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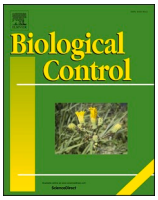

Pseudomonas azotoformans and *Pseudomonas putida*: Novel kiwifruit-native biological control agents against *Pseudomonas syringae* pv. *actinidiae*

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HIGHLIGHTS

- Native bacteria from kiwifruit leaves can contribute to bacterial canker control.
- *Pseudomonas azotoformans* and *P. putida densely* colonize *Actinidia* spp. leaves and flowers.
- *P. azotoformans* and *P. putida* produce siderophores.
- *P. azotoformans* shows resistance to copper.

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ABSTRACT

Pseudomonas syringae pv. *actinidiae* (Psa), the etiological agent of the bacterial canker in *Actinidia* plants, remains the main threat to kiwifruit orchards worldwide. Though environment-friendly disease control methods based on biological control agents (BCAs) represent a promising alternative to xenobiotic pesticides, their efficacy in field conditions has often resulted erratic. The selection of beneficial microorganisms directly from the phyllosphere of the host plant is a promising approach to overcome this limitation since it ensures the adaptation of the isolates to the environment in which they are going to be applied. This work reports the screening of the kiwifruit epiphytic bacterial community from three Psa infected orchards in Portugal to identify potential bacterial BCAs capable of inhibiting Psa growth or interfering with its virulence. Strains of *Pseudomonas putida* and *Pseudomonas azotoformans* efficiently antagonized Psa on flowers and leaves and colonized all susceptible organs with high surviving rates in glasshouse conditions. *In vitro* metabolic analysis together with genome sequencing and annotation revealed siderophore production, in particular pyoverdine, which may limit iron availability to the pathogen. Moreover, several biosynthetic gene clusters of secondary metabolites, were predicted in the genome of both strains, including non-ribosomal peptides, and the bacteriocin pyocin was predicted in the genome of BG1. Overall, these results open new perspectives to develop commercial products for Psa management based on kiwifruit-native bacteria, well-adapted to common orchard management practices, with a high efficiency of host plant colonization, at Psa-conducive temperatures, and point out possible mechanisms of action for these two BCA candidates, supporting further steps to assess their effectiveness in orchard conditions.

1. Introduction

Crop pathogens and pests represent the major burden for the agricultural sector, being responsible for up to 40 % of the food losses every year (FAO, 2022; Savary et al., 2019). The global use of pesticides in 2019 was about 4.19 million tonnes (Mt), with 968.68 thousand tonnes for fungicides and bactericides. Europe's pesticide consumption is around 11 % of the worldwide consumption with an estimated cost of 12.1 billion US dollars (FAOSTAT, 2021). The European Union (EU) regulations are limiting the use of pesticides ((EU)2018/1981) aiming at

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reducing by 50 % the overall use of chemical products by 2030 through the application of sustainable, biological, and other non-chemical alternatives, as long as they provide satisfactory pest and disease control.

The biological control of plant diseases consists in suppressing plant pathogenic populations using living organisms [biological control agents (BCAs)] (Heimpel and Mills, 2017). BCAs can have two main modes of action in crop protection: direct, through hyperparasitism or antibiosis, or indirect, by inducing plant resistance or competing with pathogens for nutrients and space (Köhl et al., 2019). Several formulations have been registered both at national and international levels based on different fungal and bacterial antagonists, but their efficacy is dependent on multiple factors, such as climatic conditions encountered in the field (e.g. temperature, humidity, solar radiation) and ecological competences (competitive and adaptive traits, colonization ability of a specific host in a specific environment, interaction with the native phytobiota) (Bardin et al., 2015; Monchiero et al., 2015). Moreover, the phenotypical diversity of natural populations (which may include strains with different degrees of sensitivity to treatments) may explain the low efficacy of control strategies. For example, Mariz-Ponte et al. (2022) have characterized the genetic diversity of *Pseudomonas syringae* pv. *actinidiae* (Psa) from Portugal kiwifruit orchards and found negligible differences among the tested isolates. However, when testing a set of antimicrobial peptides, the different strains showed different levels of sensitivity suggesting a phenotypic diversity in Portuguese Psa community (Mariz-Ponte et al., 2021). Another report showed that 16 out of the 19 BCA tested, were ineffective against one of the 15 studied isolates of *Streptomyces scabies*, the causative agent of potato scab (Otto-Hanson et al., 2013). All these examples clearly highlight the drawbacks of a single-strain BCA against natural populations of plant pathogens. Thus, during the development/search of new BCAs, organisms with multimode of action must be preferred, as well as isolates adapted to the environment/host where they are supposed to act (Bruisson et al., 2019). Finally, several studies highlighted that selecting BCAs from the host phytobiota may maximise the effectiveness of disease control (Banerjee et al., 2017; Santiago et al., 2017; May et al., 2021). In fact, microbes selected within a phytobiota have evolved for that specific ecological niche and are therefore more prone to form stable populations in homeostasis with the surrounding microbial biocenosis (Vishwakarma et al., 2020), which is a pre-requisite for an effective biological control strategy. Notably, the composition of the plant native microbiota is relatively stable and scarcely influenced by environmental factors (Berg et al., 2020).

Pseudomonas syringae pv. *actinidiae* (Psa) is the etiological agent of the bacterial canker of kiwifruit and threatens the kiwifruit industry worldwide (Donati et al., 2014; Scortichini et al., 2024). Five biovars genetically different have been characterized, being the biovar 3 the most aggressive and the one responsible for the most recent outbreaks worldwide (Scortichini et al., 2012; Firrao et al., 2018; Garcia et al., 2018; Sawada and Fujikawa, 2019). Moreover, the genetic diversity of Psa within the same biovar has been reported all over the globe (Prencipe et al., 2018; He et al., 2019; Figueira et al., 2020; Mariz-Ponte et al., 2022). Symptom development concentrates in the spring (April–May, leaf spots) and autumn (exudates, water-soaked leaf spots) after frost events (Donati et al., 2020). Spring is, therefore, a key season of Psa disease cycle, being flowers the main site of infection during this season, when the environmental conditions are highly favourable for the pathogen growth, and its spread is further facilitated by pollinators (Donati et al., 2018; 2020). Moreover, Psa infection through flowers cannot be controlled with chemical treatments or agronomical practices. Currently, the preventive measures to control bacterial spread and disease mostly rely on Cu-based agrochemicals, although several bio-based commercial products have been tested in controlled and field conditions (Monchiero et al., 2015). In some non-EU countries, the use of antibiotics such as streptomycin is also allowed (Lee et al., 2020). The use of antibiotics and Cu-based products have however resulted in the development of antibiotic and/or Cu resistance in Psa populations (Colombi et al., 2017; Lee et al., 2020, Cesari et al, 2023). Because of pollinator-mediated bacterial spread and conducive conditions in spring, flowers are a sensible site of infection, and cannot be protected with chemical treatments or agronomical practices (Donati et al., 2018; 2020). Based on these considerations, the development and validation of alternative control strategies, including biological control (Wicaksono et al., 2018), is urgently needed.

Epiphytic and endophytic bacteria from different *Actinidia* spp. organs have been shown to be promising alternatives to the current inefficient control treatments (Purahong et al., 2018). Recent studies were focused on the microbial community of kiwifruit plants, its relation to Psa, and its influence on the expression of disease symptoms (Purahong et al., 2018). Most of the Psa-associated bacteria in symptomatic and asymptomatic plant material were identified as *Pseudomonas* spp. by metabarcoding analysis (Purahong et al., 2018). Some were characterized as antagonists, producing antibiotics like 2,4-DAPG, phenazine and hydrogen cyanide (Wicaksono et al., 2018).

BCA functional characterization, at both biochemical and genomic levels, is essential not only for improving their efficacy as biopesticides, but also for their approval and registration under European Union (EU) regulations (COMMISSION REGULATION (EU) 2022/1439). The exponential development of next generation sequencing, making the sequencing of bacterial genomes more widely accessible and affordable to research laboratories, has significantly potentiated bacterial genome collection, further enabling the study and comparison of their properties, and the characterization of the genes potentially involved in biological control traits.

Characterizing the local microbial biocenosis associated to kiwifruit will facilitate the selection of new BCAs, which naturally evolved to colonize susceptible organs under orchard environmental conditions. Therefore, the objective of this study was to investigate the potential of kiwifruit leaf phyllosphere as a source of bacteria capable to control Psa. Since early spring and late autumn are key seasons for the epidemiological cycle of the kiwifruit bacteria canker, we performed the selection at mid-low temperatures which are characteristics of these periods. Their capacity to inhibit Psa growth *in vitro* and to reduce epiphytic Psa population on flowers and leaves was investigated. To investigate the mode of action of the most effective BCAs, the strains were functionally characterized for beneficial functions and their genomes were shotgun-sequenced. Their draft genomes were subject to multiple genomic analyses to characterize them taxonomically and to inspect their antimicrobial biosynthetic gene cluster repertoires to infer about their mode of action as BCAs. The approach adopted in this work allowed to identify BCAs specifically adapted to the growing conditions of kiwifruit able to protect the host plant in the most vulnerable phenological phases.

2. Materials and methods

2.1. Bacteria isolation

Leaf samples were collected from three commercial *Actinidia chinensis* var. *deliciosa* 'Hayward' orchards in northern Portugal located in Geraz do Minho, Santa Lucrécia and Briteiros Guimarães. From each orchard, 4 trees were randomly selected and 10 asymptomatic leaves from each tree were sampled for bacterial isolation. Leaves were chosen according to their development stage, equally sampling young, fully developed and mature leaves. The plant material collected from each orchard was split in half: one half was immediately used for bacteria isolation and the other half was kept in a plastic bag at 10 °C for one week to select the bacteria more adapted to spring/autumn temperatures (periods conducive to Psa infection). Leaf pools were washed in extraction buffer (10 mM $MgSO_4$, pH = 7.3) under moderate shaking for 30 min. Subsequently, the washing suspensions were collected and 80 μL were spread on 1.5 % agarized lysogenic broth (LA) medium containing cycloheximide (100 mg L^{-1}) to prevent fungal growth. For each biological replicate, after 24–48 h of incubation at 27 °C (standard

temperature for bacterial growth), morphologically different colonies were isolated and purified on fresh LA plates.

2.2. In vitro inhibition test on solid medium

For the pre-incubation, Psa CFBP 7286 was inoculated in LA medium by spreading 1 mL of 0.1 OD_{600} of bacterial suspension, removing the excess after 1 min and allowing drying for 15 min, according to Mariz-Ponte et al. (2021), with some modifications. Five μL of each BCA culture 0.01 OD_{600} was loaded on the previously Psa inoculated LA plates. Lysogenic Broth (LB) (Liofilchem, Italy) and chlortetracycline (1.5 mg L^{-1}) were used as negative and positive control, respectively. After 24–48 h of incubation at 27 °C, plate images were acquired on Gel Doc (BioRad, Hercules, CA, USA) and inhibition effect was determined by presence/absence of an inhibition halo around the bacterial drops. For the bacterial candidates that showed a halo, the experiment was repeated twice.

