Fitness Features Involved in the Biocontrol Interaction of Pseudomonas chlororaphis With Host Plants: The Case Study of PcPCL1606

Eva Arrebola¹,², Sandra Tienda¹,², Carmen Vida¹,², Antonio de Vicente¹,² and Francisco M. Cazorla¹,²*

¹ Departamento de Microbiología, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain, ² Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora” IHSIM, UMA-CSIC, Málaga, Spain

The goal of this mini review is to summarize the relevant contribution of some beneficial traits to the behavior of the species Pseudomonas chlororaphis, and using that information, to give a practical point of view using the model biocontrol strain P. chlororaphis PCL1606 (PcPCL1606). Among the group of plant-beneficial rhizobacteria, P. chlororaphis has emerged as a plant- and soil-related bacterium that is mainly known because of its biological control of phytopathogenic fungi. Many traits have been reported to be crucial during the multitrophic interaction involving the plant, the fungal pathogen and the soil environment. To explore the different biocontrol-related traits, the biocontrol rhizobacterium PcPCL1606 has been used as a model in recent studies. This bacterium is antagonistic to many phytopathogenic fungi and displays effective biocontrol against fungal phytopathogens. Antagonistic and biocontrol activities are directly related to the production of the compound 2-hexyl, 5-propyl resorcinol (HPR), despite the production of other antifungal compounds. Furthermore, PcPCL1606 has displayed additional traits regarding its fitness in soil and plant root environments such as soil survival, efficient plant root colonization, cell-to-cell interaction or promotion of plant growth.

Keywords: Pseudomonas chlororaphis, root colonization, biocontrol, avocado, antifungals

INTRODUCTION

Since the earliest studies, soil has been described as an infinite source of microorganisms with beneficial activities that promote plant health (Waksman and Woodruff, 1940). Inside the soil, the rhizosphere environment is considered the soil-plant root interphase where potentially beneficial rhizobacteria are established. The plant-beneficial microbial life can be actively recruited by the plant rhizosphere (Berendsen et al., 2018) and can finally result in the biological control of the disease (Babalola, 2010). These biocontrol rhizobacteria can use a wide range of mechanisms involved in the suppression of plant pathogens. A diverse range of bacterial genera, such as Bacillus, Pseudomonas, Serratia, Stenotrophomonas, and Streptomyces, has been commonly described as
beneficial rhizobacteria (Berg, 2009). Among them, representatives of the *Pseudomonas* genus have been commonly associated with the rhizosphere and soil habitats (Lugtenberg and Dekkers, 1999). This bacterial genus has also been widely studied due to its ability to produce antifungal compounds, compete for niche and/or nutrients on the rhizosphere, and elicit induced systemic resistance in plants (Haas and Défago, 2005). Currently, many strains belonging to the group of fluorescent *Pseudomonas* are known to enhance plant growth promotion and reduce the severity of various diseases (Ganesan and Kumar, 2005; Mercado-Blanco and Bakker, 2007; Weller, 2007).

The *Pseudomonas fluorescens* complex is one of the most diverse bacterial groups within the *Pseudomonas* genus and comprises more than fifty validly named species and many unclassified isolates (Garrido-Sanz et al., 2017). Many strains of this complex have been isolated from plant-related environments, and several species can be considered beneficial since many are described as plant growth-promoting rhizobacteria and/or minimize the effects of phytopathogens (PGPR; Kang et al., 2006; Raaijmakers et al., 2009). The beneficial effects displayed by some bacteria result from the expression of multiple activities that act directly and indirectly inhibiting pathogen activities and promoting plant health (McSpadden, 2007). To date, a number of studies have characterized the environmental factors that affect the abundance of different pseudomonad populations below ground (Berg et al., 2002; Ownley et al., 2003; Mazzola et al., 2004; Bergsma-Vlami et al., 2005). *Pseudomonas* species most commonly reported to include plant beneficial rhizospheric strains are *Pseudomonas aureofaciens*, *Pseudomonas brassicae*, *Pseudomonas chlororaphis*, *P. fluorescens*, *Pseudomonas Protegens*, and *Pseudomonas putida*.

**BENEFICIAL TRAITS OF RHIZOSPHERIC *Pseudomonas chlororaphis* STRAINS**

Among the beneficial *Pseudomonas* spp., *P. chlororaphis* has evolved to be a common inhabitant of the root environment of many plants. Moreover, it has been extensively reported the role of specific traits that render this bacterium able to be used as an inoculant for biofertilization, phytostimulation, and biocontrol purposes (Bloemberg and Lugtenberg, 2001).

