic

microorganism an ISR response (Pieterse et al. 2014). Finally, in some cases, both SAR and ISR have been shown to be induced in parallel (Van Wees et al. 2000). The focus of this section will be on the typical ISR response generally induced by plant-beneficial *Pseudomonas* spp.

Before considering ISR, it is however important to briefly described SAR, which has been fully reviewed throughout the years (Spoel and Dong 2012; Dangl et al. 2013; Henry et al. 2013; Pieterse et al. 2013, 2014). SAR in plants is triggered after a local activation of immunity through physical or chemical recognition of a pathogen by the plant. Then, a complex network of signals is activated which may lead to a systemic defense response in the plant. Two major types of signals: PTI ((PAMP (pathogen-associated-molecular-pattern)-triggered immunity)) and ETI (effector-triggered immunity) (Thonart et al. 2012), may be involved. Briefly, PTI is considered as the first line of defense of the plant, which is often bypassed by the plant pathogen through suppression of PTI or preventing the pathogen's detection. ETI is instead considered as a manifestation of the second line of defense, often described as a gene-for-gene resistance leading most of the time to a programmed cell death in order to stop the infection. In other words, PTI and ETI are the local defense lines of the plant, which in turn may trigger SAR if the pathogen is capable of escaping these first and second lines of defense. SAR is mediated by the plant hormone salicylic acid (SA). An increase in the SA level throughout the plant is essential for the establishment of SAR (Van Loon and Bakker 2006). According to current knowledge, SAR seems to remain active for the lifetime of the plant in spite of a lessened induced state that can be observed over time (Van Loon and Bakker 2006).

On the other hand, ISR is a typical mode of action of plant-beneficial *Pseudomonas* spp. in disease suppression. During ISR, bacterial determinants like flagella and secondary metabolites such as DAPG, lipopolysaccharides, and siderophores are detected by the plant host, leading to the secretion of hormonal mediators (Bakker et al. 2013). ISR is mediated by jasmonic acid (JA) and ethylene (ET). Many good reviews have been published on the subject (Bakker et al. 2007, 2013; Pieterse et al. 2014). According to the review by Bakker et al. (2007), noteworthy determinants of *Pseudomonas* spp. are the production of siderophores such as pseudobactin (pyoverdine) and antibiotics such as DAPG. More recently, the capacity of *P. chlororaphis* subsp. *aurantiaca* Pa40 in eliciting an ISR response in wheat infected by *Rhizoctonia cerealis* was linked to the production of phenazines rather than other bacterial determinants (Jiao et al. 2013).

Following the accumulation of JA, ET (in ISR) and/or SA (in SAR) different metabolic cascades connected with the different pathways (ISR or SAR) result in systemic chemical changes in the plant such as the release of proteins or defense strengthening physical barriers of the plant (Jones and Dangl 2006; Dangl et al. 2013). For instance, a hypersensitive response, which is associated with a programmed cell death, can be observed as the hallmark of ETI (Spoel and Dong 2012). After the activation of plant-defense pathways, the plant cell wall is reinforced by the deposition of glucan polymers (Spoel and Dong 2012). Current trends are to study a plant's transcriptome to better understand ISR-SAR activation differences. One of the first studies was on *Arabidopsis thaliana* whose resistance was induced

by beneficial *P. fluorescens* WCS417r (Verhagen et al. 2004). This study revealed that in *A. thaliana*, gene activity of the transcription factors implicated in the regulation of JA and ET-dependent defenses was upregulated in the root, but not in the leaves (Verhagen et al. 2004). Walters et al. (2013) reviewed controlling crop diseases using induced resistance and discussed notably that as induced resistance is a host response, its expression under field conditions is likely to be influenced by a number of factors, including the environment, genotype, crop nutrition and the extent to which plants are already induced.