Finally, for isolates showing plate inhibition of Psa, rifampicin resistant mutants were obtained to be used in *in planta* experiments to precisely monitor BCA colonisation. For this purpose, BCAs were grown on LB amended with rifampicin (50 mg L^{-1}) for at least 3 consecutive cycles. Mutant growth rate and inhibition capacity was tested in comparison with wild strain to verify that mutation did not alter the isolate phenotype.

2.3. Hypersensitive response (HR) test on Nicotiana tabacum leaves

Fresh cultures (grown in LA medium for 48 h) were used to prepare a cloudy suspension (~10^8 colony-forming units (CFU) mL^{-1}) in PBS. To infiltrate the isolates into *Nicotiana tabacum* leaves, a syringe without a needle was used. The suspension was infiltrated on the abaxial leaf lamina. Psa CFBP 7286 and PBS were used as positive and negative controls, respectively. The development of an HR was assessed 2–3 days after infiltration (Klement, 1963; Lelliot and Stead, 1987). The experiment was performed in four replicates consisting in separate leaves.

2.4. Identification of putative BCAs

The genomic DNA (gDNA) of putative BCAs was extracted according to Minas et al. (2011). gDNA was quantified by Nanodrop™ ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and diluted to 30 ng μL^{-1}. Isolates were identified through 16S rRNA gene sequencing using Lac16S-for (5′-AATGAGAGTTTGATCCTGGCT-3′) and Lac16S-rev (5′- GAGGTGATCCAGCCGCAGGTT-3′) primers (Perpetuini et al., 2019). The PCR was performed in a 40-μL reaction mixture, using Applied Biosystems AmpliTaq Gold™ DNA Polymerase with Gold Buffer and $MgCl_2$ (Thermo Fisher Scientific, Waltham, MA, USA), following manufacturer's instructions and using 60 ng genomic DNA. PCR reactions were carried out in a 2720 thermal cycler (Applied Biosystems) with the following amplification protocol: initial denaturation step at 94 °C for 4 min, followed by 25 cycles at 94 °C for 1 min, at 50 °C for 1.5 min and at 72 °C for 2 min and a final extension step at 72 °C for 7 min. The amplification product of 800 bp was sequenced (Bio-Fab Research, Rome, Italy) and the obtained sequence was identified using the Blast tool on the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM = blastn&PAGE_TYPE = BlastSearch&LINK_LOC = blasthome).

2.5. DNA extraction, genome sequencing and de novo assembly

The two most promising BCA strains, hereafter called BG1 and BG2, were grown in LB medium, and incubated at 25 °C ± 1°C, at 160 rpm, for 24 h. DNA was extracted from cells pellet using E.Z.N.A.1 Bacterial DNA purification Kit (Omega Biotek, USA) following the manufacturer's instructions.

Library Construction, Quality Control and Sequencing were conducted by Novogene Europe (Cambridge, United Kingdom) using Illumina Sequencing to obtain 150 bp paired-end reads (10,505,188 and 10,362,090 raw reads for BG1 and BG2, respectively). Low quality data were filtered using Fastp software (v0.20.0), clean reads (10,477,594 and 10,335,122 reads for BG1 and BG2, respectively) were obtained for the consecutive steps and quality checked using FastQC (version 0.11.9). Reads were assembled *de novo* using SPAdes (Galaxy Version 2.2.6, https://usegalaxy.org.au/) with the "–careful" parameter for reads error correction, and a set of k-mer lengths of 21, 33, 55 (default). QUAST (v5.2.0) was used to obtain basic assembly statistics (Table 1). These Whole Genome Shotgun projects have been deposited at DDBJ/ENA/GenBank under the accessions JAVIUJ000000000 (BG1) and JAUZTF000000000 (BG2).

2.6. Genotyping with multilocus sequence typing (MLST), average nucleotide identity (ANI) and amino acid identity (AAI) calculator

The assembled genomes were then analysed through ribosomal multilocus sequence typing (rMLST), using Pub MLST (https://pubmlst.org/), where a total of 53 rMLST marker genes were used for a rapid identification of the genomes (Appendix A, Table S1-S2). Then, the draft genome sequences were compared with related genomes available in the National Center for Biotechnology Information (NCBI) prokaryotic genomes database and average nucleotide identity (ANI) and amino acid identity (AAI) were determined through the Web interface for the Microbial Genomes Atlas (MiGA) (https://disc-genomics.uibk.ac.at/miga/). Typically, the ANI values between genomes of the same species are above 95 % (Rodriguez-R and Konstantinidis, 2014).

2.7. Colonization of flowers and leaves by the selected strains and inhibition of Psa growth in planta

The selected strains BG1 and BG2 were tested for their colonization ability of kiwifruit flowers and leaves and Psa growth inhibition in planta. For this purpose, flowers of *A. chinensis* var. *deliciosa* 'Hayward' and *A. chinensis* var. *chinensis* 'Hort 16A' were used. Flowering branches were harvested from a Psa-free orchard located at the Kiwifruit Breeding Centre of Bologna University (44.549065610586744, 11.412359125087713) one day prior to BCA inoculation, maintained in pots with water and hand-pollinated with a brush. BG1 and BG2 were applied by spray 24 h before inoculation with Psa. Inoculation was performed in the quarantine glasshouse of the University of Bologna. During the experiments, the plants were kept under natural light and temperature and relative humidity were, respectively, 22–24 °C and 70–80 %. After inoculation, the relative humidity was raised to 100 % for the following 24 h. Prior to bacteria inoculation, BCAs and Psa were grown in LA and incubated at 27 °C for 48 h. BCA suspensions were adjusted to 1 × 10^9 CFU mL^{-1}, and the Psa inoculum was adjusted to 1x10^7 CFU mL^{-1}. The Green Fluorescent Protein-expressing, kanamycin-resistant strain CFBP 7286-GFPuv (Spinelli et al., 2010) was used for inoculation. Flowers were inoculated by spraying till run-off. Water-treated plants were used as negative control, while the commercial product Amylo-X® (Biogard, Monza Brianza, Italy) was used as positive control, following the dose recommended by the manufacturer

Table 1
Genome assembly statistics based on contigs of size >= 500 bp.

Attribute	BG1	BG2
Genome size (bp)	6,533,111	6,188,273
%GC content	60.9	62.7
N50 (bp)	278,364	240,790
L50 (bp)	9	6
Number of contigs	48	54
Largest contig	664,130	871,593
Number of coding sequences	6,023	5,645
Number of RNAs	68	91

(corresponding to ~ 1x10^7 CFU mL^{-1}).

In all the experiments, three to five flowers were collected for each time point to evaluate epiphytic growth of BCAs and Psa at 3, 24, 48 and 120 h after inoculation. The bacterial population was obtained by washing each flower in 10 mL of 10 mM $MgSO_4$. The bacterial concentrations (CFU mL^{-1}) were determined by direct plating of three replicates of each 10-fold serial dilution on LA medium. For double cultures (BCA + Psa), the dilutions were plated on LA medium amended with rifampicin (50 mg L^{-1}) to select the BCA or kanamycin (50 mg L^{-1}) to select Psa. Both selective media also contained cycloheximide (100 mg L^{-1}) to prevent fungal growth.

The experiments on leaves were performed on one year-old *A. chinensis* var. *deliciosa* 'Hayward' potted plants, in glasshouse conditions, following the same methodology described for flowers. For enumeration of BCA and Psa epiphytic population, each leaf was washed in 30 mL of $MgSO_4$. Psa endophytic population was determined by surface disinfecting the leaves with hypochlorite 1 % and ethanol 70 %, washing 3 times in water, and grinding them in sterile $MgSO_4$ with mortar and pestle. Then, 10-fold serial dilution were plated on LA medium as previously described. All the experiments performed on *A. chinensis* var. *deliciosa* 'Hayward' were repeated for two consecutive years. For normalization purposes, for each experiment, the Psa population in BCA-treated plants was compared to the Psa population in water-treated controls and presented as the % of Psa population reduction, following Equation (1):

$$Efficacy\ (\%) = \frac{Psa(BCA)}{Psa(water)} \times 100 \quad (1)$$

where *Psa (BCA)* and *Psa (water)* corresponds to Psa bacterial population in BCA-treated plants and water-treated controls, respectively.

2.8. Characterization of selected strains

2.8.1. Bacterial growth at 10 °C and 27 °C

The selected strains (BG1 and BG2) and Psa were recovered from the glycerol stocks, plated on LA and allowed to grow for two days. Overnight cultures were thereafter made from the pure cultures of these bacteria. The concentration of each strain in the overnight culture was determined by serial dilutions and recorded. A volume of 100 μL of each bacterial culture at a concentration of 10^3 CFU/mL was then used to inoculate three replicates of 20 mL of LB. Bacteria were grown at 10 °C or 27 °C, at 160 rpm, for 96 h. The absorbance of each bacterial solution was recorded at the start of the test and later at 6 h, 24 h, 48 h, 72 h and 96 h. The concentration of the bacteria was below detection limit, through serial dilution, until 72 h. Therefore, the concentration of each strain was determined at the two last time points of this experiment (72 h and 96 h). The growth curve of each strain was prepared using the absorbance values of the corresponding bacterial solutions.

2.8.2. Biochemical characteristics

Biochemical characteristics of the selected strains BG1 and BG2 was performed according to Sangiorgio et al. (2023). In details, the production of siderophore (Pérez-Miranda et al., 2007), and the growth promoting compounds acetoin and indole acetic acid (IAA), ammonia, and 1-aminocyclopropane-1-carboxylate ACC-deaminase activity was assessed (Blomqvist et al., 1993; Cappuccino and Sherman, 1992; Penrose and Glick, 2003; Ahmad et al., 2008). Finally, the detection of HCN-production was performed according to the protocol of Bakker & Schippers (1987).

2.8.3. Sensitivity to copper

Sensitivity to copper of the selected strains BG1 and BG2 was evaluated on liquid Mannitol-Glutamate-Yeast (MGY) extract media (Abbasi et al., 2015). Copper-sensitive *Pseudomonas syringae* pv. *actinidiae* CRAFRU 8.43 strain was used as positive control. Three concentrations of copper sulphate ($CuSO_4{\cdot}5H_2O$, Merk) were used for the test (0.8, 1.6, and 3.2 mM). The experiment was performed in 24-well plate pre-inoculated with MGY + $CuSO_4{\cdot}5H_2O$, where 10 μL of bacterial culture grown overnight in LB (Luria-Bertani) was inoculated, to a final volume of 1 mL. The OD_{600} of the bacterial suspension was read 48 h after inoculation using Tecan Infinite® 200 Pro. Bacterial growth inhibition was calculated as described in Equation (2):

$$Inhibition(\%) = \frac{OD_{600}withoutcopper - OD_{600}withcopper}{OD_{600}withoutcopper} \times 100 \quad (2)$$

where OD_{600} *without copper* corresponds to the bacterial growth at 0 mM and OD_{600} *with copper* to the bacterial growth at any of the copper concentrations tested.