**Subspecies of *Pseudomonas chlororaphis***

*Pseudomonas chlororaphis*, *P. aureofaciens*, and *Pseudomonas aurantiaca* were initially included in the Approved List of Bacterial Names (Skerman et al., 1980) and considered separate species in the first edition of *Berger's Manual of Systematic Bacteriology* by Palleroni (1984). However, the results obtained by Peix et al. (2007) of fatty acid analysis, phenotypic characterization, 16S rRNA gene sequencing, and DNA-DNA relatedness together with the results obtained by Hilario et al. (2004) on the phylogenetic analysis of several housekeeping genes, support the reclassification of *P. aurantiaca* as a later heterotypic synonym of *P. chlororaphis*. The results published by Peix et al. (2007) also reveal that strains of *P. aurantiaca*, *P. aureofaciens*, and *P. chlororaphis* form three clearly distinguishable groups within *P. chlororaphis* that merit the status of subspecies. Therefore, the current classification is *P. chlororaphis* subsp. *chlororaphis* subsp. nov., *P. chlororaphis* subsp. *aureofaciens* subsp. nov., comb. nov. and *P. chlororaphis* subsp. *aurantiaca* subsp. nov., comb. nov. (Peix et al., 2007). Three years later, Burr et al. (2010) added a new subspecies to *P. chlororaphis* and placed it on a distinct branch within this species with the name *P. chlororaphis* subsp. *piscium* subsp. nov. The current reports of sequenced bacterial genomes of *P. chlororaphis* strains (Calderón et al., 2015; Deng et al., 2015; Town et al., 2016; Moreno-Avitia et al., 2017; Biessy et al., 2019) will help to refine the current classification of the *P. chlororaphis* group.

**Main Traits Involved in Biocontrol by *P. chlororaphis***

These aerobic, Gram-negative bacteria are associated with soil and plant roots (González-Sánchez et al., 2010; Calderón et al., 2015; Vida et al., 2017a). Typically, this species possesses plant-colonizing and antagonistic activities against soilborne plant pathogens. Products from secondary metabolism usually mediate antagonism, and can be regulated by the GacS-GacA two component regulatory system. GacS-GacA system governs a complex signal transduction pathway, involving regulatory RNAs and translational repression (Yan et al., 2018; Jahanshah et al., 2019). Simultaneously, Quorum Sensing (QS) is a regulatory systems which is involved in the general biology performance of *P. chlororaphis*, including biofilm formation, antifungal production or exoenzyme secretion. QS is a mechanism of intercellular signaling that makes the bacterial population to act co-ordinately, based in the secretion of diffusible signal molecules (mainly acyl homoserine lactones, or AHL; Venturi, 2006). The use of OMCs and functional studies have revealed a more complex scenario, where the presence of several QS systems can coexist inside the same bacterial cell (Morohoshi et al., 2017), but also the participation of secondary metabolites (such as the antifungals phenazines and/or the resorcinol-related compounds) in final QS regulation (Selin et al., 2010; Brameyer et al., 2015).

Recent reports using OMCs techniques, have allowed a more comprehensive understanding of the potential weaponry that *P. chlororaphis* group could uses to interact with the root plant. For example, presence of different antimicrobial and insecticidal compounds, cyclic peptides, siderophores, bacteriocins, molecules involved in beneficial plant-bacteria interactions, secretions systems, antibacterial proteins, etc., (Loper et al., 2012; Chen et al., 2015; Biessy et al., 2019). Below, the most relevant are summarized (Table 1).

Phenazines are among the most copious secondary metabolites produced by fluorescent pseudomonads, and phenazine-producing microorganisms represent a ubiquitous group of antibiotic-producing bacteria in the environment (Chin-A-Woeng et al., 2000; Mavrodi et al., 2013). Phenazine
TABLE 1 | Summary of main compounds produced by Pseudomonas chlororaphis subspecies with beneficial effects in plant pathogen control.

| Compound                        | Target/beneficial effect                                                                 | Subspecies          | Reference strain | References                      |
|---------------------------------|-------------------------------------------------------------------------------------------|---------------------|------------------|---------------------------------|
| Antibiotics                     |                                                                                          |                     |                  |                                 |
| Phenazine 1-carboxamide         | Antifungal redox-active antibiotic                                                        | Pa, Pe, Pc, Pp      | PCL1391          | Hernández et al., 2004          |
| Phenazine 1-carboxylic acid     | Antifungal redox-active antibiotic                                                        | Pc, Pp              | PCL1391          | Chin-A-Woeng et al., 1998       |
| 2-hydroxy phenazine 1-carboxylic acid | Fungistatic and bacteriostatic                                                        | Pa, Pe, Pc          | GP72             | Li et al., 2016                 |
| Pyrrolnitrin                    | Antifungal compound                                                                       | Pa, Pe, Pc,        | PA23             | Nandi et al., 2015              |
| 2-hexyl, 5-propylresorcinol     | Antifungal compound and signal molecule                                                   | Pa, Pe, Pc          | PCL1606          | Cazorla et al., 2006            |
| 2,4 Diacetylphloroglucinol      | Membrane damage, distribution of mitochondria electron transport chain and inhibition of V-ATPase activity. Antifungal | Pc                 | UFB2             | Deng et al., 2015               |
| Rhzoxin                         | Antifungal                                                                               | Pc                  | MA 342           | Loper et al., 2008              |
| Insecticidal compounds          |                                                                                            |                     |                  |                                 |
| Cyclic peptides                 | Insecticidal, surfactant and antagonistic activity                                         | Pc                  | PCL1391          | Flury et al., 2017              |
| Fit toxin                       | Insecticidal activity                                                                     | Pc, Pe, Pp          | PCL1606          | Flury et al., 2016              |
| Siderophores                    |                                                                                            |                     |                  |                                 |
| Pyoverdine                      | Fe chelation and competition                                                              | Pa, Pe, Pc          | D-TR133          | Barelmann et al., 2003          |
| Achromobactine                  | Fe chelation and competition                                                              | Pa, Pe, Pc          | PCL1606          | Calderón et al., 2015           |
| Hemophore                       | Fe chelation                                                                              | Pp                  | PCL1807          | Biessy et al., 2019             |
| Enzymes and hormones            |                                                                                            |                     |                  |                                 |
| Chitinase                       | Chitin hydrolysis enzyme and antifungal                                                   | Pc, Pe, Pp          | PCL1391          | Flury et al., 2016              |
| Protease                        | Protein hydrolysis enzyme and antifungal                                                  | Pa                  | M71              | Raio et al., 2017               |
| Phosphatase                     | Phosphorus solubilization enzyme                                                          | Pc                  | SZY6             | Ahemad, 2015                    |
| ACC deaminase                   | Plant growth promotion                                                                    | Pa, Pe, Pc, Pp      | 6G5              | Glick, 2014                     |
| PQQ                             | Plant growth promotion                                                                    | Pa, Pe, Pc          | B23              | Nishiyama et al., 1991          |
| IAA                             | Plant growth promotion                                                                    | Pa, Pe, Pc          | O6               | Kang et al., 2006               |
| Volatile                        |                                                                                            |                     |                  |                                 |
| 2,3 butanediol                  | Elcite plant resistance                                                                   | Pa, Pe, Pc          | O6               | Han et al., 2006                |
| Hydrogen cyanide                | Metalloenzymes inhibitor and antifungal                                                   | Pa, Pc, Pe, Pp      | PA23             | Nandi et al., 2015              |
| Hormones                        |                                                                                            |                     |                  |                                 |
| Indol acetic acid               | Plant growth promotion                                                                    | Pa, Pc, Pe, Pp      | O6               | Kang et al., 2006               |

