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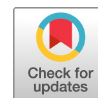

Inhibition of Three Potato Pathogens by Phenazine-Producing *Pseudomonas* spp. Is Associated with Multiple Biocontrol-Related Traits

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ABSTRACT Phenazine-producing *Pseudomonas* spp. are effective biocontrol agents that aggressively colonize the rhizosphere and suppress numerous plant diseases. In this study, we compared the ability of 63 plant-beneficial phenazine-producing *Pseudomonas* strains representative of the worldwide diversity to inhibit the growth of three major potato pathogens: the oomycete *Phytophthora infestans*, the Gram-positive bacterium *Streptomyces scabies*, and the ascomycete *Verticillium dahliae*. The 63 *Pseudomonas* strains are distributed among four different subgroups within the *P. fluorescens* species complex and produce different phenazine compounds, namely, phenazine-1-carboxylic acid (PCA), phenazine-1-carboxamide (PCN), 2-hydroxyphenazine-1-carboxylic acid, and 2-hydroxphenazine. Overall, the 63 strains exhibited contrasted levels of pathogen inhibition. Strains from the *P. chlororaphis* subgroup inhibited the growth of *P. infestans* more effectively than strains from the *P. fluorescens* subgroup. Higher inhibition was not associated with differential levels of phenazine production nor with specific phenazine compounds. The presence of additional biocontrol-related traits found in *P. chlororaphis* was instead associated with higher *P. infestans* inhibition. Inhibition of *S. scabies* by the 63 strains was more variable, with no clear taxonomic segregation pattern. Inhibition values did not correlate with phenazine production nor with specific phenazine compounds. No additional synergistic biocontrol-related traits were found. Against *V. dahliae*, PCN producers from the *P. chlororaphis* subgroup and PCA producers from the *P. fluorescens* subgroup exhibited greater inhibition. Additional biocontrol-related traits potentially involved in *V. dahliae* inhibition were identified. This study represents a first step toward harnessing the vast genomic diversity of phenazine-producing *Pseudomonas* spp. to achieve better biological control of potato pathogens.

IMPORTANCE Plant-beneficial phenazine-producing *Pseudomonas* spp. are effective biocontrol agents, thanks to the broad-spectrum antibiotic activity of the phenazine antibiotics they produce. These bacteria have received considerable attention over the last 20 years, but most studies have focused only on the ability of a few genotypes to inhibit the growth of a limited number of plant pathogens. In this study, we investigated the ability of 63 phenazine-producing strains, isolated from a wide diversity of host plants on four continents, to inhibit the growth of three major potato pathogens: *Phytophthora infestans*, *Streptomyces scabies*, and *Verticillium dahliae*. We found that the 63 strains differentially inhibited the three potato pathogens. These differences are in part associated with the nature and the quantity of the phenazine compounds being produced but also with the presence of additional biocontrol-related traits. These results will facilitate the selection of versatile biocontrol agents against pathogens.

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Potato (*Solanum tuberosum* L.) is an important food crop grown worldwide that contributes to feeding more than one billion people. While accounting for only 2% of the food energy supply (1), potato consumption has increased considerably in developing countries, and potato is regarded as a highly valuable crop that could significantly contribute to global food security (2). Approximately 21% of the attainable potato yield is lost to pathogens and viruses (3), and a large amount of money is spent to control these organisms, primarily by using synthetic pesticides (4, 5). For instance, using fungicides to control potato late blight, a disease caused by the oomycete *Phytophthora infestans*, has been estimated to represent an annual cost of 115.5 million euros in the Netherlands (5), which accounts for 14.7% of the total potato's farm gate price (5). In addition, extensive use of most synthetic pesticides has deleterious consequences on human health and the environment (6, 7). Using naturally occurring bacteria that produce antimicrobial compounds capable of altering pathogen growth, such as plant-beneficial antibiotic-producing *Pseudomonas* spp., could represent a viable and environmentally friendly alternative and/or a complement to the use of synthetic pesticides (8, 9).

Plant-beneficial *Pseudomonas* spp. are ubiquitous rod-shaped Gram-negative bacteria that aggressively colonize the rhizosphere and protect the root system against soil-dwelling plant pathogens (8). Numerous strains produce antibiotic compounds, such as 2,4-diacetylphloroglucinol, pyrrolnitrin, and phenazines, which can inhibit pathogen growth in the rhizosphere (8, 10, 11). Phenazine compounds are particularly interesting because they display broad-spectrum antibiotic activity toward many fungal, oomycete, and bacterial plant pathogens (12, 13) and also because they promote survival and persistence of bacterial cells in the rhizosphere (11, 14). Phenazine production is mediated by a seven-gene operon (15), whose organization is conserved in all phenazine-producing *Pseudomonas* spp. sequenced to date (16, 17). The enzymes encoded by these biosynthetic genes catalyze the biosynthesis of phenazine-1-carboxylic acid (PCA), which is the first and main phenazine molecule produced by phenazine-producing pseudomonads. Some strains harbor additional biosynthetic genes, such as *phzH* and *phzO*, which enable them to produce additional phenazine molecules (18, 19). Strains harboring *phzH* produce phenazine-1-carboxamide (PCN) in addition to PCA (19), while strains harboring *phzO* produce, in addition to PCA, 2-hydroxphenazine (2-OH-PHZ) and 2-hydroxyphenazine-1-carboxylic acid (2-OH-PCA) (18). Interestingly, these four compounds do not seem to possess the same antimicrobial activity depending on the targeted plant pathogen. For example, the introduction of the *phzH* gene in two PCA-producing *Pseudomonas* strains enabled them to produce PCN and to suppress tomato foot and root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (19). Conversely, a *phzH* deletion mutant of *P. chlororaphis* subsp. *piscium* PCL1391 producing only PCA inhibited *Verticillium dahliae* microsclerotia germination more effectively than the wild-type strain producing PCN (19). This suggests that PCN has less activity toward *V. dahliae* microsclerotia than PCA. More recently, Yu et al. generated mutants of *P. chlororaphis* 30-84 producing different phenazine compounds (20). Their findings also support differences in antimicrobial activity for the different phenazine compounds.

Previously, our research group performed a comparative genomic analysis of 63 plant-beneficial phenazine-producing *Pseudomonas* strains isolated from a wide diversity of host plants on four continents (17). The 63 strains were distributed among four subgroups within the *P. fluorescens* species complex. The genomic diversity of these 63 strains was large, as reflected by the size of the pangenome, accounting for more than 25,000 protein-coding genes. We identified numerous phytobeneficial traits involved in plant pathogen suppression, plant growth promotion and rhizosphere colonization. The strains harbor a myriad of biocontrol-related traits, including type III and VI

secretion systems and effectors, antibiotics, cyclic lipopeptides and siderophores. This diversity is interesting given that biocontrol-related traits could act in synergy to suppress plant diseases. For example, the importance of both phenazine and cyclic lipopeptide production by *Pseudomonas* sp. strain CMR12a in the biocontrol of *Rhizoctonia solani* has been clearly demonstrated (21). In other cases, the diversity of biocontrol-related traits could expand the biocontrol range of plant-beneficial *Pseudomonas* strains. For example, in strains producing both pyrrolnitrin and phenazines, phenazines have been shown to be more important for the biocontrol of *Fusarium oxysporum* (22) and *Sclerotium rolfsii* (23), while pyrrolnitrin has been shown to be more important for the biocontrol of *Fusarium graminearum* (24) and *Sclerotinia sclerotiorum* (25). The ability to produce these two compounds likely allows biocontrol strains to inhibit more plant pathogens than strains producing only one. Given that the biocontrol-related traits are unevenly distributed among the 63 strains under study, they will likely differ in their ability to inhibit plant pathogen growth.

The aim of this study was to characterize the abilities of 63 plant-beneficial phenazine-producing *Pseudomonas* strains to inhibit the growth of three of the most important potato pathogens: the oomycete *P. infestans*, the Gram-positive bacterium *Streptomyces scabies*, and the ascomycete *V. dahliae*. Several studies have already identified different phenazine-producing *Pseudomonas* strains as promising biocontrol agents against these three potato pathogens (26–29), but the exact biocontrol mechanisms involved, as well as the implication of additional key determinants other than phenazine production, are not fully understood. Therefore, in this study, in addition to characterizing the biocontrol potential of the 63 strains and quantifying the amount of phenazine compounds produced in different growth media, we also highlighted several biocontrol-related traits that could synergistically contribute to the inhibition of the three potato pathogens. In parallel, we also investigated the differential effects of the phenazine compounds produced by the strains toward the three potato pathogens under study. Taken together, this information will contribute to more rapidly and more efficiently selecting phenazine-producing *Pseudomonas* spp. for biocontrol.

RESULTS

***In vitro* antagonism of phenazine-producing *Pseudomonas* spp.** The ability of 63 plant-beneficial phenazine-producing *Pseudomonas* strains to inhibit the growth of *P. infestans*, *S. scabies*, and *V. dahliae* was evaluated using confrontation assays. For each of the three potato pathogens, two groups of *Pseudomonas* strains were statistically discriminated: one group encompassing the strains showing the highest pathogen suppression activity and another group composed of strains showing the lowest pathogen suppression activity. The results are presented in Fig. 1. In general, the 63 phenazine-producing *Pseudomonas* strains efficiently inhibited the growth of the oomycete *P. infestans* on V8 agar (Fig. 1A), with the width of the inhibition zones ranging from 0.5 to 22 mm (median, 16.4 mm). However, on average, strains from the *P. chlororaphis* and the CMR12a/CMR5c subgroups inhibited *P. infestans* mycelial growth more effectively than the strains belonging to the two other subgroups. In fact, the strains showing the highest activity all belong to the *P. chlororaphis* and the CMR12a/CMR5c subgroups (Fig. 1A). Against the bacterial pathogen *S. scabies*, the 63 strains showed contrasting pathogen suppression activity on oat bran agar (OBA) (Fig. 1B), with inhibition zones ranging from 0 mm to 21.4 mm (median of 9.7 mm) and no clear taxonomic segregation. Against *V. dahliae*, the 63 strains also showed contrasting pathogen suppression activity on potato dextrose agar (PDA) (Fig. 1C), with inhibition zones varying between 0 and 23.9 mm (median, 11.2 mm). The width of these inhibition zones, however, showed a clear segregation between *P. chlororaphis* strains harboring the phenazine biosynthetic gene *phzH* (responsible for the production of PCN) and those harboring *phzO* (responsible for the production of 2-OH-PHZ and 2-OH-PCA). While most *phzH*$^+$ strains were among the group showing the highest antagonistic activity, strains harboring *phzO* consistently showed very low activity against *V.*

FIG 1 *In vitro* antagonism of plant-beneficial phenazine-producing *Pseudomonas* spp. (A) Inhibition of *Phytophthora infestans*. (B) Inhibition of *Streptomyces scabies*. (C) Inhibition of *Verticillium dahliae*. For the 63 strains under study, the inhibition zone, defined as the distance between the

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dahliae. Several PCA-producing strains from the *P. fluorescens* subgroup also exhibited very high pathogen suppression activity toward *V. dahliae*.