2.8.4. Genome annotation and biosynthetic gene cluster prediction

The assembled genomes were annotated with the Rapid Annotation using Sub-system Technology (RAST) web server (http://rast.nmpdr.org) using the RASTtk pipeline and the default parameters. Since both BG1 and BG2 have previously shown biological control traits, the RAST annotated genome was further analyzed using the Antibiotics and Secondary Metabolites Analysis Shell (antiSMASH) webserver (v6.1.1) and BAGEL web server (https://bagel5.molgenrug.nl/) to predict putative biosynthetic clusters (BCs) and to mine bacterial genomic DNA for bacteriocins and post-translationally modified peptides (RiPPs), respectively (Valenzuela-Ruiz et al., 2019).

2.8.5. Pyoverdine production

For pyoverdine production assessment, each strain was cultivated in 3 mL of LB. Ten μL of overnight culture was spot-inoculated on KB-CAS agar plates as described by Owen et al. (2011). Strains that produced a yellow-orange halo around the colony area were retained as positive for siderophore production and further observed under UV light for qualitative assessment of pyoverdine (fluorescence).

2.9. Statistical analysis

For the statistical analyses GraphPad prism 6 was used. The results are presented as individual values in boxplots. The Student's *t*-test was used to assess the significance of differences between the treatment and water control at $p < 0.05$.

3. Results

3.1. Screening for antagonistic activity against Psa, hypersensitive response (HR) and molecular identification

The isolation and purification of bacterial epiphytes from kiwifruit leaves led to a collection of 94 bacterial isolates. All 94 isolates were tested for their ability to inhibit Psa growth *in vitro* (Fig. 1) leading to the selection of 9 isolates as potential BCAs for Psa control. Among them, 4 were isolated from Briteiros de Guimarães, 1 from Geraz do Minho and 4 from Santa Lucrécia plant material. Based on the absence of hypersensitive response induction in tobacco plants (Appendix A, Fig. S1) and their identification through 16S sequencing, two strains from Briteiros de Guimarães (from the leaf batch that incubated at 10 °C, BG1 10 °C D and BG2 10 °C C, hereafter named BG1 and BG2, respectively) were found to be non-phytopathogenic and initially proposed as belonging to *Pseudomonas poae* and *Pseudomonas putida* species (Table 2).

3.2. Genotyping with multilocus sequence typing (MLST), average nucleotide identity (ANI) and amino acid identity (AAI) calculator

The Ribosomal Multilocus Sequence Typing (rMLST) approach analyses the diversity in the 53 genes encoding the bacterial ribosome protein subunits (*rps* genes). The determination of *rps* gene variation

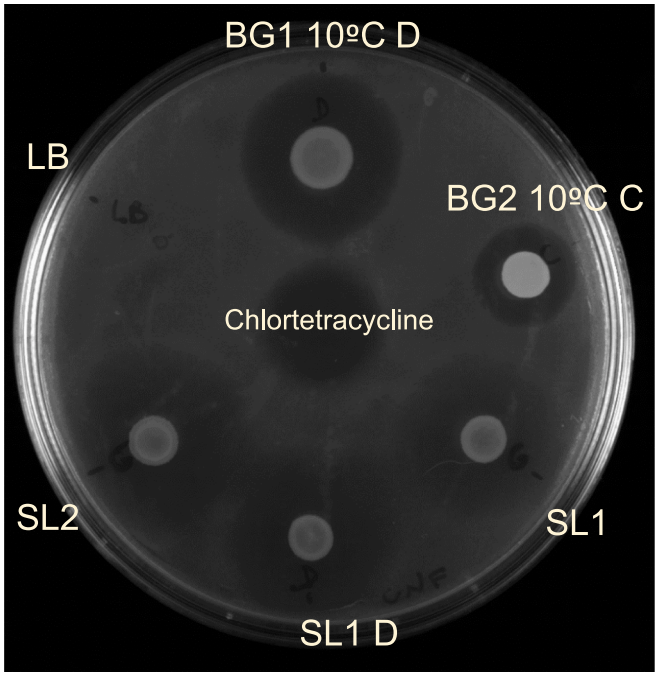

Fig. 1. Evaluation of *P. syringae* pv. *actinidiae* growth inhibition by BG1 and BG2 strain. Psa is visible on the background, while BCA antagonistic activity is represented by halo formation. LB was used as a negative control, while the antibiotic chlortetracycline in the central inhibition circle as a positive control.

Table 2
Hypersensitive response test of the potential BCAs on tobacco leaves and their identification by 16S gene sequencing. (−) indicates no HR (no necrosis); (+) indicates HR (necrosis); BG (Briteiros de Guimarães); GM (Geraz do Minho); SL (Santa Lucrécia).

Isolate	HR	Location	Molecular identification
BG1 10 °C D (BG1)	−	BG	*Pseudomonas poae*
BG2 10 °C C (BG2)	−	BG	*P. putida*
BG1 C	+	BG	*P. syringae* pv. *syringae*
BG2 B	+	BG	*P. syringae* pv. *syringae*
GM2 A	+	GM	*P. syringae* pv. *syringae*
SL1 G	+	SL	*P. syringae* pv. *syringae*
SL1 D	+	SL	*P. syringae* pv. *syringae*
SL2 G	+	SL	*P. syringae* pv. *syringae*
SL4 A	+	SL	*P. syringae* pv. *syringae*

permits the rapid identification of the phylogenetic position of any bacterial sequence at the domain, phylum, class, order, family, genus, species and strain levels. From the 53 genes queried, exact matches for 47 and 46 genes were found for BG1 and BG2, respectively, from which a taxonomic classification was obtained as *P. azotoformans* and *P. putida*, respectively (Appendix A, Table S1-S2).

The average nucleotide identity (ANI) that measures the nucleotide-level genomic similarity among the coding regions of different genomes showed that BG1 shares 90.57 % of its genome with *P. azotoformans* DSM 18862 T, with an ANI value of 96.34 % (best hit) (Table 3). The same analysis revealed that BG2 shares 87.94 % of its genome with the strain *P. putida* NBRC 14164, with an ANI value of 96.33 % (Table 3). Based on amino acid identity (AAI), another index of pairwise genomic relatedness used in prokaryotic taxonomy, consistent with the ANI analysis, revealed a great similarity between BG1 and *P. azotoformans* (AAI 97.63 %), on the one hand, and BG2 and *P. putida* (AAI 97.66) on the other hand (Table 3).

Table 3
Average nucleotide identity (ANI) and amino acid identity (AAI) values for BG1 and BG2 against *P. azotoformans* and *P. putida* genomes from NCBI, respectively.

Dataset	ANI (%)	Fraction of genome shared (%)	AAI (%)	Fraction of proteins shared (%)
P. azotoformans GCA 900624915 1 (DSM 18862T^{T})	96.34	90.57	97.63	91.03
P. putida NBRC 14164 GCA 000412675 1 (NBRC 14164^{T})	96.33	87.94	97.66	89.92

3.3. Evaluation of Psa biological control with selected bacterial isolates in kiwifruit flowers and leaves

BG1 and BG2 were tested for the inhibition of Psa growth and colonization capacity on flowers of *A. chinensis* var. *deliciosa* 'Hayward' and *A. chinensis* var. *chinensis* 'Hort 16A' and leaves of potted *A. chinensis* var. *deliciosa* 'Hayward'. Regarding flower colonization/survival capacity, both BG1 and BG2 showed very high flower colonization capacity and no symptoms of phytotoxicity under the concentration tested. The experiment was performed both in 2020 and 2021, but only the results from 2021 are shown (Fig. 2) since in 2020 the experiment was only performed in *A. chinensis* var. *deliciosa*. Nonetheless, the results obtained in 2020 were consistent with the ones shown in Fig. 2. BG2 showed a preference for *A. chinensis* var. *chinensis* flowers revealing up to 1.5 logs higher population in some of the timepoints, in comparison to *A. chinensis* var. *deliciosa* (Fig. 2B). Moreover, BCA population in flowers was almost not affected by the presence of Psa (Fig. 2). On leaves, both strains could keep a bacterial density over 10^7 CFU/gFW during the first seven days of the trial, with a low population decrease rate over time (Fig. 3). BG1 alone (without the presence of Psa) showed about 0.5 log higher population than the other treatments during this period. BG2 was not affected by Psa presence (Fig. 3).

Regarding the inhibition of Psa growth on flowers, for normalization purposes, the effect of each BCA was compared to the water-treated control of each experiment and presented as the % of Psa population reduction. In 2020 (Fig. 4A), when inoculated alone, Psa could reach a high epiphytic population (over 10^7 CFU mL^{-1}) in approximately 24 h (data not shown). However, the presence of either BG1 or BG2 significantly reduced pathogen population within 24 h. After 48 h, epiphytic Psa population reached a reduction of about 56 % and 87 % in treated flowers with BG2 or BG1, respectively. Conversely, the commercial product Amylo-X®, based on *Bacillus amyloliquefaciens* subsp. *plantarum* D747, used as positive control, did not provide any significant protection (Fig. 4A). In 2021, in *A. chinensis* var. *deliciosa* (Fig. 4B), none of the strains showed a reduction significantly different from the water-treatment, although the % of reduction in some of the timepoints was very high (>40 % for BG1 at 24 h). It is worth precising that, in that year, Psa did not well colonize *A. chinensis* var. *deliciosa* flowers and therefore, though the treatment was highly effective (~100 % reduction), the low population of Psa present in the water-treated control and the high variability negatively influenced the statistical significance of the results. By contrast, in *A. chinensis* var. *chinensis*, both BG1 and BG2 provided a significant reduction in the first 24 h (30–40 % reduction), but such effect was later reduced (Fig. 4C). As observed in *A. chinensis* var. *deliciosa*, Amylo-X® was also unable to efficiently reduce the pathogen population in *A. chinensis* var. *chinensis*. The reduction in Psa population was also visually observed in confocal laser scanning micrographs photography (Appendix A, Fig. S2).