Reference strains published are included. 1Pa: P. chlororaphis subsp. aurantiaca; Pe: P. chlororaphis subsp. aureofaciens; Pc: P. chlororaphis subsp. chlororaphis; Pp: P. chlororaphis subsp. Piscium.

Compounds are redox-active nitrogen-containing heterocyclic molecules and its beneficial role on plant biology is not limited to antibiosis against phytopathogenic microbes (Pierson and Pierson, 2010; Biessy and Filion, 2018; Biessy et al., 2019). Additional effects have been shown for this compound such as triggering induced systemic resistance in plants, reducing the expression of key pathogenicity-related genes of the phytopathogen, or its involvement in the root persistence (Biessy and Filion, 2018). In relation to the bacterial interaction with the plant root, phenazines can be crucial for biofilm formation (Selin et al., 2010). An extensive colonization of the rhizosphere is a prerequisite in efficient disease suppression by preventing pathogen form access to the root (Lugtenberg and Kamilova, 2009). The involvement of phenazines on root colonization has been strengthened because some phenazine compounds could be terminal signaling factors in the QS network of some bacteria, and are directly involved in biofilm formation on biotic surfaces (Dietrich et al., 2006; Selin et al., 2012).

Pyrrolnitrin and the volatile compound hydrogen cyanide, are also among the additional antifungal compounds typically produced by P. chlororaphis strains. Pyrrolnitrin is considered a key compound for fungal biocontrol (Hill et al., 1994) and is becoming even more relevant than phenazines extending its action to eukaryotic organisms (Nandi et al., 2015; Huang et al., 2018). The same observation can be applied to the volatile compound hydrogen cyanide, which also has a broad spectrum of prokaryotic and eukaryotic targets (Nandi et al., 2017; Kang et al., 2018). The biological importance of this broad spectrum of both active compounds would be related to its typical environmental persistence, for example, allowing them to escape from predation (Nandi et al., 2017). Related to the insecticidal activity of this bacterial species, the most studied virulence factor against insects is the Fit toxin.
which is similar to Mcf1 of the entomopathogenic bacterium Photorhabdus luminescens (Ruffner et al., 2015). Fit mutants of P. chlororaphis PCL1391 further showed reduced virulence, and the residual toxicity could be assigned to the wide range of other antimicrobial compounds produced by P. chlororaphis (previously listed) or cyclic lipopetides (Flury et al., 2017).

About Clps, these compounds can be involved in many biological functions, such as motility, biofilm formation, protection against predators and antagonists (De Souza et al., 2003; Raaijmakers et al., 2010). Clps produced by plants-beneficial bacteria were found to induce plant resistance and to contribute to plant protection against root pathogenic fungi (Olorunleke et al., 2015). But interestingly, Clps were demonstrated to be further insect pathogenicity factor in P. chlororaphis strains (Flury et al., 2016, 2017).

The production of exoenzymes has also been described to have a role in biocontrol activity (Haran et al., 1996). Enzymes such as chitinases, lipases or proteases have a broad distribution among the soil bacterial community and are probably related to general metabolism, but also inhibit the pathogen (degrading some cell structures) and stimulate plant growth by providing additional resources from the degradative activity (Vida et al., 2017a). Remarkably, P. chlororaphis strains can produce 1-aminoacyclopropane-1-carboxylate (ACC) deaminase (Nadeem et al., 2007), which is an enzyme produced by plant-associated bacteria that decrease the ethylene levels and protect the plant from its effect, which results in a general beneficial activity (Glick, 2014). In addition, the production of the biofertilizer hormone indole-3-acetic acid (IAA) has also been reported for P. chlororaphis strains (Dimkpa et al., 2012), and its production is important in microbe-microbe and microbe-plant signaling, and can also result in an promotion of plant growth (Kang et al., 2006).