Phenazine quantification in King's B broth. As a first step in understanding the differences observed in pathogen inhibition between the 63 strains, three different phenazine compounds (PCA, PCN, and 2-OH-PHZ) were quantified in King's B (KB) broth using high-performance liquid chromatography (HPLC) following a 5-day standardized growth period. 2-OH-PCA was not quantified because of the unavailability of a standard for reliable HPLC quantification. The results are presented in Fig. 2. At least one phenazine compound (PCA, PCN, or 2-OH-PHZ) was detected in the growth medium of 60 strains out of 63, at concentrations ranging from 2.8 to 181 μmol liter^{-1}. No phenazine compound of any kind was detected in the growth medium of strains 8B, B25, and K27. Two groups of strains were statistically discriminated: one group encompassing the strains producing the largest amount of phenazine compounds and another group composed of strains producing the smallest amount of phenazine compounds. In strains belonging to the *P. fluorescens* subgroup (22 strains) and to the *P. gessardii* subgroup (1 strain), only PCA was detected at concentrations ranging from 2.8 to 181 μmol liter^{-1}. Eight out of ten strains producing the largest amount of phenazines belong to the *P. fluorescens* subgroup. PCN was detected in the 15 *P. chlororaphis* strains harboring *phzH*, at concentrations ranging from 5.1 to 80.5 μmol liter^{-1}. In 11 out of 15 strains producing PCN, no PCA was detected. For the four other strains, PCA represented less than 20% of the total amount of phenazine compounds being produced, except for CMR5c, which produced four times more PCA than PCN. Conversely, for strains harboring *phzO* (except K27), PCA was always detected in the growth medium and in greater amounts than 2-OH-PHZ. 2-OH-PHZ was not detected in seven *phzO*$^{+}$ strains, while it was detected in the other *phzO*$^{+}$ strains at concentrations ranging from 4.9 to 33.5 μmol liter^{-1}. The 26 *phzO* nucleotide sequences were compared. However, we did not find any mutation that could explain why these seven strains do not produce 2-OH-PHZ under the growth conditions used in this study.

Correlation between phenazine production and pathogen inhibition. While the phenazine quantification data obtained in the previous section provided much-needed insights into the phenazine production potential of the 63 strains under study, the quantification of phenazine production under the same conditions used for the confrontation assays was necessary to better understand the differential inhibition exhibited by the strains. Thirteen representative strains were selected and grown in the presence of the potato pathogens on the three media used for the confrontation assays (V8 agar, OBA, and PDA). The amount of phenazine compounds in the medium was quantified by liquid chromatography-coupled mass spectrometry (LC-MS). The results are presented in Fig. 3. On V8 agar in the presence of *P. infestans*, phenazine compounds were detected in the medium for every strain, at concentrations ranging from 0.001 to 0.61 μmol g^{-1} of medium. In this medium, the three strains from the *P. fluorescens* subgroup stand out for their high phenazine production. On OBA in the presence of *S. scabies*, phenazine compounds were detected for every strain, at concentrations ranging from 0.002 to 0.14 μmol g^{-1} of medium. These values are mostly lower than the amounts of phenazine compounds detected on V8 agar, except for some strains belonging to the *P. chlororaphis* subgroup. On PDA and in the presence of *V. dahliae*, phenazine compounds were detected for every strain except LBUM636, at concentrations ranging from 0.04 to 1.02 μmol g^{-1} of medium.

To visualize whether the higher levels of phenazine production exhibited by some strains were associated with higher pathogen inhibitions, the width of the inhibition

FIG 1 Legend (Continued)

edges of the bacterial colonies and the pathogen vegetative tissues, was measured. The colors used for each strain correspond to the following phylogenetic groups: *P. fluorescens* subgroup (orange), *P. gessardii* subgroup (yellow), CMR12a/CMR5c subgroup (green), and *P. chlororaphis* subgroup (light blue for the *phzH*$^{+}$ strain, blue for the *phzO*$^{+}$ strain, and dark blue for B25, which does not harbor *phzH* nor *phzO*). When it was not possible to display the phylogenetic affiliation of the strain on the histogram bar, a colored symbol was added next to its name. Statistical analyses (Kruskal-Wallis test, followed by *post hoc* tests) discriminated two groups of strains significantly different from each other ($P < 0.05$). Error bars represent the standard errors.

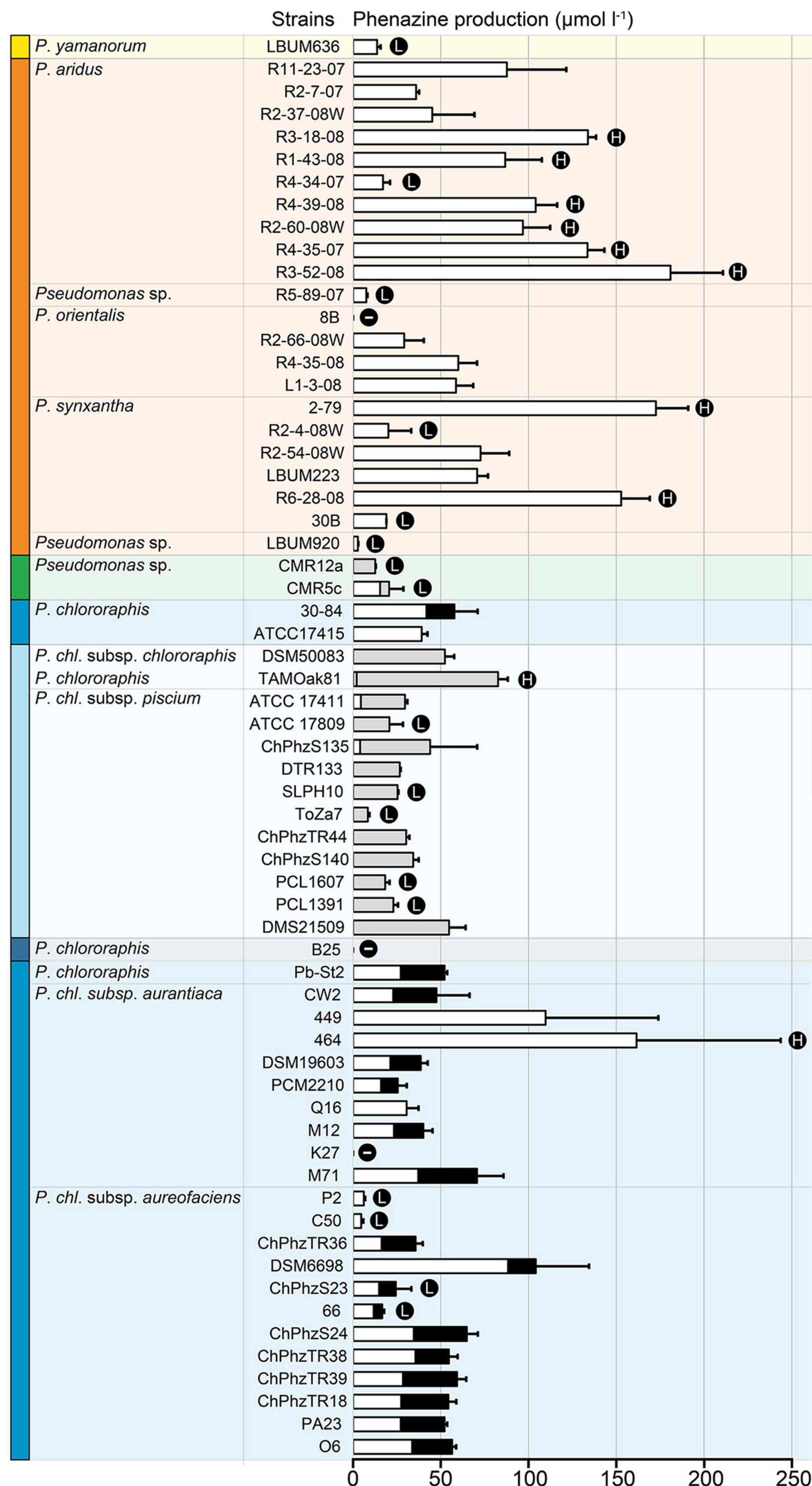

FIG 2 Phenazine production in King's B broth by the 63 strains under study. Three phenazine compounds (PCA, PCN, and 2-OH-PHZ) were quantified from 5-day-old KB broth cultures using HPLC. The

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zones obtained previously were plotted against the total amount of phenazine compounds being produced by the 13 *Pseudomonas* strains (Fig. 4). The widths of the inhibition zones were also plotted separately against the amounts of PCA/PCN/2-OH-PHZ being produced (see Fig. S1 in the supplemental material). The Kendall rank correlation coefficient τ was calculated for the three pathogens to evaluate if the total amount of phenazine compounds being produced correlates with the size of the inhibition zones. The correlation coefficient was close to zero for *P. infestans*, *S. scabies*, and *V. dahliae* inhibition data, and each test was nonsignificant ($P > 0.05$). This indicates that, overall, the amount of phenazine compounds being produced by the 13 *Pseudomonas* strains does not correlate with their ability to inhibit the three potato pathogens.

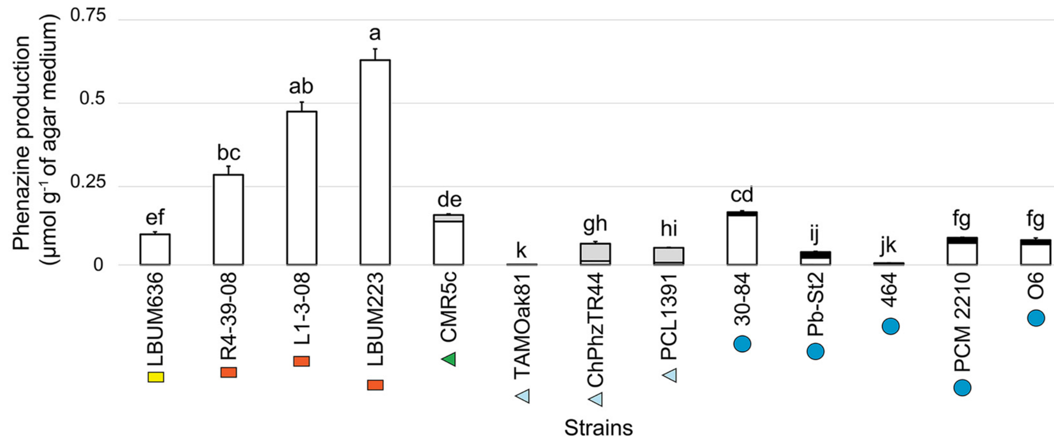
The 13 strains were grouped according to the type of phenazine compounds they produce (Fig. 5). On average, strains producing PCN or 2-OH-PHZ inhibited *P. infestans'* growth significantly better than strains solely producing PCA (Fig. 5A). On the other hand, strains producing PCN and strains producing solely PCA tended to inhibit *V. dahliae* better than strains producing 2-OH-PHZ (Fig. 5C). There was no difference for *S. scabies*, except between strains producing PCA and strains producing 2-OH-PHZ (Fig. 5B).

Antibiotic activity of phenazine compounds. To further evaluate whether the differences in terms of pathogen inhibition were related to the type of phenazine compounds being produced, inhibition tests with purified phenazine compounds were carried out. The goal was to establish whether the different phenazine molecules (PCA, PCN, and 2-OH-PHZ) have differential antibiotic activity against the three studied potato pathogens. The results are presented in Fig. 6. Against *P. infestans*, PCA was shown as the most effective phenazine compound to inhibit mycelial growth. The addition of 5 μmol of PCA (two spots of 2.5 μmol of PCA deposited at each edge of the petri dishes) led to a high inhibition of mycelial growth, while 0.5 μmol did not have any effect (Fig. 6A). Of the three pathogens under study, *S. scabies* was the most susceptible to phenazine antibiotics. We found that 2.5 μmol of PCA deposited in the center of a petri dish nearly inhibited all growth of *S. scabies* inoculated over the whole surface (Fig. 6B). In contrast, PCN and 2-OH-PHZ also exhibited antibiotic activity but to a lower level than PCA. For *V. dahliae*, only PCN was mildly inhibitory (Fig. 6C).