Further studies were done in potted 'Hayward' plant leaves. Both BG1 and BG2 could significantly reduce Psa epiphytic and endophytic population seven days after Psa inoculation (Fig. 5A). More in details, Psa invasion of inner leaf tissues was also reduced of up to 23 % (Fig. 5B). Amylo-X® could not significantly reduce either epiphytic or

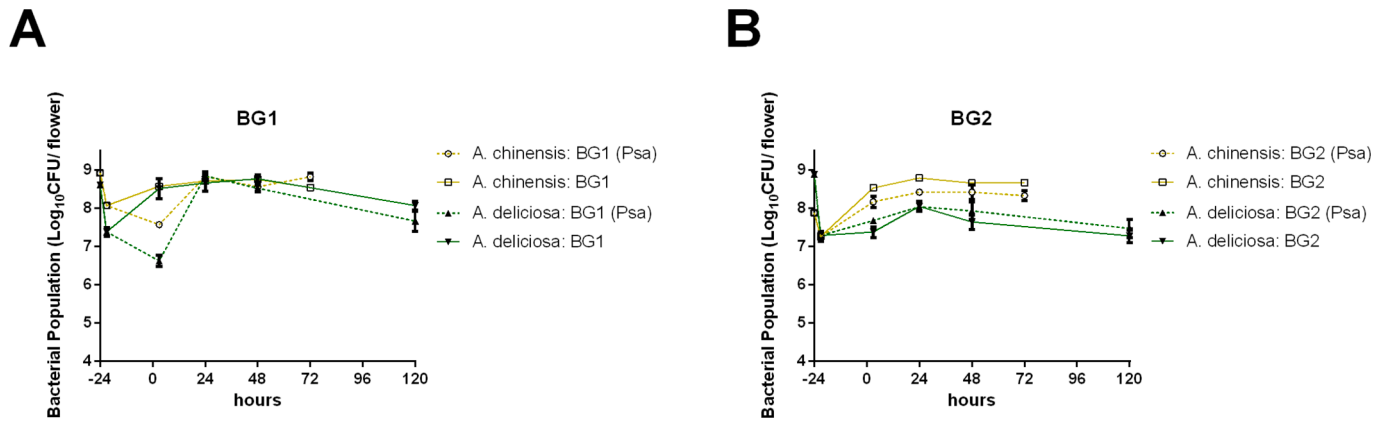

Fig. 2. Epiphytic population of *P. azotoformans* (BG1) and *P. putida* (BG2) on *A. chinensis* var. *deliciosa* and *A. chinensis* var. *chinensis* flowers. Flowers were inoculated with only the BCAs or both with BCAs and Psa (after 24 h), as indicated. The data shown correspond the average of at least 3 biological replicates ± standard errors.

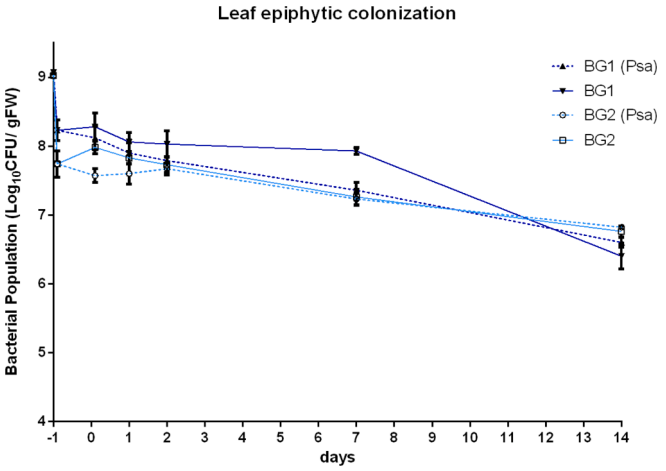

Fig. 3. Epiphytic population of *P. azotoformans* (BG1) and *P. putida* (BG2) on *A. chinensis* var. *deliciosa* leaves. Leaves were inoculated with only the BCAs or both with BCAs and Psa (after 24 h), as indicated. The data shown correspond the average of at least 3 biological replicates ± standard errors.

endophytic Psa populations.

3.4. Characterization of selected strains

3.4.1. Bacterial growth at 10 °C and 27 °C

Because spring and autumn, characterized by mid-low temperatures, are periods that are conducive to Psa infection, BG1, BG2 and Psa were grown in LB at 10 °C, a relevant temperature in the epidemiology of the disease cycle of Psa that can infect flowers early in the season (Fig. 6A). Growth curves were also performed at 27 °C, the standard temperature for bacterial growth and the temperature at which Psa grows on leaves later in the season (Fig. 6B). Both BCAs grew slower at 10 °C (Fig. 6A) than at 27 °C (Fig. 6B), displaying the exponential phase between 72 h-96 h at 10 °C, while between 24 h-48 h at 27 °C (Fig. 6). Psa grew slower than BG1 and BG2 at both temperatures, reaching at the end of the experiment, an OD_{600} that was 5 times and around 2 times lower, at 10 °C and 27 °C, respectively (Fig. 6).

3.4.2. Biochemical characterization

Microbes can improve plant growth, and overall plant health, by interacting with their metabolism through several mechanisms, including enhancing nutrient availability, producing functional volatile organic compounds (VOCs), and modulating plant hormonal balance. BG1 and BG2 were functionally characterized to identify potential plant-growth promotion characteristics and resistance induction. Both BG1 and BG2 were found to produce siderophores, belonging to two different types, namely carboxylates and hydroxamates, respectively (Table 4). None of the strains produced acetoin, IAA or HCN, or expressed ACC deaminase activity, while both isolates produced NH_4^+ (Table 4).

3.4.3. Sensitivity to copper

Copper-based agrochemicals are still widely used in orchard management. Therefore, BG1 and BG2 were tested for their ability to survive in the presence of Cu accumulation. On MGY liquid media amended with several concentrations of copper sulphate, BG1 showed a significant tolerance to the concentrations tested, with a growth inhibition ranging from 50 % to 70 %. BG2 displayed a copper-sensitivity comparable to the positive control at 1.6 and 3.2 mM (Fig. 7).

3.4.4. Genome annotation and prediction of secondary metabolite biosynthetic gene clusters (BGCs)

The genomes were annotated, following the RASTtk pipeline (Appendix A, Fig. S3). Both strains include genes coding for outer membrane efflux proteins, with a potential role in resistance/tolerance to heavy metals like arsenic, cadmium, cobalt, copper and zinc. Moreover, they also present several genes related to iron acquisition and metabolism, including siderophore-encoding genes. Regarding membrane transport, type II, V, VI, VII secretion systems have been annotated for BG1 and type I, II, VI, VII, VIII for BG2. Genes related to the type III secretion system, encountered in many phytopathogenic bacteria, were not found in either strain. Both genomes revealed also the presence of genes involved in the tolerance to abiotic factors (oxidative and water stress conditions), the biological control of phytopathogens (lipopeptides and antibiotic biosynthesis), and the promotion of plant growth (auxin biosynthesis).

Further, in line with their significant biological control activity against Psa, AntiSMASH analysis on the RAST annotated genomes of BG1 and BG2 predicted the presence of several biosynthetic gene clusters (BGC) with similarity to known clusters from the MIBiG database, including pathways involved in the production of cyclic lipopeptides (viscosin and fengycin), siderophores (pyoverdine) and other antimicrobial compounds (thiopeptide and lankacidin) (Table 5-6). Pyoverdine production was confirmed on BG1, by a qualitative test on KB-CAS agar plates (Appendix A, Fig. S4).

In addition, other gene clusters encoding for ribosomally-synthesized and post-translationally modified peptides-like (RiPP-like), as well as N-acetyl glutaminyl glutamine amide (NAGGN) were identified, though without any similarity to known antimicrobial-producing gene clusters present in the MIBiG database. From the identified putative BGCs, when observed, the similarity with known clusters ranged from 4 to 68 % for BG1 and from 5 to 50 % for BG2. Interestingly, in both strains, the BGC

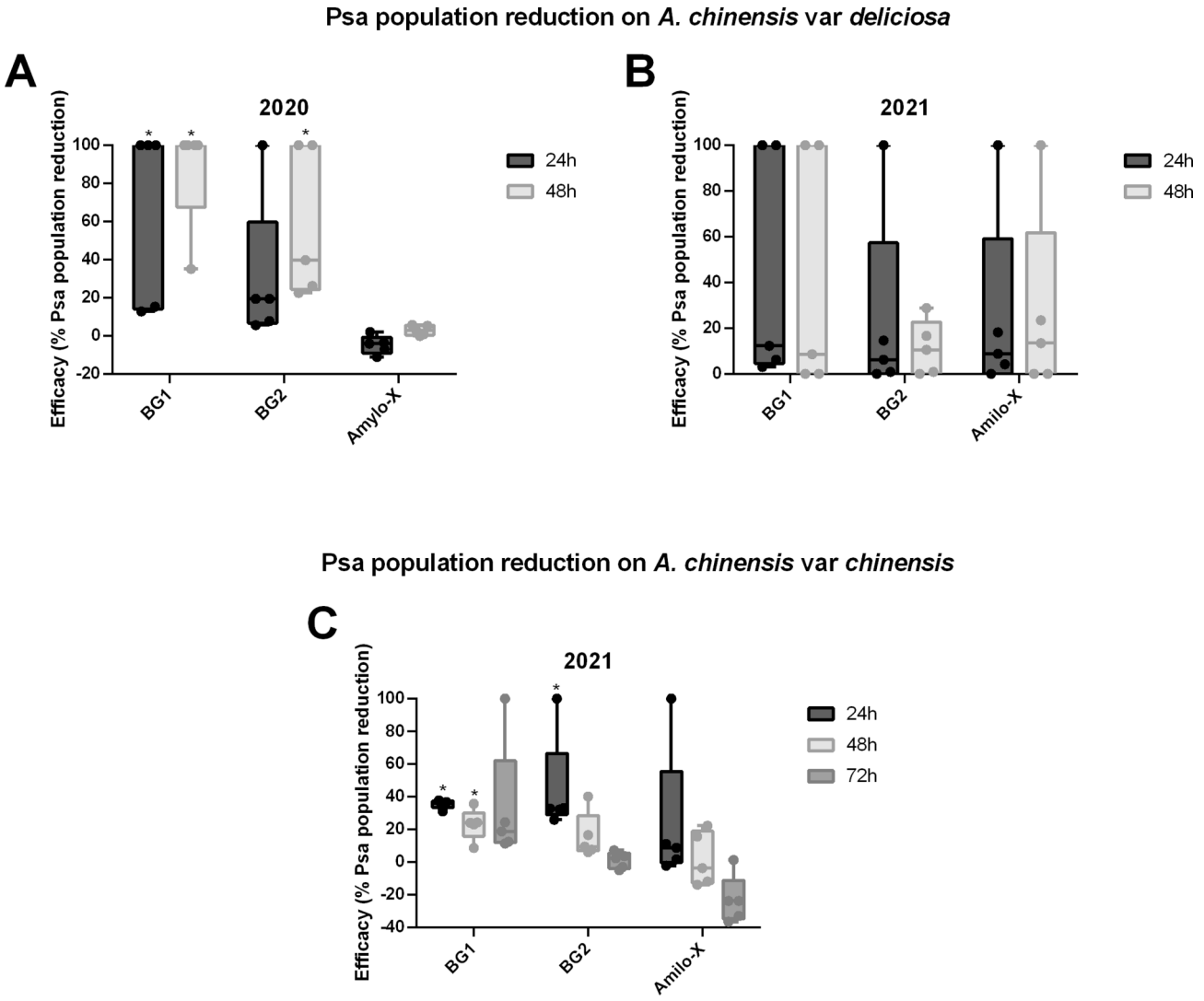

Fig. 4. Efficacy of *P. azotoformans* (BG1) and *P. putida* (BG2) in reducing *P. syringae* pv. *actinidiae* epiphytic population on *Actinidia chinensis* var. *deliciosa* (A-B) and *Actinidia chinensis* var. *chinensis* (C). The efficacy is expressed as the percentage of reduction of Psa population when compared with water-treated plants. Psa epiphytic population was assessed at 24 h, 48 h and, whenever possible, 72 h after inoculation. Boxplot showing median (line inside the box), first and third quartiles (top and bottom lines of the boxes, respectively), maximum and minimum value (top and bottom whiskers, respectively) and original data points (dots). An asterisk indicates a significant difference among the treated samples and the water-treated control, according to the Student's *t*-test (* represent significant differences for $p < 0.05$).

with the highest similarity was a non-ribosomal peptide synthetase (NRPS) similar at 68 and 50 % on BG1 and BG2, respectively, with the lipopeptide viscosin.