Other compounds can also have an important role for P. chlororaphis, such as the production of siderophores, which can be considered as a general beneficial activity, at least, for all the soil-related Pseudomonas spp. (Zhang and Rainey, 2013). These molecules are secondary metabolites involved in iron quelation. The most known is pyoverdine, a water-soluble fluorescent pigment produced by fluorescent Pseudomonas species (Barelmann et al., 2003). However, the recent comparative genomic studies of P. chlororaphis genomes, revealed the putative presence of various secondary siderophores, such as achrromobactine and hemophore (Biessy et al., 2019).

THE BENEFICIAL RHIZOBACTERIUM Pseudomonas chlororaphis PCL1606 (PcPCL1606) AS A MODEL

In order to find potential bacterial biocontrol agents against the avocado white root rot caused by Rosellinia necatrix, a collection of bacterial isolates belonging to the genera Bacillus and Pseudomonas were isolated from avocado rhizosphere (Cazorla et al., 2006, 2007; Pliego et al., 2011). Interestingly, a number of P. chlororaphis were consistently isolated from avocado roots (Cazorla et al., 2006). The management of this crop could enhance this presence on avocado roots of P. chlororaphis isolates, since it has been reported that application of organic amendments can enhance the presence of specific groups of beneficial microbes, including antagonistic P. chlororaphis (Vida et al., 2016).

PcPCL1606 as a Biological Control Agent

Nearly all the P. chlororaphis isolated from avocado roots were antagonistic and produced a broad range of antimicrobials including phenazines. Among them, the strain PcPCL1606 do not produce phenazines; otherwise produce proteases, lipases and the antifungal metabolite 2-hexyl 5-propylresorcinol (HPR; Figure 1). Another unusual characteristic of this strain is the absence of plant growth promotion in the assayed plant models; however, siderophore production and phosphorous solubilization were detected (among other PGPR-related traits; Vida et al., 2017a). This strain displayed strong antagonism to many phytopathogenic fungi and showed biocontrol of crown and root rot of tomato, caused by Fusarium oxysporum f. sp. radicis-lycopersici and avocado white root, caused by R. necatrix (Cazorla et al., 2006; Gonzalez-Sanchez et al., 2013). Effectiveness of biocontrol was directly related to the compound HPR (Cazorla et al., 2006; Calderon et al., 2013). HPR production was led by three biosynthetic genes located in a cluster (darA, darB, and darC) followed by two independent regulatory genes (darS and darR; Nowak-Thompson et al., 2003; Calderon et al., 2013). Further experiments revealed that HPR production was also under transcriptional regulation of the GacS-GacA two-component regulatory system, as previously described for other antifungal antibiotics (Haas and Keel, 2003), and also modulated by different growth parameters such as temperature, pH and the presence of salts in the medium (Calderon et al., 2014a).

Main Features of PcPCL1606 Involved in Pathogen and Plant Interaction

PcPCL1606 showed strong antifungal activity (Figure 1), and HPR production was the main determinant in the antagonistic and biocontrol phenotypes (Calderon et al., 2013). In addition to HPR, other antifungals can be produced by PcPCL1606, such as pyrrolnitrin (PRN) or hydrogen cyanide (HCN), as well as several exoenzymes such as proteases, chitinases or phosphatases (Vida et al., 2017a). Nevertheless, HPR is more than a powerful compound against pathogenic fungi in the soil and could have additional roles. It has been reported that some alkylresorcinols (to which the compound HPR belongs) can behave as quorum sensing-like signal molecules in the genus Photorhabdus (Brameyer et al., 2015), and for this, could have a similar role in HPR-producing P. chlororaphis strains. Thus, additional HPR-dependent traits, which are different from antagonism, could have an essential role in the beneficial effects of PcPCL1606 on the plant, such as the root colonization or the biofilm formation (Calderon et al., 2014b, 2019).

Related to the possibility to physically exclude the pathogen from the plant root habitat (Figure 1), biological processes, such as biofilm formation or chemotaxis, are crucial for the PcPCL1606. PcPCL1606 is strongly attracted to the avocado
As a result of this attraction, PcPCL1606 efficiently colonizes avocado roots (González-Sánchez et al., 2010) and can be found forming a biofilm on avocado root surfaces, located in the same area where R. necatrix can be found during the early stages of infection (Calderón et al., 2014b). Moreover, two bacteriocins (R-tailocins 1 and 2), recently described in PcPCL1606 would contribute to better competition against other rhizosphere-associated bacteria (Dorosky et al., 2017). However, PcPCL1606 bacterial cells also displayed a direct chemotaxis to fungal exudates and finally showed a direct contact with the fungal hyphae of R. necatrix. This cell-to-cell contact causes an increase in stress symptoms on the hyphae, among others, by the direct release of antifungal substances, which lead to an accelerated ageing process in the hyphae and hyphal death (Calderón et al., 2014b; Moore-Landecker, 1996). Moreover, the root colonization ability and biofilm formation of the wild-type strain was also related to HPR production, and the absence of HPR resulted in reduced root colonization levels and no biofilm formation by PcPCL1606 (Calderón et al., 2014b, 2019).