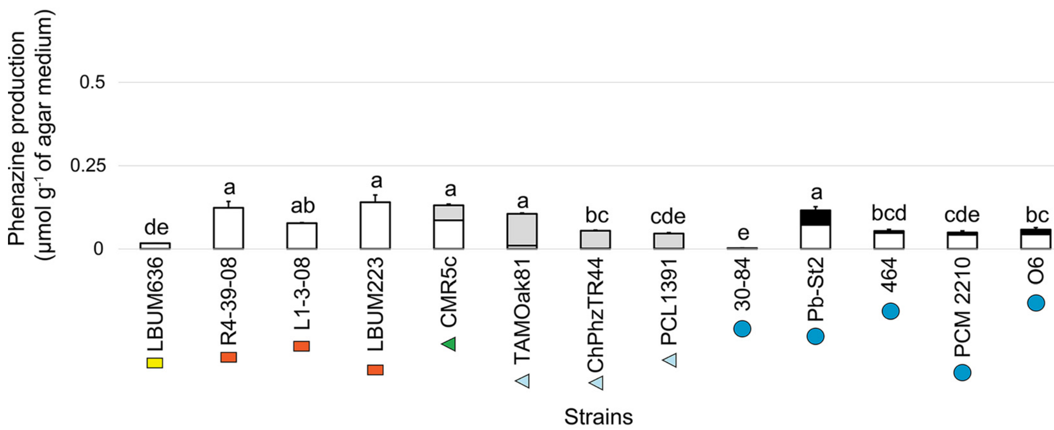
Additional biocontrol-related traits potentially involved in pathogen suppression. In addition to phenazine antibiotics, we wanted to determine whether other determinants could potentially be involved in pathogen growth suppression. Previously, we studied the distribution of genes/clusters involved in biocontrol, plant-growth promotion and rhizocompetence in the 63 *Pseudomonas* strains under study (17). These results were used together with the inhibition data to search for genes and clusters whose presence correlates with high or low pathogen inhibition. For each of the 74 genes/clusters of interest and for each pathogen, we calculated the mean inhibition for the strains harboring the gene/cluster and compared it to the mean inhibition for strains that do not harbor it (using Wilcoxon-Mann-Whitney test). We then calculated a mean inhibition ratio by dividing these two means. If this ratio is superior to 1, it means that strains harboring the gene/cluster tend to be more antagonistic than strains that do not have it. Ratios associated with their respective *P* values are presented in Fig. 7 and Table 1. We found 21 genes/clusters associated with higher or lower *P. infestans* inhibition, with mean inhibition ratios ranging from 0.74 to 1.35. We did not find any determinant that could be significantly associated with differential inhibition for *S. scabies*. For *V. dahliae*, 24 genes/clusters were associated with dif-

FIG 2 Legend (Continued)
colors correspond to PCA (white), PCN (gray), and 2-OH-PHZ (black). The symbol "–" indicates the absence of phenazine detection. Phenazine production by the different strains was compared between each other using Kruskal-Wallis and *post hoc* tests. Two groups of strains were statistically discriminated, one group encompassing the strains with high phenazine production (H) and the other encompassing the strains with low phenazine production (L). Error bars represent the standard errors. The colors used for each strain correspond to the following phylogenetic groups: *P. fluorescens* subgroup (orange), *P. gessardii* subgroup (yellow), CMR12a/CMR5c subgroup (green), and *P. chlororaphis* subgroup (blue).

A. V8 agar (in confrontation with *P. infestans*)



B. Oat bran agar (in confrontation with *S. scabies*)



C. Potato dextrose agar (in confrontation with *V. dahliae*)

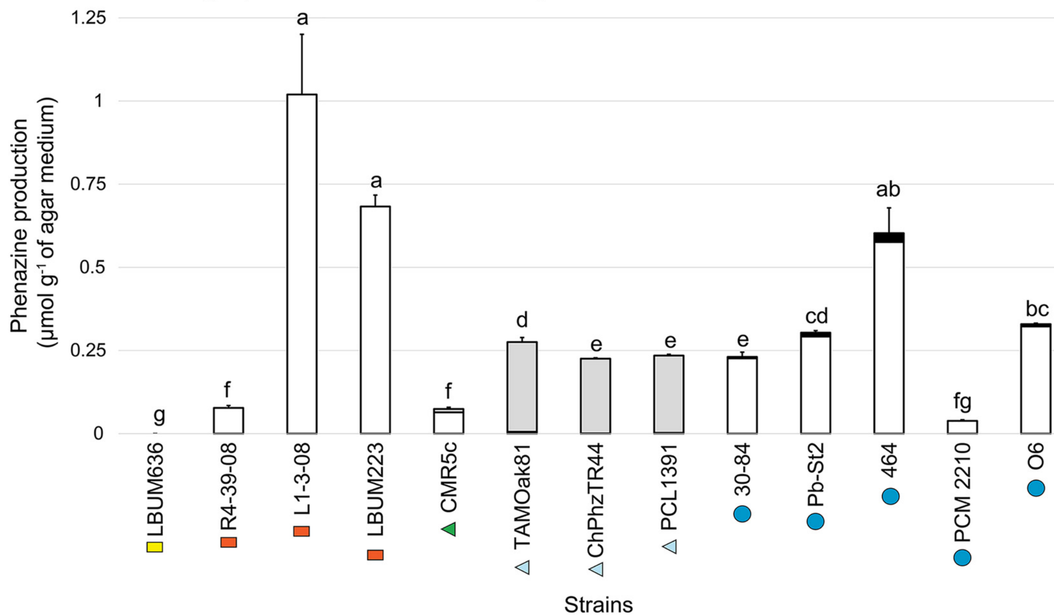


FIG 3 Phenazine production in three different agar-solidified growth media. Three phenazine compounds (PCA, PCN, and 2-OH-PHZ) were quantified in V8 agar, OBA, and PDA in the presence of the three potato pathogens. The histogram bars correspond to the amount of PCA (white), PCN (gray), and 2-OH-PHZ (black) being produced. For each medium, strains

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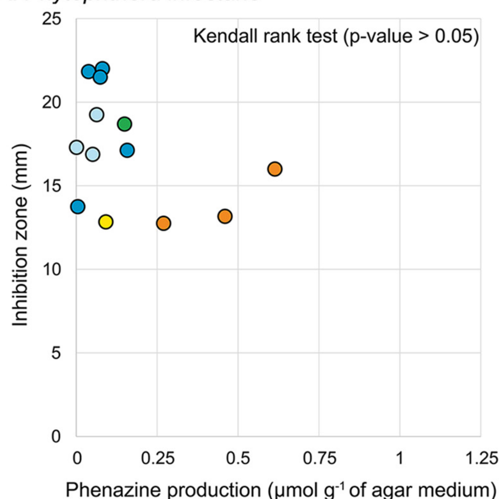
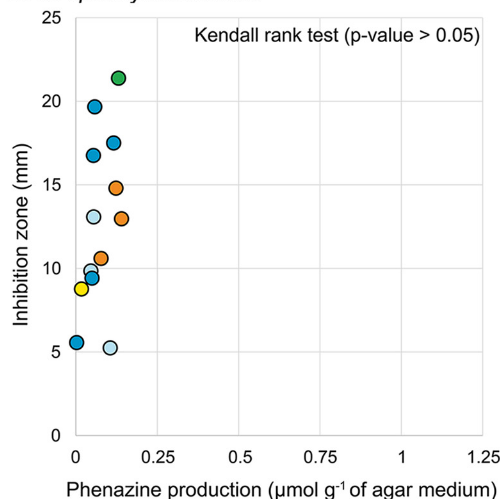




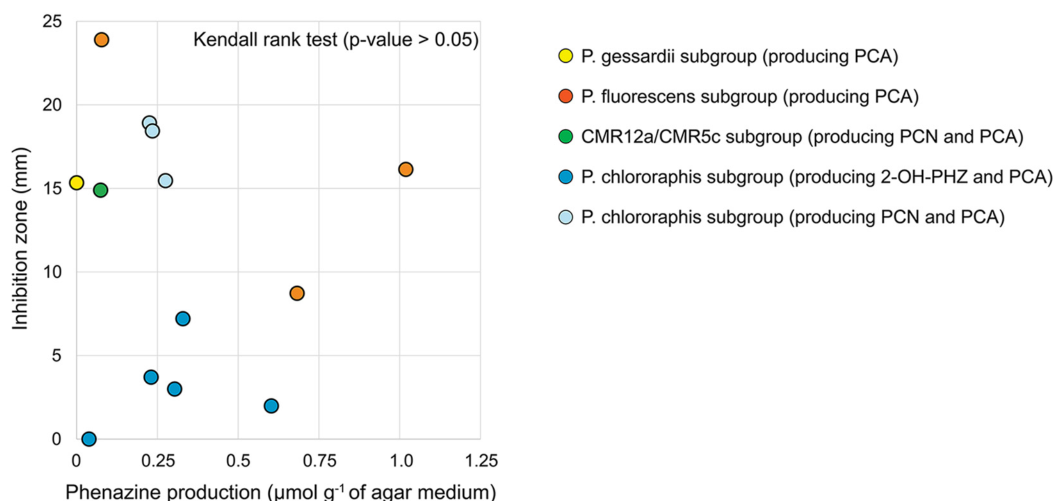


FIG 4 Correlation between pathogen inhibition and total phenazine production by *Pseudomonas* spp. (A) *Phytophthora infestans*. (B) *Streptomyces scabies*. (C) *Verticillium dahliae*. For each pathogen, correlations between the width of the inhibition zones and phenazine production in the confrontation medium was examined using Kendall rank tests.

ferential inhibition, with mean inhibition ratios ranging from 0.40 to 1.96. No common determinant was significantly associated with higher pathogen inhibition for both *P. infestans* and *V. dahliae*.

DISCUSSION

In this study, we compared the ability of 63 phenazine-producing *Pseudomonas* strains isolated from a wide diversity of host plants on four continents (17) to inhibit the growth of three different potato pathogens of economic significance: the oomy-

FIG 3 Legend (Continued)

with different letters are significantly different ($P < 0.05$). Phenazine production was not compared across the different media. Error bars represent the standard errors. For each strain, a symbol indicates the phenazine compounds likely to be produced based on the presence or absence of the two accessory phenazine biosynthetic genes in their genome: PCA producer (rectangle), PCN (and PCA) producer (triangle), and 2-OH-PHZ (and PCA) producer (circle). The color of each symbol specifies the phylogenetic group to which the strain belongs: *P. fluorescens* subgroup (orange), *P. gessardii* subgroup (yellow), CMR12a/CMR5c subgroup (green), and *P. chlororaphis* subgroup (blue).