3.3.2.2.3 Antibiosis

Antibiosis is described as the capacity to produce and secrete antibiotic compounds or other antimicrobial diffusible compounds leading to the inhibition of a pathogen's growth and, in most cases, to the reduction of the pathogen's population. Several secondary metabolites produced by fluorescent pseudomonads have been studied and their activity has been demonstrated by comparing the activity of wild-type strains to isogenic non-antibiotic producing mutant strains. These types of studies have shown that antibiosis is one of the most important mechanisms for biocontrol in fluorescent pseudomonads (Siddiqui 2005). Plant-beneficial *Pseudomonas* spp. are able to produce and secrete a wide range of antimicrobial compounds. The best example of this is *P. protegens* CHA0, which can synthetize more than ten compounds displaying antagonistic activity towards pathogens: DAPG, hydrogen cyanide (HCN), pyoluteorin (PLT), pyrrolnitrin (PRN), and multiple phenazine compounds (Haas et al. 1991; Haas and Défago 2005). For a description of the nature, the biosynthesis, and the function of antibiotic compounds produced by fluorescent pseudomonads, readers are referred to several excellent reviews on this topic (Keel et al. 1992; Raaijmakers et al. 2002; Haas and Défago 2005; Fernando et al. 2006; Mavrodi et al. 2006, 2010). As one of the most studied group of antibiotics involved in plant-beneficial *Pseudomonas* spp./pathogen interactions, we will describe in more detail the phenazine derivatives group, their production and their action in the field.

3.3.2.2.4 Phenazines

Phenazines play a vital role in the biocontrol of plant diseases (Tambong and Höfte 2001; Chin-A-Woeng et al. 2003; Mavrodi et al. 2006; D'aes et al. 2011; Hua and Höfte 2015) and may also contribute to biofilm formation and virulence (Price-Whelan et al. 2006; Pierson and Pierson 2010; Selin et al. 2010). The most common phenazine derivatives are pyocyanin, phenazine-1-carboxylic acid (PCA) and phenazine-1-carboxamide (PCN). These compounds appear to improve the stability of colonies by producing a biofilm that allows the bacteria to attach to roots or seeds of plants (Mavrodi et al. 2006). The genes responsible for the production of PCA are organized in an operon of seven genes: *phzABCDEFG* (Mavrodi et al. 2006, 2010). This operon is accompanied by genes involved in the regulation, transport, resistance and PCA conversion to other phenazine derivatives. The phenazine operon is well conserved as the loss of the ability to produce phenazines is usually associated with a reduced ability to survive in the environment (Mavrodi et al. 2013).

As previously indicated, fluorescent pseudomonads can produce a large range of antimicrobial secondary metabolites, however, the capacity to produce a greater number of antibiotics is not necessarily associated with a better biocontrol response (Perneel et al. 2007). The production of HCN, PCA, PCN, PLT and PRN by *Pseudomonas* sp. CMR5c would suggest that this strain could be a perfect biocontrol agent with a broad spectrum activity. However, despite the myriad of secondary metabolites being produced, *Pseudomonas* sp. CMR5c was not as effective as *Pseudomonas* sp. CMR12a, which produces phenazine derivatives, against *Pythium myriotylum* in cocoyam (Perneel et al. 2007). The authors concluded that in this system, phenazines are key factors in the biological control of cocoyam root rot rather than pyrrolnitrin and pyoluteorin.