In addition to the AntiSMASH, BAGEL, a software more specialised in bacteriocin cluster prediction, identified genes that may be involved in the synthesis of colicin in BG1. The query sequence obtained for the bacteriocin gene cluster was predicted to include two genes encoding for regulatory proteins, one for Immunity/Transport protein, one core peptide that would correspond to the bacteriocin itself, and several genes encoding for proteins with BLAST hit with UniRef90 (composed of sequences displaying at least a 90 % sequence identity) or unknown function (Fig. 8). A BLASTP analysis conducted on the protein sequence of the predicted core peptide revealed an identity of 99 % with an S-type pyocin produced by *Pseudomonas* sp. CYM-20–01 and an identity of more than 95 % with pyocins produced by other strains of the *Pseudomonas* genus.

4. Discussion and conclusion

Kiwifruit leaf and flower phyllosphere bacterial community is mostly composed of the genera *Acinetobacter*, *Actinobacteria*, *Bacillus*, *Pseudomonas*, *Massilia* and *Sphingomonas* (Purahong et al., 2018). Several of these genera include antagonistic species against *P. syringae* (Ye et al., 2014; Nikolić et al., 2019). However, by imposing a stricter selection condition, with an incubation of the plant material at 10 °C for 7 days, the only isolates able to inhibit Psa belonged to the genus *Pseudomonas* confirming the adaptation of these bacteria to low temperature (Moreno and Rojo, 2014; Chauhan et al., 2023). *Pseudomonas* spp. can be isolated from various natural sources, such as plants and soil, and its use in biological control has been widely reviewed (Haas and Défago, 2005; Höfte, 2021). It is a diverse genus with more than 220 described species, including, along with plant pathogens such as Psa, also many beneficial plant-associated strains with the capacity to control plant diseases (Peix et al., 2018; Höfte, 2021). Many *Pseudomonas* isolates have been found to produce antimicrobial compounds, like phenazines, polyketides, cyclic lipopeptides, pyrrolnitrin and hydrogen cyanide (Gross and Loper, 2009; Höfte, 2021), and to promote plant growth by increasing the availability and uptake of mineral nutrients or through root growth enhancement via the production or manipulation of phytohormones, being thus most commonly used as root colonizers (Lugtenberg et al.,

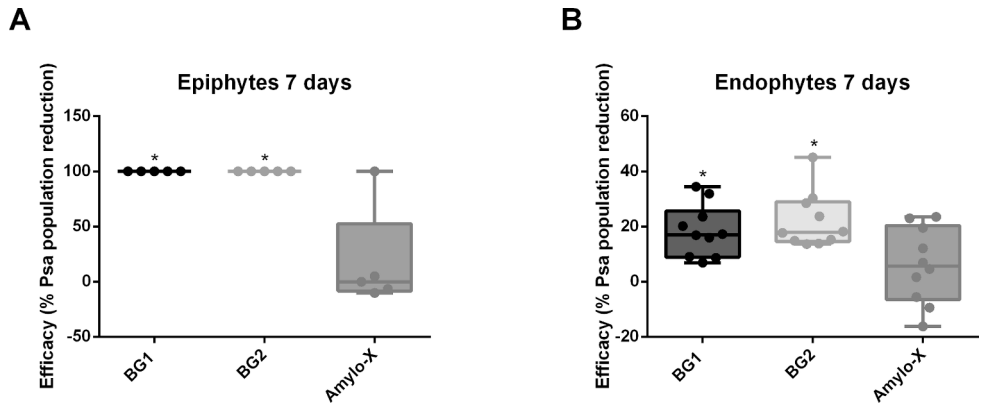

Fig. 5. Efficacy of *P. azotoformans* (BG1) and *P. putida* (BG2) in reducing *P. syringae* pv. *actinidiae* epiphytic (A) and endophytic (B) populations on *Actinidia chinensis* var *deliciosa*. The efficacy is expressed as the percentage of reduction of Psa population when compared with water-treated plants. Psa population was assessed 7 days after the inoculation. Boxplot showing median (line inside the box), first and third quartiles (top and bottom lines of the boxes, respectively), maximum and minimum value (top and bottom whiskers, respectively) and original data points (dots). An asterisk indicates a significant difference among the treated samples and the water-treated control, according to the Student's *t*-test (* represent significant differences for $p < 0.05$).

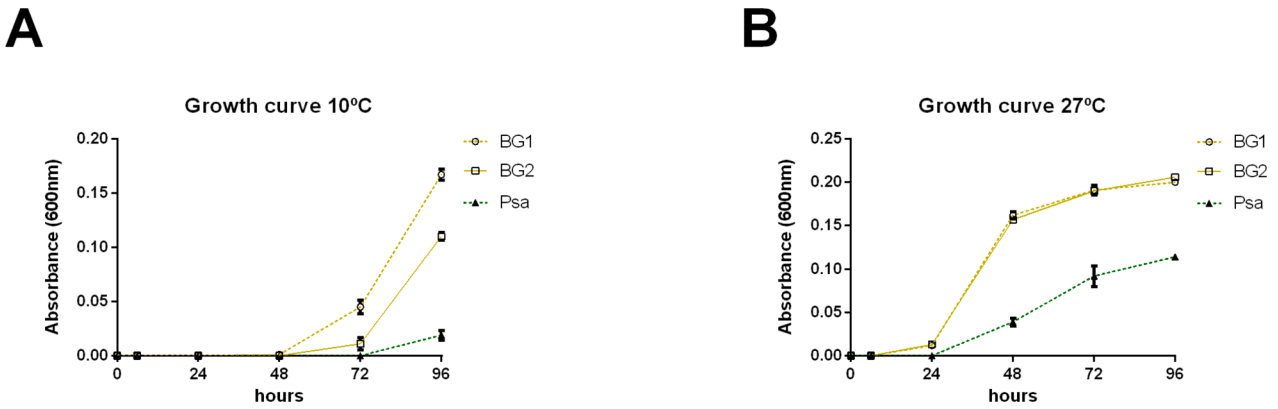

Fig. 6. Growth curve of *P. azotoformans* (BG1), *P. putida* (BG2) and *P. syringae* pv. *actinidiae* (Psa) at 10 °C (A) and 27 °C (B), in LB liquid medium. The data shown correspond to the average of 3 biological replicates ± standard errors.

Table 4
Plant-growth promoting activities.

	Siderophore production	Acetoin production	IAA production	HCN production	NH_4^+ production	ACC deaminase activity
BG1	carboxylates	–	–	–	+	0
BG2	hydroxamates	–	–	–	+	0

2001). Their efficacy as BCAs may also be achieved by nutritional competition and preemptive exclusion (Stockwell et al., 2010).

In the case of kiwifruit phyllosphere, Purahong et al (2018) found that bacterial canker disease was observed when Psa was present in a syndemic association with *Pseudomonas syringae* pv. *syringae* and *Pseudomonas viridiflava*. In addition, the disease was associated to a reduced microbial biodiversity and an overall modified, "pathogenic" microbiome (pathobiome). Thus, it can be assumed that the microbial community plays an important role in disease development. Indeed, it may account for the survival of a pathogen, whose relative abundance can lead to disease onset or greater severity (Lv et al., 2023). From our screening, seven of the nine strains showing antimicrobial activity were identified as *Pseudomonas syringae* pv. *syringae* but since they have shown anti-Psa activity, these isolates may not directly contribute to this potential pathobiome.

Biological control products based on antagonistic bacteria, such as Amylo-X® (containing *Bacillus amyloliquefaciens* ssp. *plantarum* D747), have been used in the management of kiwifruit bacterial canker, though with a poor success (Monchiero et al., 2015). Accordingly, in our study, this commercial product was ineffective in controlling Psa population on the flowers of both *A. chinensis* var. *deliciosa* and *A. chinensis* var. *chinensis*. On the contrary, BG1 and BG2 showed promising results. However, it is worth noticing that *B. amyloliquefaciens* ssp. *plantarum* D747 was used at 10^7 CFU/mL, according to the manufacturer's recommendation for application on kiwifruit, and, therefore, we cannot rule out the possibility that the greater efficacy for BG1 and BG2 could be due to the 100-fold greater concentration used for the experiments. Based on 16S sequencing, BG1 and BG2 were initially proposed as *P. poae* and *P. putida,* which are species well known as antagonists of several plant pathogens (e.g., *Erwinia amylovora*, *Penicillium expansum*, *Fusarium oxysporum*) (Dagher et al., 2020; Jayamohan et al., 2020; Ren et al., 2021), and can also be used as plant-growth promoters (Sun et al., 2019; Chuen et al., 2021). However, following genomic studies, based on the rMLST analysis using a query of 53 genes and considering an ANI value threshold of 95 % (Goris et al., 2007; Jain et al., 2018), BG1 was reclassified as *P. azotoformans,* another *Pseudomonas* species with reported potential for biological control (Fang et al., 2016). The effect of BG1 and BG2 on *A. chinensis* var. *deliciosa* was not very consistent

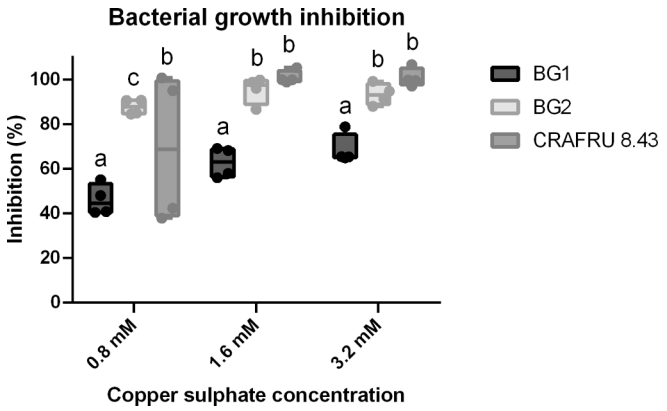

Fig. 7. Inhibition of *P. azotoformans* (BG1), *P. putida* (BG2) and *P. syringae* pv. *actinidiae* (CRAFRU 8.43) by copper sulphate. Boxplot showing median (line inside the box), first and third quartiles (top and bottom lines of the boxes, respectively), maximum and minimum value (top and bottom whiskers, respectively) and original data points (dots). Data were subjected to two-way ANOVA and then Tukey's HSD as post-hoc test to evaluate statistical differences among treatments ($p < 0.05$). Different letters indicate a significant difference among the strains within a treatment.