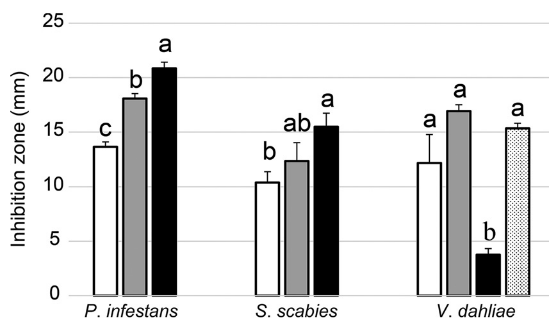
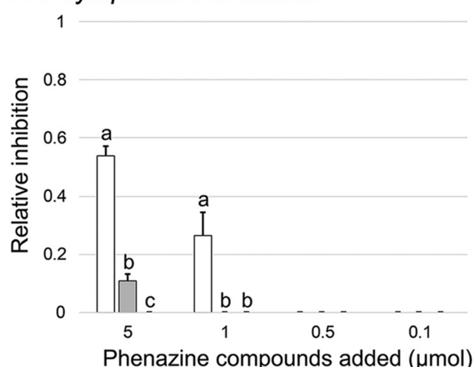
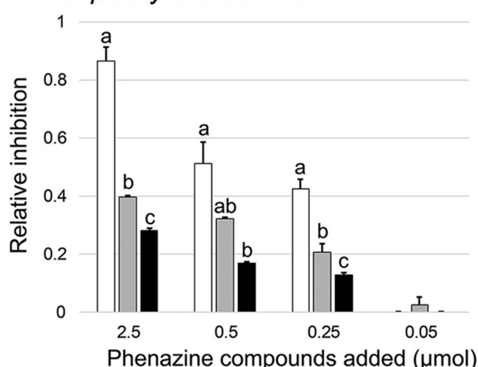


FIG 5 Mean inhibition of the three studied potato pathogens by phenazine-producing *Pseudomonas* spp. The 13 strains were grouped according to the phenazine compounds they produce in the three media used for the confrontation assays. The colors correspond to strains producing only PCA (white), strains producing PCN (gray), strains producing 2-OH-PHZ (black), and strains producing no phenazine compound of any kind (dotted). For each pathogen, groups with different lowercase letters are significantly different ($P < 0.05$). Groups were not compared across pathogens. Error bars represent the standard errors.

cete *P. infestans*, the Gram-positive bacterium *S. scabies*, and the ascomycete *V. dahliae*. We also measured the amount of phenazine molecules being produced by 13 representative strains in the confrontation assay media, to determine whether the differences observed in pathogen inhibition correlate with different phenazine production levels or whether the capacity to produce a specific phenazine compound correlates with high or low inhibition values. In parallel, the antibiotic activity of different concentrations of the purified phenazine molecules under study was also measured. Finally, pathogen inhibition values were analyzed in light of genomic data obtained in a previous study (17) to identify positive or negative associations between pathogen inhibition and the presence of specific phytobeneficial traits.

Phenazine antibiotics have been previously shown to inhibit the vegetative growth of *P. infestans* (27, 30) and other oomycetes (31, 32). Notably, we established in a previous study that an isogenic mutant of *P. yamanorum* LBUM636 impaired in phenazine production inhibited the growth of *P. infestans* less effectively than the wild type (27). It is therefore not surprising that nearly every strain under study inhibited the growth of *P. infestans*, to a certain extent. However, on average, strains from the *P. chlororaphis* and CMR12a/CMR5c subgroups performed better than strains from the *P. fluorescens* subgroup (Fig. 1). We did not find any correlation between the level of phenazine production and the width of the inhibition zones observed for *P. infestans* (Fig. 4), suggesting that the differences in *P. infestans* inhibition do not originate from differences in phenazine production between the different strains. In fact, on V8 agar, the three strains from the *P. fluorescens* subgroup produced much more phenazine compounds than any other strains (Fig. 3). On the other hand, while strains from the *P. fluorescens* subgroup only produce PCA, most *P. chlororaphis* strains produce additional phenazine compounds, namely, 2-OH-PHZ and PCN. PCN producers and 2-OH-PHZ producers inhibited *P. infestans* more, on average, than strains producing only PCA (Fig. 5). This suggests that the differences in *P. infestans* growth inhibition could originate from the type of phenazine compounds being produced. Nonetheless, when the three purified phenazine compounds were tested for their antibiotic activity against *P. infestans*, PCA had the highest activity, whereas PCN had very low activity, and 2-OH-PHZ no detectable activity at all (Fig. 6). In addition, the amounts of purified phenazine compounds added to the agar-solidified media were comparable to the amounts produced and quantified in these media when phenazine-producing *Pseudomonas* spp. were present. Together, these findings suggest that, whereas PCA probably plays an important role in the inhibition of *P. infestans* for PCA-producing strains, strains from the *P. chlororaphis* and CMR12a/CMR5c subgroups likely rely on other biocontrol-related traits to inhibit the growth of *P. infestans*. Fourteen phytobeneficial traits were found to be





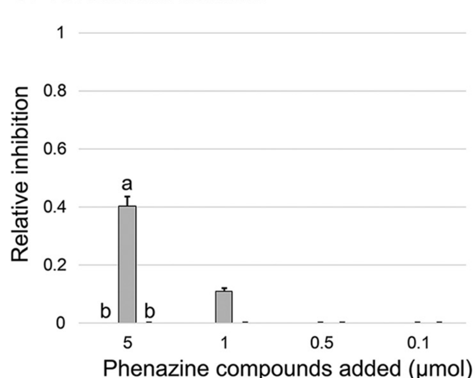


FIG 6 Antibiotic activities of PCA, PCN, and 2-OH-PHZ against three potato pathogens. Phenazine compounds dissolved in DMSO were added to culture media inoculated with one of the three potato pathogens: *Phytophthora infestans* (A), *Streptomyces scabies* (B), and *Verticillium dahliae* (C). The colors correspond to PCA (white), PCN (gray), and 2-OH-PHZ (black). Statistical analyses (Kruskal-Wallis, followed by *post hoc* tests) were conducted to compare each concentration, and significant differences ($P < 0.05$) are indicated with different lowercase letters. Relative inhibitions were not compared across different concentrations or pathogens. Error bars represent the standard errors.

associated with greater *P. infestans* inhibition when present in the strains under study (Table 1). This includes biosynthetic clusters involved in antibiotic production (hydrogen cyanide, pyrrolnitrin, and 2-hexyl 5-propyl resorcinol), cyclic lipopeptide production (viscosin), and siderophore production (achromobactin), as well as several antibacterial proteins. Most of these genes/clusters are present in almost all strains belonging to the *P. chlororaphis* subgroup but not outside this subgroup. While their association with a greater *P. infestans* inhibition may only originate from their taxonomic distribution, some are likely to contribute to *P. infestans* growth suppression. For example, every strain belonging to the *P. chlororaphis* and CMR12a/CMR5c subgroups harbors the *hcnABC* gene cluster (17), which is responsible for the production of the respiratory toxin hydrogen cyanide (HCN) (33). In our study, this cluster was associated with a higher pathogen inhibition by the strains harboring it (+33%), and several *P. chlororaphis* strains under study are known to produce HCN (34–36). *P. infestans* has been previously reported to be susceptible to this molecule (37). However, in a recent study, HCN was shown to play only a minor role in the inhibition of *P. infestans* mycelial growth by the phenazine-producing strain R47 (38). This strain belongs to *P. chlororaphis* subsp. *aureofaciens* and is closely related to several strains under study. Reverse genetic approaches will need to be used to evaluate the importance of these biocontrol-related traits in the inhibition of *P. infestans* growth.

The phenazine-producing strain *P. synxantha* LBUM223 has previously been shown to inhibit *S. scabies* growth *in vitro* (28) and to reduce potato common scab symptoms

FIG 7 Mean pathogen inhibition ratios associated with the presence or absence of phytobeneficial traits. Ratios for each phytobeneficial trait were calculated by dividing the mean inhibition achieved by *Pseudomonas* strains harboring it by the mean inhibition achieved by strains not harboring it. For example, if a gene/cluster has a ratio of 1.5 for a given pathogen, it means that strains harboring this gene/cluster inhibit the pathogen 50% more than strains not harboring it. Only phytobeneficial traits that significantly correlate ($P < 0.05$) with higher or lower pathogen inhibition and are present in five strains or more are presented.

under soil conditions, both in growth chambers (28) and in the field (29). In our assay, LBUM223-mediated *S. scabies* growth inhibition was intermediate, with a mean inhibition zone of 13 mm. Twenty strains exhibited higher inhibition against *S. scabies* than LBUM223, with inhibition zones ranging from 13.1 to 21.4 mm (Fig. 1). Unlike *P. infestans* inhibition, there was no clear phylogenetic pattern, with strains from three different subgroups included in the high inhibition group (Fig. 1). In addition, we did not find any correlation between the total amount of phenazine compounds being produced and the width of the inhibition zones (Fig. 4). PCA has already been identified as an important determinant in *S. scabies* growth inhibition by *P. synxantha* LBUM223 (28). Indeed, an isogenic mutant of *P. synxantha* LBUM223 impaired in phenazine production was shown to inhibit *S. scabies* growth and to protect potato plants against common scab less effectively than the wild type (28, 39). In this study, the addition of 2.5 μmol of PCA was shown to inhibit the growth of *S. scabies* drastically (Fig. 6), and we found that the two other phenazine compounds, PCN and 2-OH-PHZ, inhibited *S. scabies* growth, even though PCA inhibitory activity was higher (Fig. 6). Based on these results, the amount of phenazine compounds being produced by several strains in OBA (Fig. 3) are sufficient to inhibit *S. scabies* growth. Interestingly, the three strains that did not produce any phenazine compounds in KB broth under our conditions (although they harbor the *phz* biosynthetic operon) had no effect on *S. scabies* growth, reinforcing the importance of phenazine production for *S. scabies* growth inhibition. Unlike *P. infestans* inhibition, we did not find any phytobeneficial trait that correlates with a high *S. scabies* growth inhibition, which is partly related to the absence of phylogenetic patterns in the differential inhibition exhibited by the 63 strains. However, this

TABLE 1 Phytobeneficial traits associated with *P. infestans* or *V. dahliae* differential inhibition by phenazine-producing *Pseudomonas* strains[a]

		Phytophthora infestans		*Verticillium dahliae*	
Gene/cluster	**Category**	***P***	**MI ratio**	***P***	**MI ratio**
Triglyceride lipase	Antibacterial protein	>0.05	1.09	2.19E–03	1.96
Unknown siderophore 1	Siderophore	2.61E–03	0.80	3.96E–04	1.78
Hrp1	Type III secretion system	2.88E–05	0.76	7.35E–04	1.74
Putative Rhs toxin	Antibacterial protein	1.38E–04	0.74	3.16E–03	1.70
phzO	Antibiotic	6.17E–03	1.15	1.49E–06	0.40
Pore-forming pyocin	Antibacterial protein	>0.05	0.88	2.10E–02	1.63
Type III secretion system	Type III secretion system	1.56E–04	0.80	1.01E–02	1.54
HCN	Antibiotic	1.79E–05	1.33	1.90E–03	0.60
PAA catabolism	Plant-bacterium interaction	7.78E–05	1.28	8.26E–04	0.57
Tae4-like	Antibacterial protein	3.37E–05	1.35	1.04E–02	0.65
Pyrrolnitrin	Antibiotic	6.56E–03	1.16	1.12E–05	0.47
HPR	Antibiotic	9.60E–03	1.15	3.37E–05	0.48
NRPS 9	Orphan gene cluster	>0.05	1.13	4.42E–04	0.46
2,3-btd biosynthesis	Plant-bacterium interaction	1.82E–03	1.24	1.17E–03	0.58
iaa biosynthesis	Plant-bacterium interaction	1.86E–04	1.27	3.10E–03	0.61
phzH	Antibiotic	>0.05	1.16	1.45E–02	1.45
DUF4150/Tox-GHH2	Antibacterial protein	2.89E–04	1.25	8.14E–03	0.64
Achromobactin	Siderophore	1.73E–04	1.26	1.15E–02	0.65
Tox-REase-5	Antibacterial protein	5.03E–04	1.29	2.64E–02	0.69
S-type pyocin (DNase)	Antibacterial protein	5.34E–04	1.23	1.37E–02	0.66
NRPS-PKS 5	Orphan gene cluster	>0.05	1.16	3.94E–02	1.40
SPI-1 (Inv/Mixi/Spa)	Type III secretion system	2.72E–02	0.84	>0.05	1.38
iaa catabolism	Plant-bacterium interaction	>0.05	0.97	6.74E–03	0.51
Rhs protein (nuclease)	Antibacterial protein	>0.05	0.93	3.41E–02	1.41
Acetoin catabolism	Plant-bacterium interaction	>0.05	0.96	5.99E–03	0.61
Viscosin	Cyclic lipopeptides	3.10E–02	1.19	>0.05	0.87
NRPS 2	Orphan gene cluster	4.28E–02	0.75	>0.05	1.02
Nor	Denitrification cluster	1.33E–02	1.16	>0.05	0.97

[a]Only phytobeneficial traits that significantly correlate ($P < 0.05$) with higher or lower pathogen inhibition in at least one of the two pathogens under study and are present in five *Pseudomonas* strains and more are presented. MI ratio, mean inhibition ratio.

does not mean that other biocontrol-related traits not highlighted in this study but found in some strains do not play a role in *S. scabies* inhibition.