To obtain insight into the features of PcPCL1606, its complete genome sequencing was completed. Phylogenetic studies clustered this strain into the P. chlororaphis clade which is placed into the fluorescent Pseudomonas complex, however, as previously mentioned, PcPCL1606 it is not a typical P. chlororaphis strain (Biessy et al., 2019). Thus, phylogenetic analysis revealed clear differences with the genomes of other biocontrol P. chlororaphis, such as PcPCL1601 or PcPCL1607, also isolated from avocado root (Calderón et al., 2015; Vida et al., 2017b; Biessy et al., 2019). Analysis of PcPCL1606 genome confirmed a lack of phenazine biosynthetic genes, cyclic lipopeptides that are related to the surfactant and insecticidal properties, which are typical for P. chlororaphis.
Biessel, A., Novinscak, A., Blom, J., Léger, G., Thomashow, L., Cazorla, M. (2015). Phosphate-solubilizing bacteria-assisted phytoremediation of Pseudomonas Biessy, A., and Filion, M. (2018). Phenazines in plant-beneficial Berg, G. (2009). Plant-microbe interactions promoting plant growth and health: Berg, G., Roskot, N., Steidle, A., Eberl, L., Zock, A., and Smalla, K. (2002). Plant- P. chlororaphis reached a population density that was enough to reduce isolates from avocado roots, displayed high persistence and facts pointed out to a promising future for the use of biocontrol agents belonging to the specie P. chlororaphis. Regarding the model bacterium PcPCL1606, studies revealed that PcPCL1606, as well as other P. chlororaphis isolates from avocado roots, displayed high persistence and reached a population density that was enough to reduce disease (González-Sánchez et al., 2013). Under commercial greenhouse conditions, applications of PcPCL1606 cells resulted in biocontrol against R. necatrix. Moreover, some other P. chlororaphis isolates from avocado roots, that have different beneficial traits (such as phenazine production or plant growth promotion), could also provide plant protection. These finding suggest that a promising approach to improve P. chlororaphis based biocontrol would be to develop consortia which combine strains with complementary traits resulting in more stable or even enhanced beneficial effects on plants.

**AUTHOR CONTRIBUTIONS**

EA and FC designed the review content. EA, ST, CV, AV, and FC wrote the manuscript. All authors read and approved the final manuscript.

**FUNDING**

This research was supported by the Spanish Plan Nacional I + D + I. Grant AGL2017-83368-C2-1-R and partially supported by the European Union (FEDER). CV and ST were supported by a grant from FPI, Ministerio de Ciencia e Innovación, Spain.

**REFERENCES**

Ahemad, M. (2015). Phosphate-solubilizing bacteria-assisted phytoremediation of metalliferous soils: a review. 3 Biotech 5, 111–121. doi: 10.1007/s13205-014-0206-0 Babalola, O. O. (2010). Beneficial bacteria of agricultural importance. Biotechnol. Lett. 32, 1559–1570. doi: 10.1007/s10529-010-0347-0 Barelmann, I., Fernández, D. U., Budziszewicz, H., and Meyer, J. M. (2003). The pyoverdine from Pseudomonas chlororaphis D-TR133 showing mutual acceptance with the pyoverdine of Pseudomonas fluorescens CHA0. Biometals 16, 263–270. doi: 10.1023/A:1020615830765 Berendes, R. L., Vismans, G., Yu, K., Song, Y., de Jonge, R., Burgman, W. P., et al. (2018). Disease-induced assemblage of a plant-beneficial bacterial consortium. ISME J 12, 1496–1507. doi: 10.1038/s41396-018-0093-1 Berg, G. (2009). Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biotechnol. 84, 11–18. doi: 10.1186/AEBM.68.7.3328-3338.2002 Berg, G., Roskot, N., Steidle, A., Eberl, L., Zock, A., and Smalla, K. (2002). Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different Verticillium host plants. Appl. Environ. Microbiol. 68, 3328–3338. doi: 10.1128/AEM.68.7.3328-3338.2002 Bergsma-Vlami, M., Prins, M. E., and Raaijmakers, J. M. (2005). Influence of plant species on population dynamics, genotypic diversity and antibiotic production in the rhizosphere by indigenous Pseudomonas spp. FEMS Microbiol. Ecol. 52, 59–69. doi: 10.1016/femsme.2004.10.007 Biessy, A., and Filion, M. (2018). Phenazines in plant-beneficial Pseudomonas spp.: biosynthesis, regulation, function and genomes. Environ. Microbiol. 20, 3905–3917. doi: 10.1111/1462-2920.14395 Biessy, A., Novinscak, A., Blom, J., Léger, G., Thomashow, L., Cazorla, M. F., et al. (2019). Diversity of phytobeneficial traits revealed by whole-genome analysis of worldwide-isolated phenazine-producing Pseudomonas spp. Environ. Microbiol. 21, 437–455. doi: 10.1111/1462-2920.14476 Bloemberg, G. V., and Lugtenberg, B. J. (2001). Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr. Opin. Plant Biol. 4, 343–350. doi: 10.1016/S1369-5266(00)00183-7 Brameyer, S., Kresovic, D., Bode, H. B., and Heermann, R. (2015). Dialkylresorcinols as bacterial signaling molecules. Proc. Natl. Acad. Sci. U.S.A. 112, 572–577. doi: 10.1073/pnas.1417685112 Burr, S. E., Gobeli, S., Kuhnert, P., Goldschmidt-Clermont, E., and Frey, J. (2010). Pseudomonas chlororaphis subsp. Piscium subsp. nov., isolated from freshwater fish. Int. J. Syst. Evol. Microbiol. 60, 2753–2757. doi: 10.1099/ijs.0.011692-0 Calderón, G. E., Carrión, V. J., de Vicente, A., and Cazorla, F. M. (2014a). darR and dar are regulatory genes that modulate 2-hexyl, 5-propyl resorcinol transcription in Pseudomonas chlororaphis PCL1606. Microbiology 160, 2670–2680. doi: 10.1099/mic.0.082677-0 Calderón, G. E., de Vicente, A., and Cazorla, F. M. (2014b). Role of 2-hexyl, 5-propyl resorcinol production by Pseudomonas chlororaphis PCL1606 in the multiotic interactions in the avocado rhizosphere during the biocontrol process. FEMS Microbiol. Ecol. 89, 20–31. doi: 10.1111/1574-6941.12319 Calderón, C. E., Pérez-García, A., de Vicente, A., and Cazorla, F. M. (2013). The dar genes of Pseudomonas chlororaphis PCL1606 are crucial for biocontrol activity via production of the antifungal compound 2-hexyl, 5-propyl resorcinol. Mol. Plant Microbe Interact. 26, 554–565. doi: 10.1094/MPMI-01-13-0012-R Calderón, C. E., Ramos, C., de Vicente, A., and Cazorla, F. M. (2015). Comparative genomic analysis of Pseudomonas chlororaphis PCL1606 reveals new insight into antifungal compounds involved in biocontrol. Mol. Plant Microbe Interact. 28, 249–260. doi: 10.1094/MPMI-10-14-0326-FI Calderón, C. E., Tienda, S., Heredia-Ponce, Z., Arezebola, E., Cárcamo-Oyarce, G., Eberl, L., et al. (2019). The compound 2-hexyl, 5-propyl resorcinol has a key role in biofilm formation by the biocontrol rhizobacterium Pseudomonas chlororaphis PCL1606. Front. Microbiol. 10:396. doi: 10.3389/fmicb.2019.00396 Cazorla, F. M., Duckett, S. D., Bergström, E. T., Odijk, R., Lugtenberg, B. J. J., Thomas-Oates, J. E., et al. (2006). Biocontrol of avocado dermatophora root rot by antagonistic Pseudomonas fluorescens PCL1606 correlates with the production of 2-hexyl, 5-propyl resorcinol. Mol. Plant Microbe Interact. 19, 418–428. doi: 10.1094/MPMI-19-0418-C Cazorla, F. M., Romero, D., Pérez-García, A., Lugtenberg, B. J. J., de Vicente, A., and Blomberg, G. (2007). Isolation and characterization of antagonistic Bacillus subtilis strains from the avocado rhizoplane
González-Sánchez, M. A., Pérez-Jiménez, R. M., Pliego, C., Ramos, C., de Glick, B. R. (2014). Bacteria with ACC deaminase can promote plant growth and Garrido-Sanz, D., Arrebola, E., Martínez-Granero, F., García-Méndez, S., Pseudomonas fluorescens Flury, P., Aellen, N., Ruffner, B., Péchy-Tarr, M., Fataar, S., Metla, Z., et al. Deng, P., Wang, X., Baird, S. M., and Lu, S. E. (2015). Complete genome of Dorosky, R. J., Yu, J. M., Pierson, L. S. III, and Pierson, E. A. (2017). Frontiers in Microbiology | www.frontiersin.org 7