Another important point to consider concerning phenazines implication in biocontrol is the quantity or the dose being produced by a given BCA. Pathogen destruction is linked to high levels of antibiotics (Haas and Keel 2003) and a decrease in the pathogen population may depend on the concentration of PCA produced by a BCA (Arseneault et al. 2014). This hypothesis is supported by the fact that the level and the timing of antibiotic biosynthetic gene expression depends on the bacterial population density. The higher a bacterial concentration is, the more antibiotic accumulation will occur in soil (Mavrodi et al. 2012a). In general, the scientific community agrees on the necessity to have a minimal threshold of BCA present in order for biocontrol to occur. For phenazine-producing plant-beneficial *Pseudomonas* spp., this level has been estimated between 10^4 and 10^6 CFU/g of root (Raaijmakers and Weller 1998; Haas and Défago 2005). The ability to quantify antibiotics directly in soils is increasingly interesting to scientists. For example, it was observed that for PCA, 100 µM localized produced amounts were sufficient for the inhibition of pathogens (Mendes et al. 2011). It has, however, been suggested that a sub-inhibitory concentration of antibiotics might, in some cases, suppress disease development through the alteration of the transcriptional activity of key pathogenesis genes in the pathogen (Davies et al. 2006; Raaijmakers and Mazzola 2012). Arseneault et al. (2013) have suggested that transcriptional changes in a pathogen leading to reduced virulence due to the exposure to sub-inhibitory concentration of antibiotics is a key factor in biocontrol and could be considered as an independent mechanism of antibiosis (Arseneault et al. 2013). More specifically, the reduction of potato common scab disease symptoms was not linked to a reduction in *Streptomyces scabies* following the inoculation of potato plants with PCA-producing *P. fluorescens* LBUM 223, but to a significant alteration of gene expression, notably genes involved in pathogenicity, suggesting a novel biocontrol mechanism (Arseneault et al. 2017, unpublished results).

3.3.3 Regulation of Biocontrol Mechanisms

As previously mentioned, the production of secondary metabolites is usually dependent on bacterial population density, a phenomenon known as quorum sensing (QS). Once the quorum is reached, bacteria are able to modify the expression of some

operons involved in secondary metabolite biosynthesis by "sensing" the accumulation of small signaling molecules called autoinducers. For *Pseudomonas* spp., N-acyl-L-homoserine lactones (AHL) have been identified as key signal molecules and their synthesis and recognition generally involves the LuxI/LuxR-like protein family system (Lee et al. 2010). QS is involved in many cellular processes such as antibiotic synthesis and biofilm formation. In *P. aeruginosa*, this is the main mechanism for regulating the production of PCA (Pierson and Pierson 1996; Chin-A-Woeng et al. 2003). More recently, in *P. chlororaphis* subsp. *aurantiaca* StFRB508, multiple AHLs produced via two different quorum-sensing systems demonstrated the regulation of a same QS-regulated-function; PCA production (Morohoshi et al. 2013). Biosynthesis of phenazines seems to occur late during the growth phase of *Pseudomonas* spp. (Mavrodi et al. 2006) as it depends on the population density and certain environmental conditions such as temperature, pH, and the availability of certain nutrients (Chin-A-Woeng et al. 2003). Throughout the years, a lot of excellent review articles that have focused on quorum sensing have been published and we refer the reader to these for more details on the subject (Miller and Bassler 2001; Schauder and Bassler 2001; Compant et al. 2005; Williams 2007; Ng and Bassler 2009).

3.4 Conclusions and Future Perspectives

Fluorescent pseudomonads are proven to be an important group of plant growth promoters and biocontrol agents. They are able to utilize various mechanisms to increase plant growth, protect plants from disease, and to colonize and maintain significant populations in the rhizosphere of many different plants. However, many questions remain as to how to utilize our knowledge of root colonization, plant growth promotion and biocontrol activities of *Pseudomonas* species to use these strains in large-scale agricultural contexts. The advent of next-generation sequencing technologies will allow future research to investigate the accessory genomes of many *Pseudomonas* species to better understand their unique plant growth promotion and biocontrol activities. Next-generation sequencing will also allow researchers to focus on the rhizosphere as a whole and better understand the interactions of *Pseudomonas* species with the indigenous rhizosphere population and the plant through transcriptome and metagenome analyses. Better understanding of these complex interactions may gain insight to overcome inconsistent disease control, which remains a major impediment to widespread use and commercialization of plant growth promoting *Pseudomonas* species.

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