Table 5
AntiSMASH-predicted biosynthetic gene clusters (BGCs) in *P. azotoformans* (BG1) genome.

Type	Length (bp)[a]	Most similar known cluster name	Similarity (%)[b]
Betalactone	23 079	fengycin	13
RiPP-like	10 824	–	–
NRPS	62 243	Pyoverdine SMX-1	25
NAGGN	14 717	–	–
NRPS	52 878	Pf-5 pyoverdine	9
Thiopeptide	29 625	Lipopolysaccharide	5
RiPP-like	10 845	–	–
Arylpolyene	43 575	APE Vf	40
Redox-cofactor	22 147	Lankacidin	13
RiPP-like	10 875	–	–
NRPS	46 209	MA026	14
NRPS;NRP-metallophore	36 496	Viscosin	68
NRPS-like	26 317	Pyoverdine DC3000	4

[a] bp, base pair.
[b] , "-" indicates no similarity with known clusters.

Table 6
AntiSMASH-predicted biosynthetic gene clusters (BGCs) in *P. putida* (BG2) genome.

Type	Length (bp)[a]	Most similar known cluster	Similarity (%)[b]
Redox-cofactor	16 110	Lankacidin	13
NRP-metallophore	74 409	Pf-5 pyoverdine	5
NRPS	80 192	Viscosin	50
ranthipeptide	21 430	Pf-5 pyoverdine	7
RiPP-like	12 186	–	–
NAGGN	14 903	–	–
RiPP-like	10 833	–	–
NRPS-like	42 771	Pyoverdine SMX-1	6
NRPS	31 096	Pf-5 pyoverdine	5

[a] bp, base pair.
[b] , "-" indicates no similarity with known clusters.

between experiments, likely due to the phenological stage of the flowers used in the different experiments (higher BBCH in 2021). This may explain why water-treated plants couldn't be efficiently colonized by Psa, and thus, BG1 and BG2 couldn't disclose a statistically significant effect. Nonetheless, in *A. chinensis* var. *deliciosa* (2020) and *A. chinensis* var. *chinensis* (2021), both BG1 and BG2 showed a significant reduction in flower colonization by Psa. Moreover, despite the poor colonization efficiency of Psa on *A. chinensis* var *deliciosa* in 2021, both BG1 and BG2 showed high population densities, suggesting that they can survive with the resources available at the late stages of flower development, when stigmas start drying. Moreover, from the growth curves at 10 °C we can assume that these bacteria are well adapted to medium–low temperatures (typical from spring and autumn).

One week after Psa inoculation on leaves, the pathogen could not be detected epiphytically on leaves pre-treated with either BG1 or BG2, likely due to their fast growth and good colonization capacity, as verified on flowers and leaves. The high population density and persistence of the antagonists, even after several days, suggests that they may persist in the plant for a long time, continuously acting as pathogen inhibitors. In our experimental conditions, in which a high-density Psa inoculum was used, the density and persistence of BCAs was not sufficient to fully prevent the infection, as verified by Psa endophytic population reduction that did not exceed 23 %. However, in orchard conditions, with lower Psa inoculum density and fluctuations of temperature and humidity, infection might be less efficient, facilitating pathogen control.

The characterization of putative BCAs, using genomic, transcriptomic and proteomic approaches, is essential to improve their efficacy and understand their full potential as biopesticides (Massart et al., 2015). Moreover, the availability of sequencing techniques at affordable prices has enabled researchers to speed up the discovery of novel antimicrobial compounds reducing the time-consuming workflow for the isolation and characterization of potential candidate compounds (Nielsen et al., 2011; Naughton et al., 2017). RAST annotation showed that both BG1 and BG2 are likely tolerant to heavy metals like arsenic, cadmium, cobalt, copper and zinc, due to the presence of several genes coding for efflux outer membrane proteins. In line with this finding, the copper-sensitivity test revealed that BG1 is significantly less sensitive than BG2 and Psa CRAFRU 8.43 to copper concentrations up to 3.2 mM, which may be attributable to its isolation from a conventional orchard, where copper-based treatments and other foliar chemical applications are frequent, thus likely leading to resistance/tolerance acquisition. In addition, genes encoding for enzymes of the lipopeptide pathway and for NRPs, identified in this work, may contribute to antimicrobial capacity (Liu et al., 2020). In particular, nonribosomal peptides are bioactive compounds that gained a significant interest for their application to control phytopathogens (Ranjan et al., 2023). In this study, nine BGCs were identified for the synthesis of NRPs (five in BG1 and four in BG2), two of them (one per strain) likely producing a NRP with a high similarity to the lipopeptide viscosin. Other *P. putida* have been reported to produce lipopeptides from the viscosin family, while no viscosin production has been described in *P. azotoformans* so far. Most of the remaining NRPs were predicted to share similarity to the siderophore pyoverdine, in line with the annotation of genes involved in siderophore production (RAST), however this similarity is low, indicating that these BGCs may not be functional. Siderophores are typically classified as catecholates, hydroxamates, and α-carboxylates. However, some siderophores like pyoverdine are classified as "mixed" (e.g., containing both hydroxamate and catecholate functional groups) (Meyer and Hornsperger, 1978; Meyer and Stintzi, 1998). In this work, the siderophores detected in the biochemical experiments belong to the hydroxamate and carboxylate groups, but colour tone changes if more than one functional group is present which may lead to misinterpretation (Pérez-Miranda et al., 2007). Therefore, pyoverdine production was qualitatively assessed by growing BG1 and BG2 on KB-CAS medium (Taguki et al., 2010). Both strains produced a yellow-orange halo, which confirms their capacity to produce siderophores. However, plate observation under UV light evidenced that only BG1 produced a fluorescent blue-green halo, confirming its ability to produce pyoverdine. Similarly, the commercial product BlightBan, that uses the beneficial bacterium *Pseudomonas*

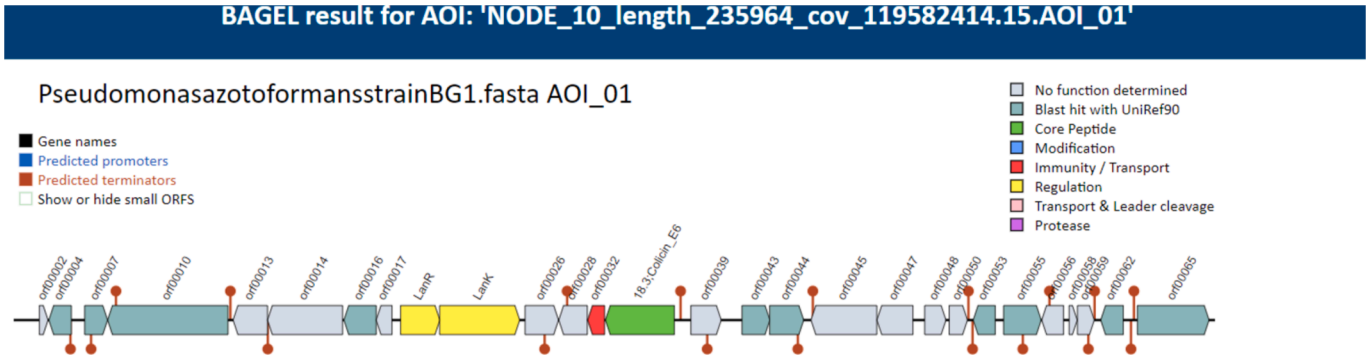

Fig. 8. BAGEL output for bacteriocin colicin predicted in *P. azotoformans* (BG1) genome.

fluorescens A506, competes with fire blight bacterium for nutrients in apple and pear tree blossoms keeping the number of harmful bacteria low to avoid severe fire blight infection (Wilson and Lindow, 1993; Lindow and Suslow, 2003).

A putative BGC for colicin was identified in BG1 genome. Colicins are a subfamily of bacteriocins (peptide toxins) produced by Gram-negative bacteria, forming pores in cell membranes, allowing ion fluxes finally resulting in membrane depolarization (Cascales et al., 2007). In this study, we identified a core peptide with colicin-like traits that shares 99 % identity with a pyocin (i.e., *Pseudomonas* spp.-specific colicin), suggesting that BG1 produces this bacteriocin, which may help it in reducing the competition from other neighbouring bacterial strains. The deciphering of their modes of action using traditional microbiological methods and a biochemical characterization (in-depth metabolic studies) may help in the interpretation of AntiSMASH and BAGEL results.

Despite the description of several putative pathways producing antimicrobial compounds, BG1 and BG2 supernatants did not display any antimicrobial activity (data not shown). Thus, the production of antimicrobial compounds may be induced by the presence of the pathogen or other environmental triggers (Zong et al., 2022), or BG1 and BG2 may control Psa mainly by competition for nutrients and space. In this scenario, siderophores, like pyoverdine, may play an important role and the prediction of several BGCs with similarity to pyoverdine may indicate diverse routes for siderophore production. Alternatively, pyoverdine biosynthetic machinery may act indirectly in the production of other antimicrobial compounds, as observed in the *P. putida* W15Oct28 (Ye et al., 2014).

Other strains of *P. putida* and *P. azotoformans* have been reported as BCAs, in particular against other *Pseudomonas* spp. (Ye et al., 2014; Lee et al., 2015; Fang et al., 2016; Daura-Pich et al., 2020), thus supporting the potential use of BG1 and BG2 as BCAs. Importantly, their isolation from kiwifruit orchard may represent an advantage for their adaptation to the environmental conditions, thus likely ensuring their efficiency in controlling Psa in field conditions.