Dimkpa, C. O., Zeng, J., McLean, J. E., Britt, D. W., Zhan, J., and Anderson, A. J. (2012). Pathway in the plant-beneficial bacterium Pseudomonas chlororaphis O6 is inhibited by ZnO nanoparticles but enhanced by CuO nanoparticles. Appl. Environ. Microbiol. 78, 1404–1410. doi: 10.1128/AEM.07424-11

Dorosky, R. J., Yu, J. M., Pierson, L. S. III, and Pierson, E. A. (2017). Pseudomonas chlororaphis produces two distinct R-tailocins that contribute to bacterial competition in biofilm and on roots. Appl. Environ. Microbiol. 83:706–17. doi: 10.1128/AEM.00706-17

Flury, P., Aellen, N., Ruffner, B., Péchy-Tarr, M., Fataa, S., Metla, Z., et al. (2016). Insect pathogenicity in plant-beneficial pseudomonads: phylogenetic distribution and comparative genomics. ISME J. 10, 2525–2542. doi: 10.1038/ismej.2016.5

Flury, P., Vesga, P., Péchy-Tarr, M., Aellen, N., Dennert, F., Hofer, N., et al. (2017). Antimicrobial and insecticidal: cyclic lipopeptides and hydrogen cyanide produced by plant-beneficial Pseudomonas strains CHA0, CMR12a, and PCL1391 contribute to insect killing. Front. Microbiol. 8:100. doi: 10.3389/fmicb.2017.00100

Ganesan, G., and Kumar, A. M. (2005). Pseudomonas fluorescens a potential bacterial antagonist to control plant diseases. J. Plant Interact. 13, 123–134. doi: 10.1080/1742910600907043

Garrido-Sanz, D., Arrebola, E., Martínez-Granero, F., García-Méndez, S., Muriel, C., Blanco-Romero, E., et al. (2017). Classification of isolates from bacterial antagonist to control plant diseases. Microbiol. Cell Fact. 16: doi: 10.1186/s40793-015-0106-x

Hilario, E., Buckley, T. R., and Young, J. M. (2004). Improved resolution on the phylogenetic relationships among Pseudomonas by the combined analysis of atpD, carA, recA and 16S rDNA. A van Leeu. J. Microbiol. 86, 51–64. doi: 10.1023/B:ANTO.000002910.15711.17