The 63 strains exhibited major differences in their ability to inhibit *V. dahliae* growth. The group displaying the highest inhibition was composed of PCN-producing strains from the *P. chlororaphis* subgroup and PCA-producing strains from the *P. fluorescens* subgroup (Fig. 1). Conversely, 2-OH-PHZ-producing strains were particularly inefficient at inhibiting *V. dahliae* growth, even though many of those strains were good at inhibiting the growth of *P. infestans* and *S. scabies*. We did not find any correlation between total phenazine production and inhibition values (Fig. 4). However, we found that the three phenazine molecules display different inhibitory activity. Only PCN inhibited mycelial growth under our conditions (Fig. 6). These results differ from those obtained by Debode et al. (26). These authors found that a PCL1391 mutant impaired in PCN production and producing only PCA inhibited *V. dahliae* microsclerotia germination slightly more effectively than did the wild type. Furthermore, a PCL1391 mutant impaired in phenazine production was as effective as the wild type in inhibiting microsclerotia germination. We hypothesize that this discrepancy regarding PCN antibiotic activity might originate from the physiological differences between actively growing mycelium and the melanized resting structures that are microsclerotia. While the antibiotic activity of PCN might explain, to a certain extent, why *phzH*$^+$ strains are efficient at inhibiting mycelial growth, it does not explain the higher inhibition exhibited by PCA-producing strains, considering that PCA had no antibiotic activity under our conditions. This suggests that other biocontrol-related traits are likely involved in the high *V. dahliae* inhibition by the strains belonging to the *P. fluorescens* subgroup. Nine phytobeneficial traits were found to be associated with a higher *V. dahliae* inhibition (Table 1). For example, strains harboring at least one copy of a gene, identified

previously (17) and encoding a putative type VI effector with a triglyceride lipase domain (PF01764), were 96% more effective in inhibiting mycelial growth than strains not harboring it (Table 1). However, given that this effector is likely injected in neighboring cells using the type VI secretion system, it is unlikely to play a role under our conditions. Similarly, type III secretion systems and antibacterial proteins are unlikely to be involved in pathogen growth inhibition under our conditions, even though these phytobeneficial traits are associated with a higher inhibition. These associations rather originate from their taxonomic distribution. Strains from the *P. fluorescens* subgroup harbor a cluster involved in the biosynthesis of an undescribed siderophore (17). This siderophore is associated with a higher inhibition (+78%). This siderophore could be involved in *V. dahliae* inhibition as siderophores have already been demonstrated to engage in *V. dahliae* inhibition (40). Finally, the orphan gene cluster NRPS-PKS 5 was also associated with a higher inhibition (+40%) for strains harboring it. This cluster is only found in strains from the *piscium* subspecies and might contribute, in addition to PCN, to the superior inhibition exhibited by PCN-producing strains from the *P. chlororaphis* subgroup.

It is possible that phenazine production by *phzO*$^+$ *Pseudomonas* strains was slightly underestimated in our study given that 2-OH-PCA was not quantified because of the unavailability of a standard for reliable HPLC quantification. However, we believe that the total amount of 2-OH-PCA is likely negligible compared to the amount of PCA and 2-OH-PHZ produced by the strains. The conversion of 2-OH-PCA from PCA is mediated by the flavin-diffusible monooxygenase PhzO (18). 2-OH-PCA subsequently undergoes a spontaneous decarboxylation leading to the production of 2-OH-PHZ (18, 41). Several authors have reported a nearly total conversion of 2-OH-PCA to 2-OH-PHZ in solutions buffered at pH 7 (18, 41), which is close to the pH of the different media used in our study. Nevertheless, several media used in this study (PDA and OBA) are not buffered, and it is possible that the growth of the pathogen/biocontrol agent resulted in the acidification of the growth medium. In this case, the conversion of 2-OH-PCA to 2-OH-PHZ would remain incomplete.

In this study, phenazine production was quantified in four different media, namely, KB broth, V8 agar, OBA, and PDA. A considerable variability in the amounts of phenazine compounds being produced by the 13 strains across the different media used for the confrontation assays was observed. This was expected, considering the differences in the growth media composition. Indeed, phenazine production has been shown to fluctuate greatly depending on many environmental factors, such as pH, temperature and the presence of glucose or amino acids (42–44). For example, phenazine production is increased in *P. synxantha* 2-79, in *P. chlororaphis* subsp. *aureofaciens* O6 and in *P. chlororaphis* subsp. *piscium* PCL1391 in the presence of glucose (42, 43, 45). In addition, it has been shown that phenazine-producing *Pseudomonas* strains can be affected differently by the presence of some compounds (42). This explains why phenazine production for some strains appears to remain stable across the different media, while for others it varies substantially. In addition, some factors, such as growth time, may impact the amount of phenazine compounds being quantified. For example, the phenazine-producing *Pseudomonas* strains were incubated in the confrontation media for different periods of time, ranging from 6 days on OBA to 20 days on PDA. It is possible that the incubation time influenced phenazine production, resulting in the accumulation of more or less phenazine compounds in the growth media. In addition, it is also possible that the presence of the potato pathogens in the petri dish could influence phenazine production. For example, several soilborne *Streptomyces* strains have been reported to interfere with quorum-sensing signaling in the phenazine-producing opportunistic pathogen *Pseudomonas aeruginosa*, leading to a reduction in phenazine production (46, 47). In addition, fusaric acid produced by the ascomycete *Fusarium* spp. has been shown to inhibit PCN production by *P. chlororaphis* subsp. *piscium* PCL1391, even under conditions that are favorable to its production (42, 48). It might be possible that similar mechanisms are used by the three potato pathogens under

study to interfere with phenazine production. These all represent interesting research questions to address in future studies.

In this study, we identified several plant-beneficial phenazine-producing *Pseudomonas* strains that excel in inhibiting the growth of some of the three potato pathogens under study. However, *P. chlororaphis* subsp. *piscium* ChPhzTR44, a PCN-producing strain isolated from the rhizosphere of tomato grown in the Fusarium wilt suppressive soils of Châteaurenard in France (49), is the only strain included in the higher inhibition group for all three potato pathogens under study (Fig. 1). In addition to the phenazine biosynthetic operon, this strain harbors the HCN biosynthetic cluster *hcnABC*, the viscosin biosynthetic cluster, three siderophore biosynthetic clusters and two orphan NRPS/NRPS-PKS gene clusters (17). Moreover, this strain was shown to be a good colonizer of the potato rhizosphere, being detected by quantitative PCR at 6.54×10^6 *phzD* copies per g of rhizosphere soil (50). This is interesting because biocontrol agent population size in the rhizosphere has been shown to correlate with disease incidence reduction (51, 52) and with antibiotic accumulation (53, 54). While this strain appears to be an excellent candidate to suppress the three different potato pathogens under study, further investigations in the presence of the plant, grown under controlled and field conditions, are needed to confirm its effectiveness as a versatile biocontrol agent.

In conclusion, we compared the ability of 63 phenazine-producing *Pseudomonas* strains to inhibit the growth of three potato pathogens of economic significance. Overall, the strains exhibited contrasted levels of pathogen inhibition and phenazine production. While several differences may be explained by the nature or the quantity of phenazine compounds being produced, our results suggest that other biocontrol traits are likely involved. Reverse genetic approaches will need to be applied in order to validate the implication of those biocontrol-related traits in pathogen suppression. This study represents a first step in harnessing the tremendous genomic diversity of phenazine-producing *Pseudomonas* spp. with the objective of strengthening biological control of potato pathogens.

MATERIALS AND METHODS

Bacterial strains and plant pathogens. The 63 *Pseudomonas* strains used in this study are listed in Table 2. The strains were routinely grown in KB broth (55) at 25°C for 24 h under continuous shaking (120 rpm). Populations were estimated using spectrophotometer readings at 600 nm and standard curves. Three different pathogens were used. *Phytophthora infestans* (genotype US-8) was grown on 10% unclarified V8 (Campbell, Camden, NJ) agar plates at 20°C for 10 days. *Streptomyces scabies* (LBUM848) was grown on oat bran broth (56) at 28°C for 6 days with continuous shaking (200 rpm). *Verticillium dahliae* (717.96) was grown on PDA (BD, Franklin Lakes, NJ) at 25°C for 3 weeks.

Confrontation assay. The 63 strains of phenazine-producing *Pseudomonas* spp. were tested for their ability to inhibit three potato pathogens (*P. infestans*, *V. dahliae*, and *S. scabies*) using *in vitro* confrontation assays. For *P. infestans* and *V. dahliae*, 8.9-cm petri dishes containing 20 ml of growing medium (10% unclarified V8 agar for *P. infestans* and PDA for *V. dahliae*) were inoculated in the center with agar plugs (5 mm) taken from the margin of actively growing mycelium. Four drops (10 μl each) of 24-h-old bacterial cultures were added at the edges of the inoculated petri dishes. The plates were incubated at 20°C during 10 days for assays involving *P. infestans* and at 25°C during 20 days for those involving *V. dahliae*. The petri dishes were placed according to a randomized block design. Subsequently, the distances between the mycelium and the edge of the bacterial colonies were measured. For *S. scabies*, 100 μl of 6-day-old culture of *S. scabies* was spread to cover 8.9-cm petri dishes filled with 20 ml of OBA, and the plates were left to dry. One drop (20 μl) of phenazine-producing *Pseudomonas* culture was spotted in the center of the plates. After 6 days at 28°C, the inhibition zone was measured from the edge of the phenazine-producing *Pseudomonas* colonies to the limits of the area where *Streptomyces* vegetative growth was inhibited. For the three pathogens, values superior to 20 mm correspond to nearly complete inhibition. For each pair of potato pathogens/phenazine-producing *Pseudomonas* strains, four replicates were used.