Overall, our results support the idea that selected epiphytes may be considered as potential BCAs against Psa, particularly considering that such candidates belong to the natural phyllosphere of the host plant. *Pseudomonas* spp. may be particularly effective competitors of Psa due to their similar metabolic needs and niche requirements. The selection of BCAs able to grow at low temperature, such as the one occurring at blooming and/or autumn, may be particularly useful to control Psa in the most vulnerable phenological stages of kiwifruit growth. Moreover, this study allowed the accurate taxonomic assignment of BG1 and BG2 as *P. azotoformans* and *P. putida*, respectively, two species with previous reported BCA potential. RAST, AntiSMASH and biochemical analyses indicated that both strains produce siderophores and pyoverdine production was confirmed for BG1, possibly contributing to the inhibition of Psa growth. The characterization of their precise mode of action, together with the evaluation of their effectiveness in orchard conditions, the safety for pollinators, the possible insect-mediated spread in the orchard and comprehensive analysis of human health risks, still need further investigations.

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CRediT authorship contribution statement

Cristiana Correia: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Antonio Cellini:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Irene Donati:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Panagiotis Voulgaris:** Methodology. **Adebayo Ebenezer Obafemi:** Methodology. **Elia Soriato:** Methodology. **Elodie Vandelle:** Writing – review & editing, Funding acquisition, Formal analysis, Conceptualization. **Conceição Santos:** Writing – review & editing, Funding acquisition, Formal analysis, Conceptualization. **Francesco Spinelli:** Writing – review & editing, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2025.105706.

References

Abbasi, P.A., Khabbaz, S.E., Weselowski, B., Zhang, L., 2015. Occurrence of copper-resistant strains and a shift in Xanthomonas spp. causing tomato bacterial spot in Ontario. Can. J. Microbiol. 61, 753–761.

Ahmad, F., Ahmad, I., Khan, M., 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol. Res. 163, 173–181.

Bakker, A.W., Schippers, B.O.B., 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp-mediated plant growth-stimulation. Soil Biol. Biochem. 19, 451–457.
Banerjee, A., Donald, A.B., Joshi, S.R., 2017. Native microorganisms as potent bioinoculants for plant growth promotion in shifting agriculture (Jhum) systems. J. Soil Sci. Plant Nutr. 17, 127–140.
Bardin, M., Ajouz, S., Comby, M., Lopez-Ferber, M., Graillot, B., Siegwart, M., Nicot, P.C., 2015. Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides? Front. Plant Sci. 6, 566.
Berg, G., Rybakova, D., Fischer, D., Cernava, T., Vergès, M.C.C., Charles, T., Chen, X., Cocolin, L., Eversole, K., Corral, G.H., Kazou, M., Kinkel, L., Lange, L., Lima, N., Loy, A., Macklin, J.A., Maguin, E., Mauchline, T., McClure, R., Mitter, B., Ryan, M., Sarand, I., Smidt, H., Schelkle, B., Roume, H., Kiran, G.S., Selvin, J., de Souza, R.S.C., van Overbeek, L., Singh, B.K., Wagner, M., Walsh, A., Sessitsch, A., Schloter, M., 2020. Microbiome definition re-visited: Old concepts and new challenges. Microbiome 8, 103.
Blomqvist, K., Nikkola, M., Lehtovaara, P., Suihko, M.L., Airaksinen, U., Stråby, K.B., Penttilä, M.E., 1993. Characterization of the genes of the 2, 3-butanediol operons from Klebsiella terrigena and Enterobacter aerogenes. J. Bacteriol. 175, 1392–1404.
Bruisson, S., Zufferey, M., L'Haridon, F., Trutmann, E., Anand, A., Dutartre, A., De Vrieze, M., Weisskopf, L., 2019. Endophytes and epiphytes from the grapevine leaf microbiome as potential biocontrol agents against phytopathogens. Front. Microbiol. 10, 2726.
Cappuccino, J.G., Sherman, N., 1992. Microbiology. A Laboratory Manual. The Benjamin/Cummings Publishing Co., California, USA, p. 76.
Cascales, E., Buchanan, S.K., Duché, D., Kleanthous, C., Lloubes, R., Postle, K., Riley, M., Slatin, S., Cavard, D., 2007. Colicin biology. Microbiol. Mol. Biol. Rev. 71, 158–229.
Cesari, E., Marocchi, F., L'Aurora, A., Pucci, N., Scala, V., Loreti, S., Scortichini, M., 2023. Occurrence of copper-resistant *Pseudomonas syringae* pv. *actinidiae* strains in kiwifruit orchards of Central Italy. J. Phytopathol. 171, 768–774.
Chauhan, M., Kimothi, A., Sharma, A., Pandey, A., 2023. Cold adapted *Pseudomonas*: ecology to biotechnology. Front. Microbiol. 14, 1218708.
Chuen, N.L., Muda, R.H., Ahmad, K., 2021. Effectiveness of indigenous fluorescent pseudomonads in suppressing *Rhizoctonia solani* root rot disease and promoting plant growth in chilli seedlings. Malasyan J. Microbiol. 17, 200–211.
Colombi, E., Straub, C., Künzel, S., Templeton, M.D., McCann, H.C., Rainey, P.B., 2017. Evolution of copper resistance in the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae* through acquisition of integrative conjugative elements and plasmids. Environ. Microbiol. 19, 819–832.
Dagher, F., Olishevska, S., Philion, V., Zheng, J., Déziel, E., 2020. Development of a novel biological control agent targeting the phytopathogen *Erwinia amylovora*. Heliyon 6, e05222.
Daura-Pich, O., Hernandez, I., Pinyol-Escala, L., Lara, J.M., Martínez-Servat, S., Fernandez, C., López-García, B., 2020. No antibiotic and toxic metabolites produced by the biocontrol agent *Pseudomonas putida* strain B2017. FEMS Microbiol. Lett. 367, fnaa075.
Donati, I., Cellini, A., Buriani, G., Mauri, S., Kay, C., Tacconi, G., Spinelli, F., 2018. Pathways of flower infection and pollen-mediated dispersion of *Pseudomonas syringae* pv. *actinidiae*, the causal agent of kiwifruit bacterial canker. Hortic. Res. 5.
Donati, I., Cellini, A., Sangiorgio, D., Vanneste, J.L., Scortichini, M., Balestra, G.M., Spinelli, F., 2020. *Pseudomonas syringae* pv. *actinidiae*: Ecology, infection dynamics and disease epidemiology. Microb. Ecol. 80, 81–102.
Fang, Y., Wu, L., Chen, G., Feng, G., 2016. Complete genome sequence of *Pseudomonas azotoformans* S4, a potential biocontrol bacterium. J. Biotechnol. 227, 25–26.
FAO (Food and Agriculture Organization of the United Nations), 2022. Five ways climate change is intensifying the threats to plant health. Available online: https://www.fao.org/fao-stories/article/en/c/1507753/.
FAOSTAT (Food and Agriculture Organization of the United Nations - Statistic division), 2021. Available online: https://www.statista.com/statistics/1263369/global-pesticide-use-per-area/; accessed on 1/06/2023.
Figueira, D., Garcia, E., Ares, A., Tiago, I., Veríssimo, A., Costa, J., 2020. Genetic diversity of *Pseudomonas syringae* pv. *actinidiae*: Seasonal and spatial population dynamics. Microorganisms 8, 931.
Firrao, G., Torelli, E., Polano, C., Ferrante, P., Ferrini, F., Martini, M., Marcelletti, S., Scortichini, M., Ermacora, P., 2018. Genomic structural variations affecting virulence during clonal expansion of *Pseudomonas syringae* pv. *actinidiae* biovar 3 in Europe. Front. Microbiol. 9, 656.
Garcia, E., Moura, L., Abelleira, A., Aguín, O., Ares, A., Mansilla, P., 2018. Characterization of *Pseudomonas syringae* pv. *actinidiae* biovar 3 on kiwifruit in north-west Portugal. J. Appl. Microbiol. 125, 1147–1161.
Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P., Tiedje, J. M., 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int. J. Syst. Evol. Microbiol. 57, 81–91.
Gross, H., Loper, J.E., 2009. Genomics of secondary metabolite production by *Pseudomonas* spp. Nat. Prod. Rep. 26, 1408–1446.
Haas, D., Défago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat. Rev. Microbiol. 3, 307–319.
He, R., Liu, P., Jia, B., Xue, S., Wang, X., Hu, J., Shoffe, Y.A., Gallipoli, L., Mazzaglia, A., Balestra, G.M., Zhu, L., 2019. Genetic diversity of *Pseudomonas syringae* pv. *actinidiae* strains from different geographic regions in China. Phytopathology 109, 347–357.
Heimpel, G.E., Mills, N.J., 2017. Biological control. Cambridge University Press.
Höfte, M., 2021. The use of *Pseudomonas* spp. as bacterial biocontrol agents to control plant disease. *Microbial Bioprotectants for Plant Disease Management*. Burleigh Dodds.
Jain, C., Rodriguez-R, L.M., Phillippy, A.M., Konstantinidis, K.T., Aluru, S., 2018. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat. Commun. 9, 5114.
Jayamohan, N.S., Patil, S.V., Kumudini, B.S., 2020. Seed priming with *Pseudomonas putida* isolated from rhizosphere triggers innate resistance against *Fusarium* wilt in tomato through pathogenesis-related protein activation and phenylpropanoid pathway. Pedosphere 30, 651–660.
Klement, Z., 1963. Rapid detection of the pathogenicity of phytopathogenic *Pseudomonas*. Nature 199, 299–300.
Köhl, J., Kolnaar, R., Ravensberg, W.J., 2019. Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. Front. Plant Sci., 845.
Lee, Y.S., Kim, G.H., Song, Y.R., Oh, C.S., Koh, Y.J., Jung, J.S., 2020. Streptomycin resistant isolates of *Pseudomonas syringae* pv. *actinidiae* in Korea. Research in Plant Disease 26, 44–47.
Lee, C.J., Moon, J.W., Yoo, Y.M., Han, J.Y., Cheong, J.C., Kong, W.S., 2015. Optimum cultivation conditions for mass production of antagonistic bacterium *Pseudomonas azotoformans* HC5 effective in antagonistic of brown blotch disease caused by *Pseudomonas tolaasii*. J. Mushroom 13, 97–102.
Lelliot, R.A., Stead, D.E., 1987. Methods for the diagnosis of bacterial disease of plants. British Society for Plant Pathology, Blackwell Scientifc Publications, Oxford (UK).
Lindow, S.E., Suslow, T.V., 2003. Temporal dynamics of the biocontrol agent *Pseudomonas fluorescens* strain A506 in flowers in inoculated pear trees. Phytopathology 93, 727–737.
Liu, Y., Teng, K., Wang, T., Dong, E., Zhang, M., Tao, Y., Zhong, J., 2020. Antimicrobial *Bacillus velezensis* HC6: production of three kinds of lipopeptides and biocontrol potential in maize. J. Appl. Microbiol. 128, 242–254.
Lugtenberg, B.J., Dekkers, L., Bloemberg, G.V., 2001. Molecular determinants of rhizosphere colonization by *Pseudomonas*. Annu. Rev. Phytopathol. 39, 461–490.
Lv, T., Zhan, C., Pan, Q., Xu, H., Fang, H., Wang, M., Matsumoto, H., 2023. Plant pathogenesis: Toward multidimensional understanding of the microbiome. iMeta e129.
Mariz-Ponte, N., Regalado, L., Gimranov, E., Tassi, N., Moura, L., Gomes, P., Tavares, F., Santos, C., Teixeira, C., 2021. A synergic potential of antimicrobial peptides against *Pseudomonas syringae* pv. *actinidiae*. Molecules 26, 1461.
Mariz-Ponte, N., Gimranov, E., Rego, R., Moura, L., Santos, C., Tavares, F., 2022. Distinct phenotypic behaviours within a clonal population of *Pseudomonas syringae* pv. *actinidiae*. PLoS One 17, e0269343.
Massart, S., Perazzolli, M., Höfte, M., Pertot, I., Jijakli, M.H., 2015. Impact of the omic technologies for understanding the modes of action of biological control agents against plant pathogens. BioControl 60, 725–746.
May, A., Coelho, L.F., Pedrinho, A., Batista, B.D., Mendes, L.W., Mendes, R., Morandi, M. A., Barth, G., Viana, R.S., Vilela, E.S.D., 2021. The use of indigenous bacterial community as inoculant for plant growth promotion in soybean cultivation. Arch. Agronomy and Soil Sci. 69, 135–150.
Meyer J M, Stintzi A. 1998. Iron metabolism and siderophores in *Pseudomonas* and related species. In: Montie, T.C. (Ed.), Biotechnology Handbooks, vol. 10: Pseudomonas. Plenum Publishing Co., New York, N.Y., 201–243.
Meyer, J.M., Hornsperger, J.M., 1978. Role of pyoverdine Pf, the iron-binding fluorescent pigment of *Pseudomonas fluorescens*, in iron transport. J. General Microbiol. 107, 329–331.
Meyer, J.M., Hornsperger, J.M., 1978. Role of pyoverdine Pf, the iron-binding fluorescent pigment of *Pseudomonas fluorescens*, in iron transport. J. General Microbiol. 107, 329–331.
Minas, K., McEwan, N.R., Newbold, C.J., Scott, K.P., 2011. Optimization of a high-throughput CTAB-based protocol for the extraction of qPCR-grade DNA from rumen fluid, plant and bacterial pure cultures. FEMS Microbiol. Lett. 325, 162–169.
Monchiero, M., Gullino, M.L., Pugliese, M., Spadaro, D., Garibaldi, A., 2015. Efficacy of different chemical and biological products in the control of *Pseudomonas syringae* pv. *actinidiae* on kiwifruit. Australas. Plant Pathol. 44, 13–23.
Moreno, R., Rojo, F., 2014. Features of pseudomonads growing at low temperatures: another facet of their versatility. Environ. Microbiol. Rep. 6, 417–426.
Naughton, L.M., Romano, S., O'Gara, F., Dobson, A.D., 2017. Identification of secondary metabolite gene clusters in the *Pseudovibrio* genus reveals encouraging biosynthetic potential toward the production of novel bioactive compounds. Front. Microbiol. 8, 1494.
Nielsen, K.F., Månsson, M., Rank, C., Frisvad, J.C., Larsen, T.O., 2011. Dereplication of microbial natural products by LC-DAD-TOFMS. J. Nat. Prod. 74, 2338–2348.
Nikolić, I., Berić, T., Dimkić, I., Popović, T., Lozo, J., Fira, D., Stanković, S., 2019. Biological control of *Pseudomonas syringae* pv. *aptata* on sugar beet with *Bacillus pumilus* SS-10.7 and *Bacillus amyloliquefaciens* (SS-12.6 and SS-38.4) strains. J. Appl. Microbiol. 126, 165–176.
Otto-Hanson, L.K., Grabau, Z., Rosen, C., Salomon, C.E., Kinkel, L.L., 2013. Pathogen variation and urea influence selection and success of *Streptomyces* mixtures in biological control. Phytopathology 103, 34–42.
Owen, J.G., Ackerley, D.F., 2011. Characterization of pyoverdine and achromobactin in *Pseudomonas syringae* pv. *phaseolicola* 1448a. BMC Microbiol. 11, 1–16.
Peix, A., Ramírez-Bahena, M.H., Velázquez, E., 2018. The current status on the taxonomy of *Pseudomonas* revisited: an update. Infection, Genetics and Evolution 57, 106–116.
Penrose, D.M., Glick, B.R., 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. Physiol. Plant. 118, 10–15.
Pérez-Miranda, S., Cabirol, N., George-Téllez, R., Zamudio-Rivera, L.S., Fernández, F.J., 2007. O-CAS, a fast and universal method for siderophore detection. J. Microbiol. Methods 70, 127–131.
Perpetuini, G., Donati, I., Cellini, A., Orrú, L., Giongo, L., Farneti, B., Spinelli, F., 2019. Genetic and functional characterization of the bacterial community on fruit of three raspberry (*Rubus idaeus*) cultivars. J. Berry Res. 9, 227–247.