Hill, D. S., Stein, J. I., Torkerwitz, N. R., Morse, A. M., Howell, C. R., Pachlatko, J. P., et al. (1994). Cloning of genes involved in the synthesis of pyrrolnitrin from Pseudomonas fluorescens and role of pyrrolnitrin synthesis in biological control of plant disease. Appl. Environ. Microbiol. 60, 78–85. doi: <doi>

Huang, R., Feng, Z., Chi, X., Sun, X., Lu, Y., Zhang, B., et al. (2018). Pyrrolnitrin is more essential than phenazines for Pseudomonas chlororaphis G05 in its suppression of Fusarium graminearum. Microbiol. Res. 215, 55–64. doi: 10.1016/j.micres.2018.06.008

Jahanshah, G., Yan, Q., Gerhardt, H., Patai, Z., Lämmerhofer, M., Pianet, I., et al. (2019). Discovery of the cyclic lipopeptide gacamide A by genome mining and repair of the defective GacA regulator in Pseudomonas fluorescens PB1-1. J. Nat. Prod. 82, 301–308. doi: 10.1021/acs.canmet.9b00074

Kang, B. R., Anderson, A. J., and Kim, Y. C. (2018). Hydrogen cyanide produced by Pseudomonas chlororaphis O6 exhibits nematocidal activity against Meloidogyne hapla. Plant Pathol. J. 34, 35–43. doi: 10.4253/PPJ.OA.2016.0115

Kang, B. R., Yang, K. Y., Cho, B. H., Han, T. H., Kim, I. S., Lee, M. C., et al. (2006). Production of indole-3-acetic acid in the plant-beneficial strain Pseudomonas chlororaphis O6 is negatively regulated by the global sensor kinase GacS. Curr. Microbiol. 52, 473–476. doi: 10.1007/s00284-005-0427-x

Liu, K., Hu, H., Wang, W., and Zhang, X. (2016). Genetic engineering of Pseudomonas chlororaphis GP72 for the enhanced production of 2-hydroxyphenazine. Microb. Cell Fact. 15: 13. doi: 10.1186/s12931-016-0529-0

Loper, J. E., Hassan, K. A., Mavrodi, D. V., Davis, E. W. I. I., Lim, C. K., Shaffer, B. T., et al. (2012). Comparative genomics of plant-associated Pseudomonas spp.: insight into diversity and inheritance of traits involved in multitrophic interactions. PLoS Genet. 10:e1002784. doi: 10.1371/journal.pgen.1002784

Loper, J. E., Henkels, M. D., Shaffer, B. T., Valieroti, F. A., and Gross, H. (2008). Isolation and identification of rhizoxin analogs from Pseudomonas fluorescens Pf-5 by using a genomic mining strategy. Appl. Environ. Microbiol. 74, 3085–3093. doi: 10.1128/AEM.02848-07

Lugtenberg, B. J. J., and Dekkers, L. C. (1999). What makes Pseudomonas bacteria rhizosphere competent? Environ. Microbiol. 1, 9–13. doi: 10.1046/j.1462-2920.1999.00005.x

Lugtenberg, B. J. J., and Kamioiva, F. (2009). Plant growth-promoting rhizobacteria. Annu. Rev. Microbiol. 63, 541–556. doi: 10.1146/annurev.micro.62.081307.162918

Mavrodi, D. V., Pareiko, J. A., Mavrodi, O. V., Kwak, Y. S., Weller, D. M., Blankenfeld, W., et al. (2013). Recent insights into the diversity, frequency and ecological roles of phenazines in fluorescent Pseudomonas spp. Environ. Microbiol. 15, 675–686. doi: 10.1111/1462-2920.2012.02846.x

Mazzola, M., Funnell, D. L., and Raaijmakers, J. M. (2004). Wheat cultivar-specific selection of 2,4-diacetylphloroglucinol-producing fluorescent Pseudomonas species from resident soil populations. Microbiol. Ecol. 48, 338–348. doi: 10.1007/s00248-003-0167-y

McSpadden, B. (2007). Diversity and ecology of biocontrol Pseudomonas spp. in agricultural systems. Phytopathology 97, 221–226. doi: 10.1094/PHYTO-97-2-0221
Mercado-Blanco, J., and Bakker, P. A. H. M. (2007). Interactions between plants and beneficial Pseudomonas spp.: exploiting bacterial traits from crop protection. Antonie Van Leeuwen. 92, 367–389. doi: 10.1007/s10482-007-9167-1

Moore-Landecker, E. (1996). Fundamentals of the Fungi, 4th Edn. Saddle River, NJ: Prentice Hall. doi: <doi>

Moreno-Avía, F., Lozano, L., Utrilla, J., Bolívar, F., and Escalante, A. (2017). Draft genome sequence of Pseudomonas chlororaphis ATCC 9446, a nonpathogenic bacterium with bioremediation and industrial potential. Genome Announc. 5: e474–17. doi: 10.1128/genomeA.00474-17

Morohoshi, T., Yamaguichi, T., Xie, X., Wang, W. Z., Takeuchi, K., and Someya, N. (2017). Complete genome sequence of Pseudomonas chlororaphis subsp. Aurantiaca reveals a triplicate quorum-sensing mechanism for regulation of phenazine production. Microbes Environ. 32, 47–53. doi: 10.1264/jmem2.16162