Phenazine compounds quantification in King's B broth. The 63 *Pseudomonas* strains were inoculated in 9 ml of KB broth in triplicate and incubated for 5 days at 25°C under continuous shaking. After incubation, 1 ml of bacterial culture was transferred to a 2-ml microcentrifuge tube and centrifuged for 10 min at $10{,}000 \times g$. The supernatant was recovered and filtered on a 0.2-μm nylon filter (Microliter Analytical Supply, Mississauga, Ontario, Canada). Standards of PCA and PCN were obtained from Ryan Scientific (Mount Pleasant, SC), and standards of 2-OH-PHZ from Angene (London, UK). Standards of PCA, PCN, and 2-OH-PHZ at a concentration of 500 ng μl^{-1} were prepared by adding 100 μl of a phenazine solution (0.01 g ml^{-1}) to 9 ml of KB broth. HPLC analyses were conducted using a reverse-phase C_{18}

TABLE 2 *Pseudomonas* strains used in this study

Pseudomonas strain(s)	Origin	Genome sequence (reference)	Reference(s) or source
P. yamanorum (*P. gessardii* subgroup)			
LBUM636	Strawberry rhizosphere, Canada	60	60
P. aridus (*P. fluorescens* subgroup)			
R11-23-07, R2-7-07, R2-37-08W, R3-18-08, R1-43-08, R4-34-07, R4-39-08, R2-60-08W, R4-35-07, R3-52-08	Wheat rhizosphere, USA	17	16, 61
P. orientalis (*P. fluorescens* subgroup)			
8B	Wheat rhizosphere, Iran	17	62
R2-66-08W, R4-35-08, L1-3-08	Wheat rhizosphere, USA	17	61
P. synxantha (*P. fluorescens* subgroup)			
2-79	Wheat rhizosphere, USA	17	63
LBUM223	Strawberry rhizosphere, Canada	64	28
30B	Wheat rhizosphere, Iran	17	62
R2-54-08W, R2-4-08W, R6-28-08	Wheat rhizosphere, USA	17	61
Pseudomonas sp. (*P. fluorescens* subgroup)			
R5-89-07	Wheat rhizosphere, USA	17	61
LBUM920	Spruce rhizosphere, Canada	17	Richard Hamelin
Pseudomonas sp. (CMR12a/CMR5c subgroup)			
CMR5c, CRM12a	Cocoyam rhizosphere, Cameroon	17	65
P. chlororaphis subsp. *aurantiaca* (*P. chlororaphis* subgroup)			
DSM19603	Unknown	17	66
CW2	Radish rhizosphere, Germany	17	67
449	Maize rhizosphere, Ukraine	17	68
464	Beet rhizosphere, Ukraine	17	69
PCM2210	Beet rhizosphere, Poland	17	PCM[a]
Q16	Alfalfa rhizosphere, Serbia	17	70
M12	Maize rhizosphere, Serbia	17	Dragana Josic
K27	White clover rhizosphere, Serbia	17	Dragana Josic
M71	Tomato rhizosphere, Italy	17	71
P. chlororaphis subsp. *aureofaciens* (*P. chlororaphis* subgroup)			
DSM6698^T	River clay, The Netherlands	17	72
P2	Potato rhizosphere, Algeria	17	73
C50	Maize rhizosphere, Serbia	17	Dragana Josic
ChPhzTR18, ChPhzTR36, ChPhzTR38, ChPhzTR39	Tomato rhizosphere, France	17	49
ChPhzS23, ChPhzS24	Soil, France	17	49
66	Alfalfa rhizosphere, Uzbekistan	17	69
PA23	Soja root, Canada	74	75
O6	Soil, USA	36	76
P. chlororaphis subsp. *chlororaphis* (*P. chlororaphis* subgroup)			
DSM 50083^T	Plate contaminant	17	77
P. chlororaphis subsp. *piscium* (*P. chlororaphis* subgroup)			
DSM 21509^T	Perch intestine, Switzerland	17	78
ATCC 17411	Unknown	17	77
ATCC 17809	Unknown	17	77
ChPhzS135, ChPhzS140	Soil, France	17	49
DTR133	Tomato rhizosphere, France	17	79
SLPH10	Take-all decline soil, The Netherlands	17	16
ToZa7	Tomato rhizosphere, Greece	17	80
ChPhzTR44	Tomato rhizosphere, France	17	49
PCL1607	Avocado rhizosphere, Spain	17	81
PCL1391	Tomato root, Spain	17	35

(Continued on next page)

TABLE 2 (Continued)

Pseudomonas strain(s)	Origin	Genome sequence (reference)	Reference(s) or source
P. chlororaphis (*P. chlororaphis* subgroup)			
Pb-St2	Sugarcane stem, Pakistan	17	82
B25	Clover rhizosphere, Serbia	17	70
30-84	Soil, USA	36	83
ATCC 17415	Soil, USA	17	77
TAMOak81	Oak, USA	17	84

[a]PCM, Polish Collection of Microorganisms.

Hydro-RP column (4 μM; 100 $\times$ 2 mm; Phenomenex, Torrance, CA). Chromatography was performed using an Agilent 1100 series HPLC system consisting of a quaternary pump, a refrigerated sample holder, and a photodiode array. The samples were maintained at 4°C, and the injection volume was 10 μl. The solvent flow rate was 750 μl min^{-1}; solvent A consisted of 0.1% trifluoroacetic acid (TFA) in water, and solvent B consisted of 0.1% TFA in acetonitrile. Elution consisted of a 5-min linear gradient from 90% solvent A to 75% solvent A, followed by 4.8 min at 75% solvent A. Absorption of phenazine compounds (PCA, PCN, and 2-OH-PHZ) was measured at 254 nm, and the retention times of the phenazine standards were used to confirm the presence of phenazine compounds in the liquid cultures of the bacteria.

Phenazine compound quantification in agar-solidified growth media. Thirteen phenazine-producing *Pseudomonas* strains were selected, and confrontation assays were performed as described previously. Four agar plugs were collected from within the growth inhibition zone of each confrontation plate using a 7-mm-diameter cork borer. Care was taken to ensure plugs were taken as close as possible to *Pseudomonas* sp. growth. To ensure that phenazines were not produced by the plant pathogen and were not present in the growth medium prior to inoculation, plugs were also collected from plates inoculated solely with the pathogen, as well as from uninoculated plates. Plugs were then transferred to a 2-ml safe-lock microcentrifuge tube containing a single acid-washed, 5-mm-diameter stainless steel bead and ground by bead beating for 3 min at 30 Hz using a TissueLyser II (Qiagen, Venlo, The Netherlands). The resulting slurry was resuspended in 1 ml of acetonitrile by vortexing at maximum speed for 10 s, and the suspension was incubated at room temperature for 10 min with constant shaking on a tube rotisserie to allow passive diffusion of the phenazines into the organic solvent. The suspension was centrifuged at 10,000 $\times$ *g* for 5 min, and the resulting supernatant was collected and filtered through a 13-mm-diameter, 0.2-μm nylon membrane. Then, 250 μl of filtered supernatant was mixed with 750 μl of water containing 0.1% (vol/vol) formic acid. The dilution was incubated at room temperature for ~13 h to allow insoluble components to precipitate. Then, the 1-ml dilution was filtered through a 0.2-μm nylon membrane into a 9-mm-diameter autosampler glass vial (12 $\times$ 32 mm). When appropriate, 100 μl of dilution was mixed with 900 μl of diluent (25% [vol/vol] acetonitrile and 0.075% [vol/vol] formic acid in water) prior to filtration. Phenazines were quantified by LC-MS within 2 days of the extraction.

Extracted phenazines were separated using a 1100 Series Capillary LC System (Agilent Technologies, Santa Clara, CA), equipped with a vacuum degasser, quaternary pump and autosampler. Samples were resolved at 30°C in a 100 mm $\times$ 2 mm Synergi 4 μm Hydro-RP 80 Å LC column (Phenomenex, Torrance, CA). Mobile-phase solvents comprised water (solvent A) and acetonitrile (solvent B), both supplemented with 0.1% (vol/vol) formic acid. The sample injection volume was 1 μl. Phenazines were eluted using a linear gradient increasing from 5% solvent B to 100% solvent B over the course of 8 min, followed by a steady state of 100% solvent B for 7 min. The solvent flow rate was 400 μl min^{-1}. Phenazines were then detected using a time-of-flight mass spectrometer (model G6230B; Agilent Technologies) equipped with a dual electrospray ionization (ESI) ion source and operating in positive mode. A mass spectrum was acquired every second over a mass range of 100 to 1,700 *m/z*. PCA, PCN, and 2-OH-PHZ have mass-to-charge ratios of 225.058, 224.074, and 197.063 *m/z*, respectively. Their retention times were 9 to 7.0, 6.5 to 6.6, and 6.1 to 6.2 min, respectively.

Data acquisition, ion chromatogram extraction and chromatographic peak detection were carried out using the MassHunter Workstation software, version B.08.00 (Agilent Technologies). The area under the curve of each phenazine was noted and converted into concentration by comparison to the appropriate phenazine standard. Phenazine yields were ultimately normalized by medium weight. PCA (Ryan Scientific), PCN (AA Blocks, San Diego, CA), and 2-OH-PHZ (Angene) standards, as well as appropriate blanks, were included in each run. Phenazine standards comprised 800 ng ml^{-1} in an aqueous solution of 25% (vol/vol) dimethyl sulfoxide (DMSO) and 0.075% (vol/vol) formic acid, whereas blanks consisted of aqueous solutions of either 25% (vol/vol) DMSO (for standards) or 25% (vol/vol) acetonitrile (for phenazine extracts) and 0.075% (vol/vol) formic acid. Phenazine standards and DMSO blanks were centrifuged at 10,000 $\times$ *g* for 5 min, and the supernatant was transferred to an autosampler vial. Acetonitrile blanks were filtered through a 0.2-μm nylon membrane into an autosampler vial.

***In vitro* sensitivity to phenazine compounds.** The effect of three purified phenazine compounds (PCA, PCN, and 2-OH-PHZ) on the growth of the three potato pathogens studied was evaluated under *in vitro* conditions. The three pathogens were inoculated in 8.9-cm petri dishes containing 20 ml of growth medium (V8 agar for *P. infestans*, OBA for *S. scabies*, and PDA for *V. dahliae*) as previously described in

this work. The three phenazine compounds were dissolved and diluted in DMSO at the following final concentration: 50 μM, 10 μM, 5 μM, and 1 μM. For *P. infestans* and *V. dahliae*, two drops (50 μl each) were deposited at the edge of the petri dish. For *S. scabies*, one drop (50 μl) was deposited in the center of the petri dish. DMSO without phenazine compounds was used as a negative control. After 10 days (*P. infestans*) and 20 days (*V. dahliae*), the diameter of the fungal mycelium was measured. For each concentration/phenazine compound, the diameter of the fungal mycelium was compared to the negative control and expressed as a relative inhibition. For *S. scabies*, the diameter of the inhibition zone was measured after 6 days. The diameter of the inhibition zone was compared to the inner diameter of the petri dish and expressed as a relative inhibition. For each combination of concentration/phenazine compound/potato pathogen, three replicates were used.

Statistical analyses. The software R (57) and R Studio version 1.1.453 (58) was used to perform statistical analyses. The function "kruskal" from the R package "agricolae" version 1.2-8 (59) was used to perform a Kruskal-Wallis test, followed by multiple comparisons with the Bonferroni correction. Correlations between inhibition values and phenazine production were calculated using the Kendall rank correlation coefficient τ (R function cor.test [*x*, *y*, method="kendall"]). The Wilcoxon-Mann-Whitney test was used to generate *P* values related to the associations between inhibition values and the presence or absence of phytobeneficial traits. Only traits present in five or more strains were considered.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, DOCX file, 0.7 MB.