Prencipe, S., Gullino, M.L., Spadaro, D., 2018. *Pseudomonas syringae* pv. *actinidiae* isolated from *Actinidia chinensis* var. *deliciosa* in Northern Italy: Genetic diversity and virulence. Eur. J. Plant Pathol. 150, 191–204.

Purahong, W., Orrù, L., Donati, I., Perpetuini, G., Cellini, A., Lamontanara, A., Michelotti, V., Tacconi, G., Spinelli, F., 2018. Plant microbiome and its link to plant health: Host species, organs and *Pseudomonas syringae* pv. *actinidiae* infection shaping bacterial phyllosphere communities of kiwifruit plants. Front. Plant Sci. 9, 1563.

Ranjan, A., Rajput, V.D., Prazdnova, E.V., Gurnani, M., Bhardwaj, P., Sharma, S., Sushkova, S., Mandzhieva, S.S., Minkina, T., Sudan, J., Zargar, S.M., Chauhan, A., Jindal, T., 2023. Nature's antimicrobial arsenal: Non-Ribosomal Peptides from PGPB for plant pathogen biocontrol. Fermentation 9, 597.

Commission Implementing Regulation (EU) 2018/1981 of 13 December 2018 renewing the approval of the active substances copper compounds, as candidates for substitution, in accordance with regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. 2018. Available online: http://data.europa.eu/eli/reg_impl/2018/1981/oj.

Commission Regulation (EU) 2022/1439 of 31 August 2022 amending Regulation (EU) No 283/2013 as regards the information to be submitted for active substances and the specific data requirements for micro-organisms. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R1439.

Ren, Y., Yao, M., Chang, P., Sun, Y., Li, R., Meng, D., Xia, X., Wang, Y., 2021. Isolation and characterization of a *Pseudomonas poae* JSU-Y1 with patulin degradation ability and biocontrol potential against *Penicillium expansum*. Toxicon 195, 1–6.

Rodriguez-R, L.M., Konstantinidis, K.T., 2014. Bypassing cultivation to identify bacterial species. Microbe 9, 111–118.

Sangiorgio, D., Cellini, A., Spinelli, F., Donati, I., 2023. Promoting strawberry (*Fragaria*× *ananassa*) stress resistance, growth, and yield using native bacterial biostimulants. Agronomy 13, 529.

Santiago, C.D., Yagi, S., Ijima, M., Nashimoto, T., Sawada, M., Ikeda, S., Asano, K., Orisaka, Y., Ohwada, T., 2017. Bacterial compatibility in combined inoculations enhances the growth of potato seedlings. Microbes Environ. 32, 14–23.

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., Nelson, A., 2019. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 3, 430–439.

Sawada, H., Fujikawa, T., 2019. Genetic diversity of *Pseudomonas syringae* pv. *actinidiae*, pathogen of kiwifruit bacterial canker. Plant Pathol. 68, 1235–1248.

Scortichini, M., Marcelletti, S., Ferrante, P., Petriccione, M., Firrao, G., 2012. *Pseudomonas syringae* pv. *actinidiae*: A re-emerging, multi-faceted, pandemic pathogen. Mol. Plant Pathol 13, 631–640.

Spinelli, F., Donati, I., Vanneste, J.L., Costa, M., Costa, G., 2010. Real time monitoring of the interactions between *Pseudomonas syringae* pv. *actinidiae* and *Actinidia* species. In vii International Symposium on Kiwifruit 913, 461–465.

Stockwell, V.O., Johnson, K.B., Sugar, D., Loper, J.E., 2010. Control of fire blight by *Pseudomonas fluorescens* A506 and *Pantoea vagans* C9-1 applied as single strains and mixed inocula. Phytopathology 100, 1330–1339.

Sun, H., Kong, L., Du, H., Chai, Z., Gao, J., Cao, Q., 2019. Benefits of *Pseudomonas poae* s61 on *Astragalus mongholicus* growth and bioactive compound accumulation under drought stress. J. Plant Interact. 14, 205–212.

Valenzuela-Ruiz, V., Robles-Montoya, R.I., Parra-Cota, F.I., Santoyo, G., del Carmen, O.-M., Rodríguez-Ramírez, R., de Los, S.-V., 2019. Draft genome sequence of *Bacillus paralicheniformis* TRQ65, a biological control agent and plant growth-promoting bacterium isolated from wheat (*Triticum turgidum* subsp. *durum*) rhizosphere in the Yaqui Valley, Mexico. 3 Biotech 9, 1–7.

Vishwakarma, K., Kumar, N., Shandilya, C., Mohapatra, S., Bhayana, S., Varma, A., 2020. Revisiting plant–microbe interactions and microbial consortia application for enhancing sustainable agriculture: A review. Front. Microbiol. 11, 560406.

Wicaksono, W.A., Jones, E.E., Casonato, S., Monk, J., Ridgway, H.J., 2018. Biological control of *Pseudomonas syringae* pv. *actinidiae* (Psa), the causal agent of bacterial canker of kiwifruit, using endophytic bacteria recovered from a medicinal plant. Biol. Control 116, 103–112.

Wilson, M., Lindow, S.E., 1993. Interactions between the biological control agent *Pseudomonas fluorescens* strain A506 and *Erwinia amylovora* in pear blossoms. Phytopathology 83, 117–123.

Ye, L., Hildebrand, F., Dingemans, J., Ballet, S., Laus, G., Matthijs, S., Berendsen, R., Cornelis, P., 2014. Draft genome sequence analysis of a *Pseudomonas putida* W15Oct28 strain with antagonistic activity to Gram-positive and *Pseudomonas* sp. pathogens. PLoS One 9, e110038.

Zong, G., Fu, J., Zhang, P., Zhang, W., Xu, Y., Cao, G., Zhang, R., 2022. Use of elicitors to enhance or activate the antibiotic production in Streptomyces. Crit. Rev. Biotechnol. 42, 1260–1283.