Nadeem, S. M., Zahir, Z. A., Naveed, M., and Arshad, M. (2007). Preliminary investigation on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Can. J. Microbiol. 53, 1141–1149. doi: 10.1139/W07-081

Nandi, M., Selin, C., Brassinga, A. K. C., Belmonte, M. F., Fernando, W. G. D., Loewen, P. C., et al. (2015). Pyrorrinitin and hydrogen cyanide production by Pseudomonas chlororaphis PA23 exhibits nematicidal and repellent activity against Caenorhabditis elegans. PLoS One 10:e0123184. doi: 10.1371/journal.pone.0123184

Nandi, M., Selin, C., Brawerman, G., Fernando, W. G. D., and de Kevit, T. (2017). Hydrogen cyanide, which contributes to Pseudomonas chlororaphis strain PA23 biocontrol, is upregulated in the presence of glycine. Biol. Control 108, 47–54. doi: 10.1016/j.biocontrol.2017.02.008

Nishiyama, M., Hirouchi, M., Kohayashi, M., Yamada, H., and Beppu, T. (1991). Cloning and characterization of genes responsible for metabolism of nitrile compounds from Pseudomonas chlororaphis B23. J. Bacteriol. 173, 2465–2472. doi: 10.1128/jb.173.8.2465-2472.1991

Nowak-Thompson, B., Philip, E., Hammer, D., Hill, D. S., Staffords, J., Nowak-Thompson, B., Philip, E., Hammer, D., Hill, D. S., Staffords, J., Torkewitz, N., et al. (2003). 2, 5-diakylresorcinol biosynthesis in Pseudomonas chlororaphis subsp. Aureofaciens strain M71 and Seiridium cardinal in vivo. Microb. Environ. 19, 49–56. doi: 10.1006/mier.2017.03.003

Ruffner, B., Pék, T., Hötte, M., Blumberg, B., Grunder, J., Keel, C., et al. (2015). Evolutionary patchwork of an insecticidal toxin shared between plant-associated pseudomonads and the insect pathogens Photorhabdus and Xenorhabdus. BMC Genomics 16:609. doi: 10.1186/s12869-015-1763-2

Selin, C., Florido, D., and de Kevit, T. R. (2012). The Pfhl/Phr2 quorum-sensing system is required for pyrorrinitin and phenazine production, and exhibits cross-regulation with Rpops in Pseudomonas chlororaphis PA323. Microbiology 158, 896–907. doi: 10.1099/mic.0.05425-0

Selin, C., Habibian, R., Poritsanos, N., Athukorala, S. N., Fernando, D., and de Kevit, T. R. (2010). Phenazines are not essential for Pseudomonas chlororaphis PA23 biocontrol of Sclerotinia sclerotiorum, but do play a role in biofilm formation. FEMS Microbiol. Ecol. 71, 73–83. doi: 10.1111/j.1574-6941.2009.00792.x

Skerman, V. B. D., McGowan, V., and Sneath, P. H. A. (1980). Approved lists of bacterial names. Int. J. Syst. Bacteriol. 30, 225–420. doi: 10.1099/0027713-30-1-225

Town, J., Audy, P., Boyetchko, S. M., and Dumonceaux, T. J. (2016). Genome sequence of Pseudomonas chlororaphis strain 189. Genome Announc. 5:e581–16. doi: 10.1128/genomeA.00581-16

Venturi, V. (2006). Regulation of quorum sensing in Pseudomonas. FEMS Microbiol. Rev. 30, 274–291. doi: 10.1111/j.1574-6976.2005.00012.x

Vida, C., Bonilla, N., de Vicente, A., and Cazorla, F. M. (2016). Microbial profiling of a suppressiveness-induced agricultural soil amended with composted almond shells. Front. Microbiol. 7:4. doi: 10.3389/fmicb.2016.00004

Vida, C., Cazorla, F. M., and de Vicente, A. (2017a). Characterization of biocontrol bacterial strains isolated from a suppressiveness-induced soil after amendment with composted almond shells. Res. Microbiol. 168, 583–593. doi: 10.1016/j.resmic.2017.03.007

Vida, C., de Vicente, A., and Cazorla, F. M. (2017b). Draft genome sequence of the rhizobacterium Pseudomonas chlororaphis PCL1606, displaying biocontrol against soilborne phytopathogens. Genome Announc. 5:e130–17. doi: 10.1128/genomeA.00130-17

Wakoman, S. A., and Woodruff, B. H. (1940). The soil as a source of plant pathogenic bacteria with bioremediation and industrial potential. Science 100, 141–143. doi: 10.1126/science.100.2429.353

Weller, D. (2007). Pseudomonas biocontrol agents of soilborne pathogens: looking back over 30 years. Phytopathology 97, 250–256. doi: 10.1094/PHYTO-97-2-050

Yan, Q., Lopes, L. D., Shaffer, B. T., Kidarsa, T. A., Vining, O., Philmus, B., et al. (2018). Secondary metabolism and interspecific competition affect accumulation of spontaneous mutants in the GacS-GacA regulatory system in Pseudomonas protegens. mBio 9:e1845–17. doi: 10.1128/mBio.01845-17

Zhang, X. X., and Rainey, P. B. (2013). Exploring the sociology of pyoverdin-producing Pseudomonas. Evolution 67, 3161–3174. doi: 10.1111/evo.12183

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.