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REFERENCES

1. Burlingame B, Mouille B, Charrondiere R. 2009. Nutrients, bioactive non-nutrients, and anti-nutrients in potatoes. J Food Compos Anal 22:494–502. https://doi.org/10.1016/j.jfca.2009.09.001.
2. Devaux A, Kromann P, Ortiz O. 2014. Potatoes for sustainable global food security. Potato Res 57:185–199. https://doi.org/10.1007/s11540-014-9265-1.
3. Oerke EC. 2006. Crop losses to pests. J Agric Sci 144:31–43. https://doi.org/10.1017/S0021859605005708.
4. Guenthner JF, Wiese MV, Pavlista AD, Sieczka JB, Wyman J. 1999. Assessment of pesticide use in the United States potato industry. Am J Pot Res 76:25–29. https://doi.org/10.1007/BF02853554.
5. Haverkort AJ, Boonekamp PM, Hutten R, Jacobsen E, Lotz LAP, Kessel GJT, Visser RGF, van der Vossen EAG. 2008. Societal costs of late blight in potato and prospects of durable resistance through cisgenic modification. Potato Res 51:47–57. https://doi.org/10.1007/s11540-008-9089-y.
6. Pimentel D, Burgess M. 2014. Environmental and economic costs of the application of pesticides primarily in the United States, p 47–71. *In* Pimentel D, Peshin R (ed), Integrated pest management. Springer, Dordrecht, The Netherlands.
7. Kim K-H, Kabir E, Jahan SA. 2017. Exposure to pesticides and the associated human health effects. Sci Total Environ 575:525–535. https://doi.org/10.1016/j.scitotenv.2016.09.009.
8. Haas D, Défago G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319. https://doi.org/10.1038/nrmicro1129.
9. Weller DM. 2007. *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. Phytopathology 97:250–256. https://doi.org/10.1094/PHYTO-97-2-0250.
10. Gross H, Loper JE. 2009. Genomics of secondary metabolite production by *Pseudomonas* spp. Nat Prod Rep 26:1408–1446. https://doi.org/10.1039/b817075b.
11. Biessy A, Filion M. 2018. Phenazines in plant-beneficial *Pseudomonas* spp.: biosynthesis, regulation, function, and genomics. Environ Microbiol 20:3905–3917. https://doi.org/10.1111/1462-2920.14395.
12. Chin-A-Woeng TFC, Bloemberg GV, Lugtenberg BJ. 2003. Phenazines and their role in biocontrol by *Pseudomonas* bacteria. New Phytol 157:503–523. https://doi.org/10.1046/j.1469-8137.2003.00686.x.
13. Mavrodi DV, Blankenfeldt W, Thomashow LS. 2006. Phenazine compounds in fluorescent *Pseudomonas* spp. biosynthesis and regulation. Annu Rev Phytopathol 44:417–445. https://doi.org/10.1146/annurev.phyto.44.013106.145710.
14. Mazzola M, Cook RJ, Thomashow LS, Weller D, Pierson L. 1992. Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. Appl Environ Microbiol 58:2616–2624. https://doi.org/10.1128/AEM.58.8.2616-2624.1992.
15. Mavrodi DV, Ksenzenko VN, Bonsall RF, Cook RJ, Boronin AM, Thomashow LS. 1998. A seven-gene locus for synthesis of phenazine-1-carboxylic acid by *Pseudomonas fluorescens* 2-79. J Bacteriol 180:2541–2548. https://doi.org/10.1128/JB.180.9.2541-2548.1998.
16. Mavrodi DV, Peever TL, Mavrodi OV, Parejko JA, Raaijmakers JM, Lemanceau P, Mazurier S, Heide L, Blankenfeldt W, Weller DM, Thomashow LS. 2010. Diversity and evolution of the phenazine biosynthesis pathway. Appl Environ Microbiol 76:866–879. https://doi.org/10.1128/AEM.02009-09.
17. Biessy A, Novinscak A, Blom J, Léger G, Thomashow LS, Cazorla FM, Josic D, Filion M. 2019. Diversity of phytobeneficial traits revealed by whole-genome analysis of worldwide-isolated phenazine-producing *Pseudomonas* spp. Environ Microbiol 21:437–455. https://doi.org/10.1111/1462-2920.14476.
18. Delaney SM, Mavrodi DV, Bonsall RF, Thomashow LS. 2001. *phzO*, a gene for biosynthesis of 2-hydroxylated phenazine compounds in *Pseudomonas aureofaciens* 30–84. J Bacteriol 183:318–327. https://doi.org/10.1128/JB.183.1.318-327.2001.
19. Chin-A-Woeng TFC, Thomas-Oates JE, Lugtenberg BJJ, Bloemberg GV. 2001. Introduction of the *phzH* gene of *Pseudomonas chlororaphis* PCL1391 extends the range of biocontrol ability of phenazine-1-carboxylic acid-producing *Pseudomonas* spp. strains. Mol Plant Microbe Interact 14:1006–1015. https://doi.org/10.1094/MPMI.2001.14.8.1006.
20. Yu JM, Wang D, Pierson LS, III, Pierson EA. 2018. Effect of producing different phenazines on bacterial fitness and biological control in *Pseudomonas chlororaphis* 30-84. Plant Pathol J 34:44–58. https://doi.org/10.5423/PPJ.FT.12.2017.0277.
21. D'aes J, Hua GKH, De Maeyer K, Pannecoucque J, Forrez I, Ongena M, Dietrich LE, Thomashow LS, Mavrodi DV, Höfte M. 2011. Biological control of *Rhizoctonia* root rot on bean by phenazine- and cyclic lipopeptide-producing *Pseudomonas* CMR12a. Phytopathology 101:996–1004. https://doi.org/10.1094/PHYTO-11-10-0315.
22. Upadhyay A, Srivastava S. 2011. Phenazine-1-carboxylic acid is a more important contributor to biocontrol *Fusarium oxysporum* than pyrrolnitrin in *Pseudomonas fluorescens* strain Psd. Microbiol Res 166:323–335. https://doi.org/10.1016/j.micres.2010.06.001.
23. Le C, Kruijt M, Raaijmakers J. 2012. Involvement of phenazines and lipopeptides in interactions between *Pseudomonas* species and *Sclerotium*

rolfsii, causal agent of stem rot disease on groundnut. J Appl Microbiol 112:390–403. https://doi.org/10.1111/j.1365-2672.2011.05205.x.

24. Huang R, Feng Z, Chi X, Sun X, Lu Y, Zhang B, Lu R, Luo W, Wang Y, Miao J, Ge Y. 2018. Pyrrolnitrin is more essential than phenazines for *Pseudomonas chlororaphis* G05 in its suppression of *Fusarium graminearum*. Microbiol Res 215:55–64. https://doi.org/10.1016/j.micres.2018.06.008.
25. Selin C, Habibian R, Poritsanos N, Athukorala SN, Fernando D, De Kievit TR. 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. https://doi.org/10.1111/j.1574-6941.2009.00792.x.
26. Debode J, De Maeyer K, Perneel M, Pannecoucque J, De Backer G, Hofte M. 2007. Biosurfactants are involved in the biological control of *Verticillium* microsclerotia by *Pseudomonas* spp. J Appl Microbiol 103:1184–1196. https://doi.org/10.1111/j.1365-2672.2007.03348.x.
27. Morrison CK, Arseneault T, Novinscak A, Filion M. 2017. Phenazine-1-carboxylic acid production by *Pseudomonas fluorescens* LBUM636 alters *Phytophthora infestans* growth and late blight development. Phytopathology 107:273–279. https://doi.org/10.1094/PHYTO-06-16-0247-R.
28. St-Onge R, Gadkar VJ, Arseneault T, Goyer C, Filion M. 2011. The ability of *Pseudomonas* sp. LBUM 223 to produce phenazine-1-carboxylic acid affects the growth of *Streptomyces scabies*, the expression of thaxtomin biosynthesis genes and the biological control potential against common scab of potato. FEMS Microbiol Ecol 75:173–183. https://doi.org/10.1111/j.1574-6941.2010.00992.x.
29. Arseneault T, Goyer C, Filion M. 2015. *Pseudomonas fluorescens* LBUM223 increases potato yield and reduces common scab symptoms in the field. Phytopathology 105:1311–1317. https://doi.org/10.1094/PHYTO-12-14-0358-R.
30. Roquigny R, Novinscak A, Arseneault T, Joly DL, Filion M. 2018. Transcriptome alteration in *Phytophthora infestans* in response to phenazine-1-carboxylic acid production by *Pseudomonas fluorescens* strain LBUM223. BMC Genom 19:474. https://doi.org/10.1186/s12864-018-4852-1.
31. Gurusiddaiah S, Weller D, Sarkar A, Cook R. 1986. Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. Antimicrob Agents Chemother 29:488–495. https://doi.org/10.1128/aac.29.3.488.
32. Anjaiah V, Koedam N, Nowak-Thompson B, Loper JE, Hofte M, Tambong JT, Cornelis P. 1998. Involvement of phenazines and anthranilate in the antagonism of *Pseudomonas aeruginosa* PNA1 and Tn5 derivatives toward *Fusarium* spp. and *Pythium* spp. MPMI 11:847–854. https://doi.org/10.1094/MPMI.1998.11.9.847.
33. Laville J, Blumer C, Von Schroetter C, Gaia V, Défago G, Keel C, Haas D. 1998. Characterization of the *hcnABC* gene cluster encoding hydrogen cyanide synthase and anaerobic regulation by ANR in the strictly aerobic biocontrol agent *Pseudomonas fluorescens* CHA0. J Bacteriol 180:3187–3196. https://doi.org/10.1128/JB.180.12.3187-3196.1998.
34. Poritsanos N, Selin C, Fernando WG, Nakkeeran S, Kievit T. 2006. A GacS deficiency does not affect *Pseudomonas chlororaphis* PA23 fitness when growing on canola, in aged batch culture or as a biofilm. Can J Microbiol 52:1177–1188. https://doi.org/10.1139/w06-079.
35. Chin-A-Woeng TFC, Bloemberg GV, van der Bij AJ, van der Drift K, Schripsema J, Kroon B, Scheffer RJ, Keel C, Bakker P, Tichy HV, de Bruijn FJ, Thomas-Oates JE, Lugtenberg BJJ. 1998. Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. MPMI 11:1069–1077. https://doi.org/10.1094/MPMI.1998.11.11.1069.
36. Loper JE, Hassan KA, Mavrodi DV, Davis EW, Lim CK, Shaffer BT, Elbourne LDH, Stockwell VO, Hartney SL, Breakwell K, Henkels MD, Tetu SG, Rangel LI, Kidarsa TA, Wilson NL, van de Mortel JE, Song C, Blumhagen R, Radune D, Hostetler JB, Brinkac LM, Durkin AS, Kluepfel DA, Wechter WP, Anderson AJ, Kim YC, Pierson LS, Pierson EA, Lindow SE, Kobayashi DY, Raaijmakers JM, Weller DM, Thomashow LS, Allen AE, Paulsen IT. 2012. Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. PLoS Genet 8:e1002784. https://doi.org/10.1371/journal.pgen.1002784.
37. Hunziker L, Bönisch D, Groenhagen U, Bailly A, Schulz S, Weisskopf L. 2015. *Pseudomonas* strains naturally associated with potato plants produce volatiles with high potential for inhibition of *Phytophthora infestans*. Appl Environ Microbiol 81:821–830. https://doi.org/10.1128/AEM.02999-14.
38. Anand A, Chinchilla D, Tan C, Mène-Saffrané L, L'Haridon F, Weisskopf L. 2020. Contribution of hydrogen cyanide to the antagonistic activity of *Pseudomonas* strains against *Phytophthora infestans*. Microorganisms 8:1144. https://doi.org/10.3390/microorganisms8081144.
39. Arseneault T, Goyer C, Filion M. 2013. Phenazine production by *Pseudomonas* sp. LBUM223 contributes to the biological control of potato common scab. Phytopathology 103:995–1000. https://doi.org/10.1094/PHYTO-01-13-0022-R.
40. Berg G, Ballin G. 1994. Bacterial antagonists to *Verticillium dahliae* Kleb. J Phytopathol 141:99–110. https://doi.org/10.1111/j.1439-0434.1994.tb01449.x.
41. Chen M, Cao H, Peng H, Hu H, Wang W, Zhang X. 2014. Reaction kinetics for the biocatalytic conversion of phenazine-1-carboxylic acid to 2-hydroxyphenazine. PLoS One 9:e98537. https://doi.org/10.1371/journal.pone.0098537.
42. van Rij ET, Wesselink M, Chin-A-Woeng TF, Bloemberg GV, Lugtenberg BJ. 2004. Influence of environmental conditions on the production of phenazine-1-carboxamide by *Pseudomonas chlororaphis* PCL1391. Mol Plant Microbe Interact 17:557–566. https://doi.org/10.1094/MPMI.2004.17.5.557.
43. Slininger P, Shea-Wilbur M. 1995. Liquid-culture pH, temperature, and carbon (not nitrogen) source regulate phenazine productivity of the take-all biocontrol agent *Pseudomonas fluorescens* 2–79. Appl Microbiol Biotechnol 43:794–800. https://doi.org/10.1007/BF02431910.
44. Slininger PJ, Jackson MA. 1992. Nutritional factors regulating growth and accumulation of phenazine 1-carboxylic acid by *Pseudomonas fluorescens* 2-79. Appl Microbiol Biotechnol 37:388–392. https://doi.org/10.1007/BF00210998.
45. Park JY, Oh SA, Anderson AJ, Neiswender J, Kim JC, Kim YC. 2011. Production of the antifungal compounds phenazine and pyrrolnitrin from *Pseudomonas chlororaphis* O6 is differentially regulated by glucose. Lett Appl Microbiol 52:532–537. https://doi.org/10.1111/j.1472-765X.2011.03036.x.
46. Park S-Y, Kang H-O, Jang H-S, Lee J-K, Koo B-T, Yum D-Y. 2005. Identification of extracellular *N*-acylhomoserine lactone acylase from a *Streptomyces* sp. and its application to quorum quenching. Appl Environ Microbiol 71:2632–2641. https://doi.org/10.1128/AEM.71.5.2632-2641.2005.
47. Hassan R, Shaaban MI, Abdel Bar FM, El-Mahdy AM, Shokralla S. 2016. Quorum sensing inhibiting activity of *Streptomyces coelicoflavus* isolated from soil. Front Microbiol 7:659. https://doi.org/10.3389/fmicb.2016.00659.
48. van Rij ET, Girard G, Lugtenberg BJ, Bloemberg GV. 2005. Influence of fusaric acid on phenazine-1-carboxamide synthesis and gene expression of *Pseudomonas chlororaphis* strain PCL1391. Microbiology (Reading) 151:2805–2814. https://doi.org/10.1099/mic.0.28063-0.
49. Mazurier S, Corberand T, Lemanceau P, Raaijmakers JM. 2009. Phenazine antibiotics produced by fluorescent pseudomonads contribute to natural soil suppressiveness to Fusarium wilt. ISME J 3:977–991. https://doi.org/10.1038/ismej.2009.33.
50. Zboralski A, Biessy A, Savoie M-C, Novinscak A, Filion M. 2019. Metabolic and genomic traits of phytobeneficial phenazine-producing *Pseudomonas* spp. are linked to rhizosphere colonization in *Arabidopsis thaliana* and *Solanum tuberosum*. Appl Environ Microbiol 86:e02443-19. https://doi.org/10.1128/AEM.02443-19.
51. Bull CT, Weller DM, Thomashow LS. 1991. Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2-79. Phytopathology 81:954–959. https://doi.org/10.1094/Phyto-81-954.
52. Raaijmakers JM, Leeman M, Van Oorschot MM, Van der Sluis I, Schippers B, Bakker P. 1995. Dose-response relationships in biological control of fusarium wilt of radish by *Pseudomonas* spp. Phytopathology 85:1075–1080. https://doi.org/10.1094/Phyto-85-1075.
53. Raaijmakers JM, Bonsall RF, Weller DM. 1999. Effect of population density of *Pseudomonas fluorescens* on production of 2, 4-diacetylphloroglucinol in the rhizosphere of wheat. Phytopathology 89:470–475. https://doi.org/10.1094/PHYTO.1999.89.6.470.
54. Mavrodi DV, Mavrodi OV, Parejko JA, Bonsall RF, Kwak YS, Paulitz TC, Thomashow LS, Weller DM. 2012. Accumulation of the antibiotic phenazine-1-carboxylic acid in the rhizosphere of dryland cereals. Appl Environ Microbiol 78:804–812. https://doi.org/10.1128/AEM.06784-11.
55. King EO, Ward MK, Raney DE. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 44:301–307.
56. Goyer C, Vachon J, Beaulieu C. 1998. Pathogenicity of *Streptomyces scabies* mutants altered in thaxtomin A production. Phytopathology 88:442–445. https://doi.org/10.1094/PHYTO.1998.88.5.442.
57. R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
58. RStudio Team. 2019. RStudio: integrated development for R. RStudio, PBC, v1.1.453. RStudio Team, Boston, MA.

59. de Mendiburu F. 2017. Agricolae: statistical procedures for agricultural research. R package version 1.2-8. https://CRAN.R-project.org/package=agricolae.
60. Morrison CK, Novinscak A, Gadkar VJ, Joly DL, Filion M. 2016. Complete genome sequence of *Pseudomonas fluorescens* LBUM636, a strain with biocontrol capabilities against late blight of potato. Genome Announc 4:e00446-16.
61. Parejko JA, Mavrodi DV, Mavrodi OV, Weller DM, Thomashow LS. 2012. Population structure and diversity of phenazine-1-carboxylic acid producing fluorescent *Pseudomonas* spp. from dryland cereal fields of central Washington State (USA). Microb Ecol 64:226–241. https://doi.org/10.1007/s00248-012-0015-0.
62. Shirzad A, Fallahzadeh-Mamaghani V, Pazhouhandeh M. 2012. Antagonistic potential of fluorescent Pseudomonads and control of crown and root rot of cucumber caused by *Phythophtora drechsleri*. Plant Pathol J 28:1–9. https://doi.org/10.5423/PPJ.OA.05.2011.0100.
63. Weller DM, Cook RJ. 1983. Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. Phytopathology 73:463–469. https://doi.org/10.1094/Phyto-73-463.
64. Roquigny R, Arseneault T, Gadkar VJ, Novinscak A, Joly DL, Filion M. 2015. Complete genome sequence of biocontrol strain *Pseudomonas fluorescens* LBUM223. Genome Announc 3:e00443-15.
65. Perneel M, Heyrman J, Adiobo A, De Maeyer K, Raaijmakers JM, De Vos P, Hofte M. 2007. Characterization of CMR5c and CMR12a, novel fluorescent *Pseudomonas* strains from the cocoyam rhizosphere with biocontrol activity. J Appl Microbiol 103:1007–1020. https://doi.org/10.1111/j.1365-2672.2007.03345.x.
66. Peix A, Valverde A, Rivas R, Igual JM, Ramírez-Bahena M-H, Mateos PF, Santa-Regina I, Rodríguez-Barrueco C, Martínez-Molina E, Velázquez E. 2007. Reclassification of *Pseudomonas aurantiaca* as a synonym of *Pseudomonas chlororaphis* and proposal of three subspecies, *P chlororaphis* subsp. *chlororaphis* subsp. nov., *P chlororaphis* subsp. *aureofaciens* subsp. nov., comb. nov., and *P chlororaphis* subsp. *aurantiaca* subsp. nov., comb. nov. Int J Syst Evol Microbiol 57:1286–1290. https://doi.org/10.1099/ijs.0.64621-0.
67. Salman M. 2010. Determination of antibiotic activity on plasmids from fluorescent pseudomonads isolates CW2, WB15 and WB52 against pre-emergence damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* in cucumber. Biol Control 53:161–167. https://doi.org/10.1016/j.biocontrol.2010.01.007.
68. Veselova M, Klein S, Bass I, Lipasova V, Metlitskaya A, Ovadis M, Chernin L, Khmel I. 2008. Quorum sensing systems of regulation, synthesis of phenazine antibiotics, and antifungal activity in rhizospheric bacterium *Pseudomonas chlororaphis* 449. Russ J Genet 44:1400–1408. https://doi.org/10.1134/S102279540812003X.
69. Veselova M, Lipasova V, Protsenko MA, Buza N, Khmel IA. 2009. GacS-dependent regulation of enzymic and antifungal activities and synthesis of *N*-acylhomoserine lactones in rhizospheric strain *Pseudomonas chlororaphis* 449. Folia Microbiol (Praha) 54:401–408. https://doi.org/10.1007/s12223-009-0056-z.
70. Josic D, Protolipac K, Starovic M, Stojanovic S, Pavlovic S, Miladinovic M, Radovic S. 2012. Phenazines producing *Pseudomonas* isolates decrease *Alternaria tenuissima* growth, pathogenicity and disease incidence on cardoon. Arch Biol Sci (Beogr) 64:1495–1503. https://doi.org/10.2298/ABS1204495J.
71. Puopolo G, Raio A, Pierson LS, Zoina A. 2011. Selection of a new *Pseudomonas chlororaphis* strain for the biological control of *Fusarium oxysporum* f. sp *radicis-lycopersici*. Phytopathol Mediterr 50:228–235.
72. Kluyver A. 1956. *Pseudomonas aureofaciens* nov. spec. and its pigments. J Bacteriol 72:406–411. https://doi.org/10.1128/JB.72.3.406-411.1956.
73. Mezaache-Aichour S, Gueehi A, Nicklin J, Drider D, Prevost H, Strange RN. 2012. Isolation, identification and antimicrobial activity of pseudomonads isolated from the rhizosphere of potatoes growing in Algeria. J Plant Pathol 94:89–98.
74. Loewen PC, Villenueva J, Fernando WD, de Kievit T. 2014. Genome sequence of *Pseudomonas chlororaphis* strain PA23. Genome Announc 2:e00689-14.
75. Savchuk S, Dilantha Fernando W. 2004. Effect of timing of application and population dynamics on the degree of biological control of *Sclerotinia sclerotiorum* by bacterial antagonists. FEMS Microbiol Ecol 49:379–388. https://doi.org/10.1016/j.femsec.2004.04.014.
76. Kropp BR, Thomas E, Pounder JI, Anderson AJ. 1996. Increased emergence of spring wheat after inoculation with *Pseudomonas chlororaphis* isolate 2E3 under field and laboratory conditions. Biol Fertil Soils 23:200–206. https://doi.org/10.1007/BF00336064.
77. Stanier RY, Palleron NJ, Doudorof M. 1966. The aerobic pseudomonads a taxonomic study. J Gen Microbiol 43:159–271. https://doi.org/10.1099/00221287-43-2-159.
78. Burr SE, Gobeli S, Kuhnert P, Goldschmidt-Clermont E, Frey J. 2010. *Pseudomonas chlororaphis* subsp. *piscium* subsp. nov., isolated from freshwater fish. Int J Syst Evol Microbiol 60:2753–2757. https://doi.org/10.1099/ijs.0.011692-0.
79. Landa BB, Cachinero-Díaz JM, Lemanceau P, Jiménez-Díaz RM, Alabouvette C. 2002. Effect of fusaric acid and phytoanticipins on growth of rhizobacteria and *Fusarium oxysporum*. Can J Microbiol 48:971–985. https://doi.org/10.1139/w02-094.
80. Kamou NN, Karasali H, Menexes G, Kasiotis KM, Bon MC, Papadakis EN, Tzelepis GD, Lotos L, Lagopodi AL. 2015. Isolation screening and characterization of local beneficial rhizobacteria based upon their ability to suppress the growth of *Fusarium oxysporum* f. sp *radicis-lycopersici* and tomato foot and root rot. Biocontrol Sci Technol 25:928–949. https://doi.org/10.1080/09583157.2015.1020762.
81. Cazorla FM, Duckett SB, Bergstrom ET, Noreen S, Odijk R, Lugtenberg BJJ, Thomas-Oates JE, Bloemberg GV. 2006. Biocontrol of avocado dematophora root rot by antagonistic *Pseudomonas fluorescens* PCL1606 correlates with the production of 2-hexyl 5-propyl resorcinol. Mol Plant Microbe Interact 19:418–428. https://doi.org/10.1094/MPMI-19-0418.
82. Mehnaz S, Baig DN, Jamil F, Weselowski B, Lazarovits G. 2009. Characterization of a phenazine and hexanoyl homoserine lactone producing *Pseudomonas aurantiaca* strain Pb-St2, isolated from sugarcane stem. J Microbiol Biotechnol 19:1688–1694. https://doi.org/10.4014/jmb.0904.04022.
83. Pierson LS, Thomashow LS. 1992. Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens* 30–84. Mol Plant Microbe Interact 5:330–339. https://doi.org/10.1094/mpmi-5-330.
84. Raaijmakers JM, Weller DM, Thomashow LS. 1997. Frequency of antibiotic-producing *Pseudomonas* spp. in natural environments. Appl Environ Microbiol 63:881–887. https://doi.org/10.1128/AEM.63.3.881-887.1997.